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CLENBUTEROL TREATMENT DIFFERENTLY AFFECTS MUSCLE IN EXERCISED OR SEDENTARY RATS

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ABSTRACT

CLENBUTEROL TREATMENT DIFFERENTLY AFFECTS MUSCLE IN EXERCISED OR SEDENTARY RATS. **Helian Cavalie, Remi Mounier, Eric Clottes, Veronique Bricout, Gerard Lac.** *JEP online* 2004;7(3):111-120. Three month-old male Wistar rats (8 animals/group) were assigned to four groups as follows. Initially, rats were assigned to two groups: Group 1 for eventual completion of a progressive isometric force strength training exercise program (T) and group 2 to remain sedentary (S). Each group was subdivided in two, where one sub-group received treatment with clenbuterol (TCL and SCL groups, respectively), a selective β_2 -adrenergic receptor agonist, five days a week (2 mg/kg body wt/day), and the other did not (TCON and SCON, respectively). At the end of the 8 weeks, body composition was measured. Training reduced fat mass content. This was amplified in the TCL group, whereas lean mass was increased in both SCL and TCL groups, and reduced in the SCL. Soleus and plantaris myosin heavy chain (MHC) compositions were affected by drug treatment and/or training, with a shift to a faster phenotype in both muscles. Strength endurance capacity was reduced in the TCL group. Clenbuterol administration and/or training also affected enzymatic activities in these two muscles and more specially plantaris: citrate synthase and lactate dehydrogenase activities were decreased in SCL and TCL group whereas creatine kinase activity was augmented in both TCON SCL and TCL group. In conclusion, this study showed that clenbuterol-induced muscular hypertrophy does not depend on plasma IGF-1 content whereas the decrease of strength endurance capacity observed during the sustained endurance test is mainly due to a decline on muscular oxidative and glycolytic metabolisms.

Key Words: Strength training, MHC isoforms, Enzyme activity, plasma IGF-1

INTRODUCTION

Exercise-induced asthma or allergies in high-level athletes are more and more detailed in the literature (2, 22). Clenbuterol is a selective β_2 -adrenergic receptor agonist prescribed during asthma and/or allergy pathologies (15). Clenbuterol is also known to stimulate muscle hypertrophy, reduce body fat content (35) and improve muscular functional capacity by increasing muscular strength (12, 36). However, clenbuterol

administration reduces aerobic exercise performance, as shown by the reduction in running time to exhaustion in mice (16). Moreover, several studies have established that clenbuterol could additionally induce slow-to-fast-twitch fiber conversion within extensor digitorum longus (EDL) and soleus muscles (11, 21). Thus, it was obvious that β_2 -adrenergic mechanisms played an essential role in the training-induced enzymatic adaptation in skeletal muscle (18). These modifications and putative muscle benefits could incite athletes to take this drug to ameliorate their physical efficiencies, especially those involved in strength- and power-related sports (26).

Despite numerous studies on the effects of chronic clenbuterol administration on the skeletal muscle properties of sedentary animals, few have investigated its effects on exercise performance directly. A recent study from Duncan et al. (13) showed that chronic clenbuterol administration deleteriously affected endurance and sprint exercise performance in rats, potentially due to alterations in cardiac muscle structure and function. Nevertheless, to our knowledge no study was intended to evaluate changes at the muscular level in specifically strength-trained animals submitted to a clenbuterol treatment.

In the present work, we investigated whether a high dosage of chronic clenbuterol treatment affected differently the performance of strength training in exercised or untrained rats. We also tested the hypothesis that clenbuterol administration could have a deleterious effect on muscle enzymatic activities and phenotype, and thus, decrease the performance.

METHODS

Animals and treatments

These experiments were done in accordance with current legislation on animal experiments in France. Thirty-two 12 week-old male Wistar rats (234 ± 2 g) were randomly divided in 2 groups of sixteen exercise trained (T) or untrained (S) animals. Each rat was housed in a 22 x 22 x 18 cm plastic cage, at $22 \pm 1^\circ\text{C}$, with a 12:12 hr light:dark cycle. The animals were fed *ad libitum* with a laboratory chow containing 16% protein, 3% fat, 0.8% calcium and 0.6% phosphorus (UAR, Villemoisson sur Orge, France). Among the 16 T animals submitted to an 8-week progressive isometric force training and among the 16 S animals, half of each group received clenbuterol *per os* (Sigma Chemical, St Louis, MO; 2 mg/kg body wt/day) five days/week (TCL, SCL). Control rats (TCON, SCON) received an identical volume (1 ml) of 0.9 % NaCl.

Each exercised rat was trained between 8 and 10 a.m., five days a week for 8 weeks, according to an already described protocol (20). Briefly, each rat was set on the horizontal floor of a box and then the box was put in a vertical position. The floor was made with a wire-netting which forced the animal to grip with its claws and to remain in a climbing position. This procedure was done 4-8 times during 2 x 30 s. The animals were allowed to rest for 20 s between each 30 s period and for 3 min between each set of exercise. The intensity of the training program was progressively increased by adding a load to the tail from 0 g the first day to 200 g the last week of training. All animals were weighed weekly.

Strength measures

In week 4 (W4) and W7, the thirty-two animals were submitted to an already described grip-test (10). A resistive endurance test was also performed on W4 and W8 on both TCL and TCON groups because this test was only possible with rats accustomed to exercise. Briefly, after a short warm-up, animals had to maintain the climbing position as long as possible with loads (300 g on W4 and 400 g on W8) corresponding approximately to their body weight at that time.

Body composition

On day 54, under a light chloral anesthesia, lean and fat masses were measured on each animal by dual X-ray absorptiometry (DEXA) (6), using a Hologic QDR 4500A X-ray densitometer (Hologic, Massy, France).

Tissue sampling and biochemical analyses

On day 58, rats were sacrificed by cervical dislocation. Blood was collected by cardiac puncture. After centrifugation, plasma was harvested and frozen until use. Soleus and plantaris muscles were separated from adjacent tissue, frozen in liquid nitrogen and kept at -80°C prior to use. Muscles were used to determine myosin heavy chain isoforms (MHCs), creatine kinase (CK), lactate dehydrogenase (LDH) and citrate synthase (CS) specific activities.

Tissue samples (50-100 mg) were homogenized for 30 s with an Ultra Turrax homogenizer (Janke & Kunkel) at 4°C in 19 volumes (1 volume/100 mg of tissue) of extraction buffer (50 mmol/L Tris -acetate, pH 7.5, 250 mmol/L sucrose, 1 mmol/L EDTA) containing an anti-protease mixture (Complete™ Mini, Roche, France). 500 µl of the homogenates were then centrifuged at 4°C for 10 min at 14000 g, and the rest of the homogenates were frozen for subsequent CS and CK activity measurements. The resulting supernatant produced an enzyme sample assayed for LDH activity. The LDH activity was measured spectrophotometrically using a Monotest LDH kit from Roche (Meylan, France). The reaction mixture was composed of 68 mmol/L phosphate buffer (pH 7.5), 0.73 mmol/L pyruvate, and 1.1 mmol/L NADH. After pre-incubation at 30°C for 5 min, a known volume of enzyme sample was added to the reaction mixture, and the decrease of the absorbance was monitored at 340 nm and 30°C. One unit of LDH activity was defined as 1 µmol/min of NADH oxidized at 30°C. LDH isoform distribution in soleus and plantaris was analyzed using the Hydragel iso-LDH kit (Sebia, France). The Hydrasis system (Sebia, France) allowed electrophoretic separation of LDH isoforms. The electrophoregrams were scanned and quantified using a densitometer system equipped with an integrator (GS-700, Bio-Rad). The intra- and inter-assay variations for electrophoretic quantification were 3.9 % and 5%, respectively.

Tissue homogenates of rats were assayed for CS activity as previously described (28). The reagents were prepared individually and comprised 0.1 M/L Tris-HCl buffer (pH 8.0), 10 mmol/L 5,5'-dithiobis (2-nitrobenzoic acid), 10 mmol/L oxaloacetate and 10 mmol/L acetylCoA. The absorbance at 412 nm of thio-2-nitrobenzoic acid released during the enzymatic reaction was recorded for 1 min at 30°C. One unit of CS activity was defined as 1 µmole of thio-2-nitrobenzoic acid released per minute at 30°C. Tissue homogenates used for CS assays were also assayed for CK activity. CK activity was measured spectrophotometrically at 340 nm. The reaction mixture was composed of buffer A (150 mmol/L triethanolamine-NaOH (pH 7.4), 1.58 mmol/L Mg-acetate and 30 mmol/L glucose), 15 mmol/L NADP⁺, 15 mmol/L ADP (pH 7.0) and 15 mmol/L magnesium acetate (pH 7.0). After pre-incubation at 30°C for 5 min, a hexokinase/glucose-6-phosphate dehydrogenase mixture (Roche Diagnostics, France) and a known volume of tissue homogenates were added to the reaction mixture. The reaction was carried out at 30°C for 30 s, measuring a possible unspecific NADP⁺ reduction. Thus, phosphocreatine (4 mmol/L final concentration) was added into the spectrophotometer cuvette and the reaction was followed for 30 s at 30°C, measuring the increase of NADPH absorbance. The absorbance augmentation was directly proportional to CK activity. One unit of enzyme activity was defined as 1 µmole/min of NADPH production.

In order to determine specific activities (U/mg of protein), protein concentrations were measured using a bicinchoninic acid kit (Uptima, France) with bovine serum albumin as a standard.

Plasma IGF-1 concentration was measured by homologous radioimmunoassay (Kit Nichols Institute Diagnostics, San Juan Capistrano, CA, USA); the lowest limit of detection for this assay was 0.06 ng/ml, and the intra- and inter-assay variations were 2.4 and 5.2%, respectively.

Analysis of Myosin Heavy Chains (MHC)

Muscles were analysed for MHC isoforms as previously described (30). Myosin isoforms were extracted and separated on polyacrylamide gels containing 30% glycerol, 8% acrylamide-bis acrylamide (50:1), 0.2 M Tris, 0.1 M glycine, and 0.4% sodium dodecyl sulphate. Gels were run at constant voltage (70 V) for ~28 h and then silver-stained. The MHC protein isoform bands were scanned and quantified using a densitometer system equipped with an integrator (GS-700, Bio-Rad).

Statistical analyses

Results are presented as means ± SD. Data of muscles, body composition, IGF-1 plasmatic concentration and endurance test were analysed using a two-way between groups ANOVA to detect effects of each treatment and to determine whether there was a group x treatment interaction. Post-hoc test (Scheffe) was used to detect differences among means. Finally, to measure the time impact on each treatment, data of grip tests were studied with a 3-way ANOVA (Group x Treatment x Time). Differences were considered significant at $P < 0.05$.

RESULTS

Body composition

Effects of clenbuterol treatment on both body weight and muscle wet weight of the 4 groups of rats are shown in Table 1. The body mass was higher in the sedentary rather than exercised rats ($P < 0.05$). Clenbuterol did not induce any significant body weight increase. However, body composition was affected by strength training exercise and/or clenbuterol treatment. The study of training effect (TCL and TCON combined into one group versus SCL and SCON in another) showed that fat mass (% of body weight) of animals was lower in the T group (-34.1%) than in the S group. Besides, it was further decreased by clenbuterol treatment, the lowest fat mass being measured in animals from the TCL group. On the opposite, the drug improved the lean mass of both SCL and TCL rats (Table 1).

Table 1. Body weight, muscle mass and body composition of SCON, SCL, TCON and TCL rats

	<i>SCON</i>	<i>SCL</i>	<i>TCON</i>	<i>TCL</i>
Body mass (g)	424 ± 23	432 ± 51	389 ± 28 *	392 ± 22 *
Fat mass (%)	12.6 ± 2.2	7.9 ± 3.8 *	8.3 ± 2.3 *	5.5 ± 1.5 **†
Lean mass (%)	84.7 ± 2.2	89.6 ± 3.7 *	89.0 ± 2.3 *	92.0 ± 1.5 *
<i>Soleus</i>				
Muscle weight (mg)	156 ± 22	188 ± 25 *	155 ± 12 †	179 ± 6 **†
Muscle weight/lean mass (mg/g)	0.43 ± 0.04	0.48 ± 0.04 *	0.44 ± 0.04	0.47 ± 0.03 *
<i>Plantaris</i>				
Muscle weight (mg)	355 ± 52	480 ± 32 *	367 ± 32 †	450 ± 14 **†
Muscle weight/lean mass (mg/g)	0.98 ± 0.11	1.23 ± 0.09 *	1.04 ± 0.06 †	1.18 ± 0.05 **†

Values are presented as means ± SE. Fat and lean masses are expressed as percentages of body weight. * Significantly different from SCON, $P < 0.05$; † vs. SCL, $P < 0.05$; ‡ vs. TCON, $P < 0.05$.

Soleus and plantaris muscle masses and muscle wet weight to body weight ratios were significantly higher in both SCL (20-54% vs. SCON, 15-36% vs. TCON; $P < 0.05$) and TCL (19-31% vs. SCON, and 15-23% vs. TCON; $P < 0.05$) animals (Table 1). Nevertheless, the relative skeletal muscle mass increase due to clenbuterol administration was higher in plantaris than in soleus (+35% and +20%, respectively). In both cases, relative or absolute muscle weight was not affected by the eight weeks of strength training.

Strength capacities

After 3 weeks of protocol, the animals from the TCL group were unable to increase the weekly strength training program compared to the rats from the TCON group (Figure 1). Results of exhaustive endurance tests also showed 30% and 47% decreases in the physical performances of the animals from the TCL group compared to those from the TCON group, on W4 and W8, respectively (Figure 2).

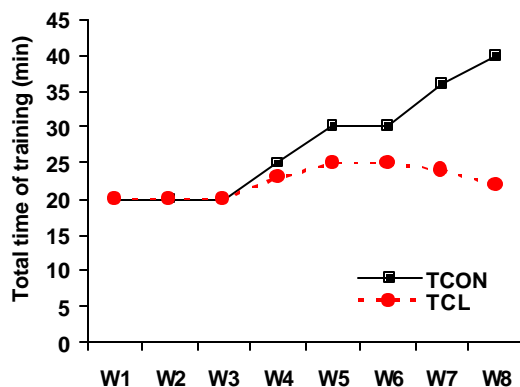


Figure 1. Total weekly time of training in TCON and TCL rats.

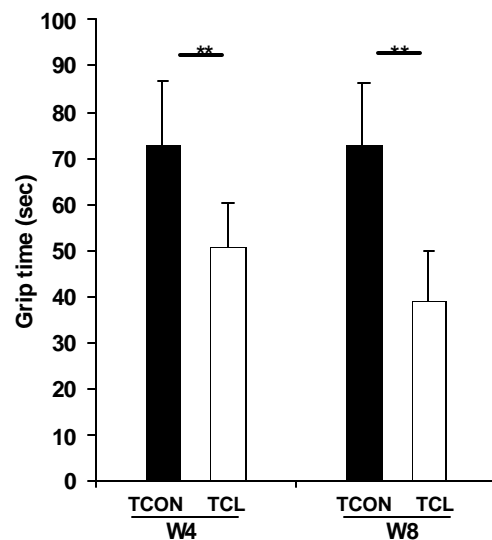


Figure 2. Results of the TCON and TCL endurance test on week 4 and week 8. Results are presented as means ± SD. ** Significantly different from TCON, $P < 0.01$.

Relative strength (expressed as a ratio of the grip test to the animal body mass) was affected by clenbuterol treatment and/or strength training (Figure 3). The strength of the TCON animals was significantly increased (+15% on W4; $P < 0.05$ and +12% on W7) compared to the one measured with the untrained rats. The SCL group presented a strength gain higher than the SCON group (+18% on W4 and +17% on W7; $P < 0.05$). TCL rats exhibited the highest strength gain, possibly indicating an additional effect of clenbuterol treatment and strength training. Finally, between W4 to W7 each rat group showed a similar strength gain (12-16%).

Biochemical measurements

In comparison to the rats from the SCON group, IGF-1 plasma concentration measured in the animals from the TCON group was significantly increased (+22%) by strength training (Figure 4). To the contrary, clenbuterol treatment alone (SCL) significantly decreased the IGF-1 plasma concentration of about 17% (vs. SCON; $P < 0.05$). The two previous observations were confirmed by the opposite effects of clenbuterol and strength training on IGF-1 plasma levels. Indeed TCL animals had a lower IGF-1 circulating concentration compared to the TCON rats.

We measured 3 enzymatic activities in soleus and plantaris (Table 2). The oxidative metabolism represented by CS activity showed that 8 weeks of clenbuterol administration decreased significantly the CS activity in soleus of SCL animals (-23%) compared to SCON animals. No difference was observed between both training groups (TCON and TCL) and the SCON one. In plantaris, clenbuterol treatment reduced dramatically the CS activity in both SCL and TCL groups compared to SCON one. Strength training alone seemed to improve the CS activity (TCON vs SCON), but the difference was not statistically significant.

CK activity measurements showed that the amounts of this enzyme were not significantly affected by clenbuterol treatment and/or training in soleus (Table 2). However, in plantaris, clenbuterol administration and/or strength training greatly increased CK activity compared to untrained animals. No additive effect of both treatment and training was observed in the TCL group.

LDH activity analysis showed that after 8 weeks of clenbuterol administration, no significant change could be measured in soleus. Training did not affect LDH activity either but surprisingly, the combination of both

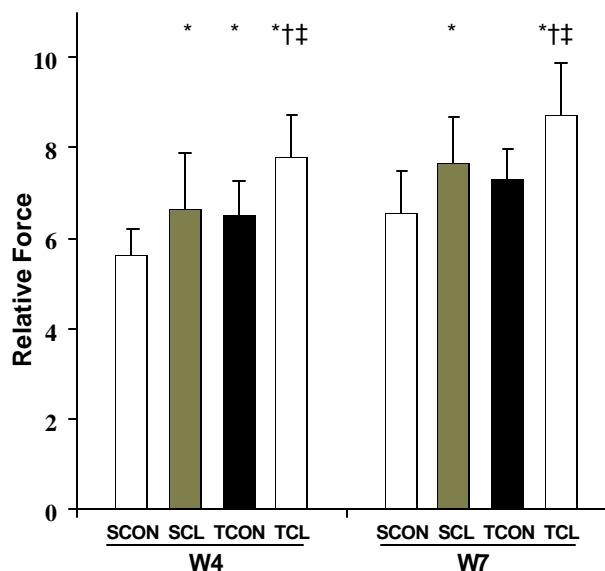


Figure 3. Measurements of relative force determined during a grip test performed on week 4 and week 7 on the four groups of rats. The relative force was normalized to animal body weights. Results are presented as means \pm SD. * Significantly different from SCON, $P < 0.05$; † vs. SCL, $P < 0.05$; ‡ vs. TCON, $P < 0.05$.

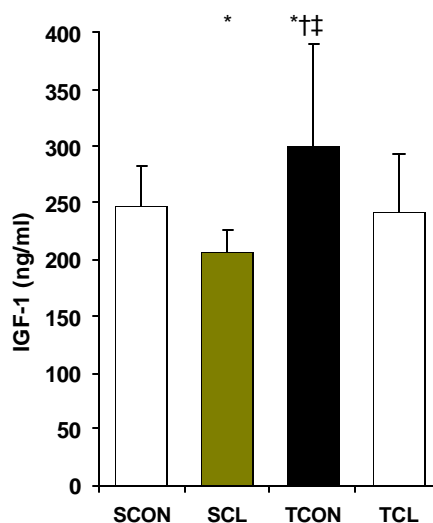


Figure 4. IGF-1 plasma concentration in SCON, SCL, TCON and TCL rats after an eight week protocol. Results are presented as means \pm SD. * Significantly different from SCON, $P < 0.05$; † vs. SCL, $P < 0.05$; ‡ vs. TCON, $P < 0.05$.

drug administration and strength training provoked a diminution of the enzyme activity. In plantaris, the drug decreased LDH total activity regardless of the strength training effect (Table 2).

LDH-A to LDH-B relative percentages were estimated. In animals that received clenbuterol (SCL group) the LDH-A subunit content is significantly augmented in soleus and decreased in plantaris. No difference was observed between TCON and TCL animals compared to SCON rats in both muscle types (Table 2).

Table 2. CS, CK, LDH enzymatic specific activities, and LDH isozyme percentage in soluble fractions of soleus and plantaris from the four different groups of rats.

	<i>SCON</i>	<i>SCL</i>	<i>TCON</i>	<i>TCL</i>
Soleus				
CS (U/mg)	0.101 ± 0.012	0.078 ± 0.010 *	0.101 ± 0.035	0.097 ± 0.013
CK (U/mg)	4.90 ± 1.70	3.96 ± 1.47	5.73 ± 1.17	4.21 ± 1.26
LDH (U/mg)	4.59 ± 1.27	5.31 ± 1.58	5.58 ± 0.43	3.68 ± 0.50 †‡
LDH-B (%)	61.7 ± 9.4	41.4 ± 13.0 *‡	58.7 ± 2.4 †	56.0 ± 5.2 †
LDH-A (%)	38.3 ± 9.4	58.6 ± 13.0 *‡	41.3 ± 2.2 †	44.0 ± 5.2 †
Plantaris				
CS (U/mg)	0.083 ± 0.011	0.034 ± 0.009 *‡	0.103 ± 0.020	0.047 ± 0.014 *‡
CK (U/mg)	5.13 ± 2.41	9.62 ± 1.96 *	14.00 ± 5.35 *	11.54 ± 2.71 *
LDH (U/mg)	10.74 ± 0.95	7.38 ± 0.88 *‡	11.28 ± 1.25	7.78 ± 0.84 *‡
LDH-B (%)	12.9 ± 0.4	24.6 ± 3.5 *‡	11.7 ± 2.0 †	13.2 ± 6.4 †
LDH-A (%)	87.1 ± 0.3	75.4 ± 3.6 *‡	88.3 ± 2.2 †	86.8 ± 8.2 †

Values are presented as means ± SD. * Significantly different from SCON, P < 0.05.

Statistical significance difference: † vs. SCL, P < 0.05; ‡ vs. TCON, P < 0.05.

Strength training alone did not affect MHC composition of soleus muscle (Table 3). In contrast, clenbuterol induced a slow to fast-twitch fiber conversion in both SCL and TCL groups (Table 3). The maximum changes were observed in SCL soleus in which the MHC 1 content was 20% lower, instead the MHC 2a was high and MHC 2x fibres, normally not expressed in soleus muscles, were detected.

In plantaris, no difference was observed between the different groups of animals concerning MHC 1 content. In this

muscle, clenbuterol treatment and/or strength training induced a shift of fast-twitch fibre to a faster phenotype (Table 3). Nevertheless, the maximum changes occurred in SCL plantaris. No difference in MHC distribution was observed between TCON and TCL groups.

DISCUSSION

The main results of this study were that clenbuterol treatment: (1) affects body composition by stimulating muscle hypertrophy and reducing body fat content, (2) improves muscular strength while it decreases training abilities and endurance performances, (3) decreases significantly the IGF-1 plasma concentration, (4) mediates the shift of MHC isoforms from slow to fast type in both soleus and plantaris muscles, (5) decreases CS and LDH activities in both muscles, while CK activity was enhanced, and finally (6) all these changes, excepted fat reduction, are lowered by the strength training program.

Table 3. MHC isoform analysis of soleus and plantaris from SCON, SCL, TCON and TCL rats.

	<i>SCON</i>	<i>SCL</i>	<i>TCON</i>	<i>TCL</i>
Soleus				
MHC 1	99.1 ± 1.1	79.5 ± 6.2 *‡	98.6 ± 2.5	91.1 ± 4.4 *†‡
MHC 2a	0.9 ± 1.1	17.2 ± 3.7 *‡	1.5 ± 2.5	8.9 ± 4.4 *†‡
MHC 2x	0.0 ± 0.0	3.3 ± 2.6	0.0 ± 0.0	0.0 ± 0.0
Plantaris				
MHC 1	2.0 ± 0.7	1.1 ± 1.3	1.1 ± 0.6	2.0 ± 1.5
MHC 2a	14.1 ± 3.5	8.5 ± 5.8 *	11.4 ± 6.6	13.4 ± 3.3
MHC 2x	57.3 ± 4.2	36.4 ± 4.3 *	43.8 ± 11.6 *	47.7 ± 1.3 *†
MHC 2b	26.6 ± 4.5	54.1 ± 10.1 *	43.8 ± 18.3 *	36.9 ± 4.6 *†

Values are presented as means ± SD. MHC amounts are expressed as percentages of total fibres. * Significantly different from SCON, P < 0.05; † vs. SCL, P < 0.05; ‡ vs. TCON, P < 0.05.

β_2 -agonists are taken by athletes who did not suffer from allergies or asthma as substitutes of anabolic steroids (26). In this study, we used a high dosage of clenbuterol (2 mg/kg body wt/day) to determine its maximum effects on skeletal muscle fibres and to exaggerate the excessive dosages that are commonly ingested by strength and bodybuilding athletes.

Concerning body composition, the main expected results were a muscular hypertrophy and a fat mass decrease (35). In our study lean mass was augmented by clenbuterol, while fat mass was reduced. Muscle clenbuterol-induced hypertrophy might result from an inhibition of proteolysis (5) and/or an increase in protein synthesis (23). The lowest fat mass composition was observed in the TCL group, apparently due to the additive effect of strength training and clenbuterol treatment. Insulin-like growth factor-1 (IGF-1) is implicated in muscular and bone metabolism especially during growth (1, 8). Its exact impact on bone metabolism is very important but its mechanism of action still remains unclear. IGF-1 plasma concentration is controlled mainly by GH and nutritional state (32). IGF factors can act by modulating the intracellular Ca^{2+} concentrations near the muscle membrane cell. Thus, IGF-1 may activate the calcineurin pathway (25) and subsequently play a role in muscular hypertrophy. Strength training is known to increase IGF-1 plasmatic concentration by 20% after 13 weeks of a high resistive training program in humans (7). This is in accordance with our results, which showed a higher IGF-1 plasma concentration in the TCON group than in SCON one (46%). Conversely, it seems that high chronic clenbuterol administration could partially reduce the plasma IGF-1 concentration. Indeed, IGF-1 level was significantly decreased in the plasma of SCL animals (-17%). This result has been already observed by Beermann et al. (4). These authors reported a decrease of IGF-1 plasma level (34%) in sheep fed for 6 weeks with cimaterol, another β_2 -agonist. Finally, concerning IGF-1, strength training seemed to protect the body against clenbuterol effects since TCL and SCON rats presented comparable IGF-1 concentrations. Therefore, in our experimental condition, one may conclude that muscular hypertrophy due to clenbuterol administration and IGF-1 plasma level was not correlated.

The most important finding of the present study was that long-term clenbuterol administration significantly increased maximal strength. Nevertheless, the ability to maintain a weekly equivalent strength training level was decreased in the TCL group. In the same way, the muscular endurance performance of TCL rats was significantly reduced as shown by the sustained endurance test. It has been shown that rats treated with clenbuterol exhibited decreased performances in endurance and sprint exercise (13). However, trained skeletal muscles showed a lower reaction than untrained muscles to a combination of exercise training and clenbuterol administration (24). These findings were consistent with the fact that in the rat, chronic treatment with β_2 -agonists deleteriously affected cardiac muscle by extensive collagen infiltration, causing reduced exercise tolerance during endurance and sprint exercise (13). It has also been reported that a 10-day clenbuterol administration in the rat could produce anabolic effects and reduce the total capillary density in left ventricle, soleus, gastrocnemius and plantaris muscles (29). These authors have suggested that clenbuterol could augment the diffusion distance of oxygen in heart left ventricle and skeletal muscles, resulting in a reduction of oxygen supply to tissues and thus, in a more important muscle fatigability.

At molecular level, in our experimental conditions, clenbuterol treatment significantly decreased citrate synthase activity in both soleus and plantaris muscles. These observations support the idea that a reduced muscle oxidative capacity may contribute to the decrease in exercise performance (16, 33). Furthermore, it appears that in the TCL group, strength training mitigates more or less a clenbuterol-induced reduction in CS activity in soleus and plantaris muscles. These findings have been already described by Torgan et al. (33) in the rat model. The authors also reported that clenbuterol administration produced a metabolic shift from oxidative to anaerobic glycolysis. The increased glycolysis was suggested to be a consequence of the administration of clenbuterol (23, 34) resulting in a raise of LDH-specific activity in rat soleus and EDL (19). Furthermore, in slow-twitch muscles, the shift of metabolic property from oxidative to glycolytic is accompanied by a decreased ratio of LDH-B on LDH-A subunits (19, 34). In our experimental conditions, LDH-A and B subunit distribution in soleus of SCL animals is in good agreement with Tsunekawa and Kitaura (34). On the opposite, a decrease in the ratio LDH-A on LDH-B in plantaris was observed. This might suggest a lower LDH-A activity, which has a higher K_M for pyruvate than LDH-B (27). Thus, a

decrease in LDH-specific activity in both soleus and plantaris muscles of SCL and TCL rats might partially be explained by LDH isozyme distribution changes. Strength training alone did not affect significantly LDH isozyme distribution and LDH specific activity. This result was also observed in human (31). At cellular level, Bakker et al. (3) demonstrated using single skinned rat skeletal muscle fibres that clenbuterol administration could cause a decrease in sarcoplasmic reticulum (SR) Ca^{2+} accumulation mainly due to an augmentation of the SR passive Ca^{2+} leak. Thus, clenbuterol might lead to permanent damages of the excitation-contraction coupling process and to a decrease in endurance performances. In mouse, long-term treatment with clenbuterol caused a decrease in the sensitivity to Ca^{2+} in fast-twitch fibres from both EDL and soleus muscles. It also triggered slow - to fast -twitch fibre conversions, which were offset by exercise training (21). In our study, untrained clenbuterol-treated animals (SCL) presented a slow-to-fast fibre conversion in both soleus and plantaris muscles as shown by the MHC isoform analysis. Strength training could only partially prevent this slow to fast fibre transition. However, in plantaris, strength training alone was able to induce changes on myosin heavy chain types from fast to faster, which is in good agreement with previous findings in human (14, 17). Interestingly, an opposite effect (shift from fast-to-slow MHC isoforms) has been demonstrated after chronic administration of either a β_2 -adrenergic antagonist or a drug that reduced muscle ATP and phosphocreatine levels (36).

In our study, skeletal muscle force was increased on W4 by strength training program or by clenbuterol administration, resulting in the same strength gain in both SCL and TCON groups. The highest muscular strength was observed in the TCL group, possibly through an additive effect of strength training and clenbuterol treatment. Strength was further increased between W4 and W7 in similar proportions for all groups, meaning that it resulted only from an age induced strength gain. The fact that CK activity was enhanced by clenbuterol administration and/or strength training in a fast muscle may explain, in part, this gain in maximal force. In the same way, transitions of muscle fibre phenotypic profiles toward faster profiles may explain improved strength gain, and, on the opposite, decreased endurance performances.

CONCLUSIONS

The results of this investigation clearly show that clenbuterol treatment alone can induce major changes in muscle enzymatic activities, with a decrease of oxidative and glycolytic pathways in both soleus and plantaris muscles, while CK content is increased in the fast muscle plantaris. Clenbuterol alone mediates the shift of MHC isoforms from a slow to a fast type distribution in both soleus and plantaris. These results could in part explain the decrease in endurance performances and the augmentation in strength. This strength improvement due to clenbuterol treatment, coupled with the decrease of bone mineral content and femoral bone density reported in a previous study (9), may lead to an increase of the fracture risk. On the opposite, strength training mitigates partly or fully the effects of the clenbuterol alone on plasma IGF-1, MHC and LDH isozyme distributions. Therefore, strength training seems to prevent to some extent the extreme modifications induced by a high chronic clenbuterol administration.

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