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メタデータ	言語: English
	出版者: 北海道教育大学冬季スポーツ教育研究センター
	公開日: 2012-11-22
	キーワード:
	作成者: 鈴木, 淳一
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	所属:
URL	https://doi.org/10.32150/00010473

冬季スポーツ研究　第4巻1号　17-22, 2002
（北海道教育大学冬季スポーツ教育研究センター紀要）

Effects of chronic clenbuterol administration on PFK and LDH activities in rat skeletal muscle

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Abstract

The effects of chronic clenbuterol (CLB) administration on glycolytic and anaerobic enzyme activities in fast-twitch muscle were studied in young (10-week-old) and middle-aged (32-week-old) male Wistar rats. Rats of the treated groups were fed a diet containing 2 mg/Kg clenbuterol hydrochloride continuously for 1 week, followed by a intermittent protocol (2-day-on / 2-day-off) for following 25 days. The CLB treatment significantly increased body weight in both young and middle-aged rats ($P<0.05$). The weight of PL muscle was significantly increased by 37% and 32%, respectively, in young and middle-aged rats after the treatment ($P<0.05$). The phosphofructokinase (PFK) activity tended to increase in young rats (by 9%) and to decrease in middle-aged rats (by 13%) after the CLB treatment. However, the differences were not significant. The CLB treatment significantly decreased the lactate dehydrogenase (LDH) activity by 13% in middle-aged rats ($P<0.05$). In young rats, however, the LDH activity did not change after the treatment. The LDH/PFK ratio, provides an index of the relative capacity of aerobic glycolysis, did not change after the CLB treatment either young or middle-aged rats. These results suggest that a decrease in anaerobic capacity in fast-twitch muscle is observed predominantly in middle-aged rats after chronic CLB treatment. The muscle hypertrophy caused by the CLB without improving glycolytic and anaerobic capacity may reduce the anaerobic performance in fast-twitch skeletal muscle.

Key words: clenbuterol; lactate dehydrogenase; phosphofructokinase; skeletal muscle

The β_2-agonicts are widely used as bronchodilators for the prevention and treatment of symptoms of exercise-induced asthma. Clenbuterol (CLB) is a β_2-adrenergic agonist known to produce hypertrophy in both skeletal and cardiac muscles in a number of species [4,7,10,12]. The muscle hypertrophy caused by the CLB treatment increased both twitch and tetanic forces, and reduced the duration of twitch contraction time in normal (C57BL/10) and dystrophic (*mdx*) mice [7]. In contrast, the CLB administration reduced an aerobic exercise performance, identified as a reduction in running time to exhaustion in ICR mice [9], and as a reduction in running activity in voluntary running wheels in *mdx* mice [6]. A reduction in oxidative enzyme (succinate dehydrogenase and ß-hydroxyacyl-CoA dehydrogenase) activities [15] and no change in muscle capillarity [16] after the CLB treatment may also reduce an aerobic performance in rats. It is therefore postulated that the muscle hypertrophy induced by the CLB treatment improves an anaerobic rather than aerobic performance if activities of glycolytic and anaerobic enzymes are increased after the treatment.

Dodd *et al* (1996) reported that the CLB treatment for 14 days significantly increased the phosphofructokinase (PFK) activity in the slow-twitch soleus (SOL) muscle, but not in the fast-twitch plantaris (PL) muscle [5]. After 5-week CLB treatment, the lactate dehydrogenase (LDH) activity was significantly increased in the SOL but not in the fast-twitch extensor digitourm longus muscle in young mice [18].

When the CLB is administered, a marked response is obtained within 1-2 weeks [1,14]. After several weeks of continuous treatment, the response is attenuated, presumably by a downregulation of the adrenoreceptors [17]. The intermittent administration could prevent an attenuation of the response

occurred while continuous treatment [11]. However, the activities of glycolytic and anaerobic enzymes have not been determined after the intermittent administration of the CLB.

In the present study, changes in the activities of PFK and LDH after the intermittent CLB administration were determined in fast-twitch plantaris muscle.

Methods

This study was approved by the Animal Care and Use Committee of Hokkaido University of Education and performed in accordance with the "Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences" of the Physiological Society of Japan.

Animals Sixteen young (9 week-old) and 15 middle-aged (32-week-old) male Wistar rats were purchased from Clea Japan Inc. (Tokyo, Japan). After the rats were fed for 7 days to allow adaptation to the new environment, rats of each age group were randomly divided into control and CLB fed groups. The four groups of rats in this study were young control (YC; n=8), young CLB (YCL; n=8), middle-aged control (MC; n=7) and middle-aged CLB (MCL; n=8). All rats were housed under conditions of control temperature (24±1 °C) and a relative humidity of about 50%. Lighting (7:00-19:00) was controlled automatically. All rats were given tap water *ad libitum*. Control groups were fed a standard commercial laboratory chow (MF-type, Oriental East Co., Tokyo, Japan). The CLB fed groups were fed a powdered diet (MF-type, Oriental East Co.) containing 2 mg /kg clenbuterol hydrochloride (C-5423, Sigma Co., St. Louis, MO, USA) continuously for 1 week, followed by a intermittent protocol (2-day-on / 2-day-off) for following 25 days. The intermittent administration could prevent an attenuation of the response occurred while continuous treatment [11]. The average CLB intake, estimated from food intake, was 116 and 80 µg /Kg/day in the YCL and MCL groups, respectively. Because the rats were housed two or three per cage, the food intake per individual rat was estimated from the food intake per cage.

Sample preparation Under pentobarbital anaesthesia (50 mg/kg i. p.), plantaris (PL) muscle was rapidly excised, washed in cold saline, made free of surrounding connective tissue, weighed and frozen in liquid nitrogen. The muscle samples were stored at -80 °C until biochemical analyses. Muscle homogenates (5% (W/V)) were obtained from ~ 50 mg of frozen tissue homogenized for two interrupted 15-s bursts with Polytron homogenizer (set at 15,000 rpm) in ice-cold medium (20 mM sodium phosphate buffer, pH 7.4; 5 mM ß-mercaptoehanol; 2 mM EDTA; 0.02% bovine serum albumin (BSA)). After centrifugation at 13,000 g for 15 min at 0 °C, the supernatant was divided into two aliquotes. One aliquote was used for phosphofructokinase (PFK) assays on that day. Other aliquotes were stored at –80 °C.

Enzyme activity determination The activity of PFK was assayed according to Passonneau & Lowry (1993)[13]. A volume of 5 µL supernatant was added to 45 µL of dilution [8] (50 mM Tris-HCl buffer, pH 8.1, containing 0.02% BSA, 5 mM ß-mercaptoethanol, 0.5 mM EDTA and 10 mM K_2HPO_4). The diluted sample solution was then added to 940 µL of reagents (50 mM Tris-HCl buffer, pH 8.1, containing 1mM ATP, 10 mM K_2HPO_4, 2 mM $MgCl_2$, 1mM 5'AMP, 1mM ß-mercaptoethanol, 0.1 mM NADH, 0.05% BSA, 0.1 U rabbit muscle aldolase, 15 U rabbit muscle triose phosphate-isomerase, 1.7 U rabbit muscle alpha-glycero-phosphate dehydrogenase). The reaction was started with addition of substrate (10 µL of 100 mM fructose-6-phosphate).

The activity of LDH was assayed according to Bass *et al* (1969)[2]. A volume of 5 µL supernatant was added to 45 µL of dilution (200 mM imidazole-HCl buffer, pH 7.0, containing 0.1% BSA) [8]. The diluted sample solution was then added to 940 µL of reagents (50 mM triethanolamine-HCl buffer, pH 7.6, containing 5mM EDTA, 0.3 mM NADH). The reaction was started with addition of substrate (10 µL of 240 mM sodium pyruvate).

All measurements were carried out at 25 °C with a spectrophotometer (U-2001, Hitachi Co., Tokyo, Japan). All activities were measured at 340 nm by following the disappearance of NADH. The extinction coefficient for NADH was 6.22 /mM/cm. Specific activities were expressed in international units (µmol /min) per gram of tissue wet mass.

Statistical Analysis All results are express-

Table 1. Effects of clenbuterol feeding on body and muscle weights.

	YC (n=8)	YCL (n=8)	MC (n=7)	MCL (n=8)	Significance (two-way ANOVA) Age	Drug	Age*Drug
Initial body weight (g)	294.3 ± 4.9	298.0 ± 4.9	500.6 ± 5.9#	501.4 ± 6.8‡	§	NS	NS
Final body weight (g)	374.9 ± 8.9	414.0 ± 6.6*	528.0 ± 9.1#	574.6 ± 8.6*‡	§	§	NS
Plantaris weight	413.1 ± 16.7	566.4 ± 17.8*	550.9 ± 23.4#	729.3 ± 23.7*‡	§	§	NS

Values are means ± SE. *, significantly different from age-matched control group at P<0.05; # and ‡, significantly different from YC and YCL, respectively, at P<0.05; §, significant at P<0.05; NS, not significant.

ed as means ± SE. Using the Kolmogrov-Smirnoff test, we first tested the distribution of all parameters to determine whether it was compatible with a normal distribution. In the present study, all data sets showed a normal distribution. Unpaired Student's t test was used for parametric two sample comparison. The two-way analysis of variance (ANOVA) was used to test the effects of age, drug and their interaction. If the two-way ANOVA was significant, differences among the four groups were analyzed using one-way ANOVA and the Fisher PLSD post-hoc test. Differences were considered to be statistically significant at P<0.05.

Results

Table 1 shows body and PL muscle weights. At the end of the treatment, body weights were significantly greater in the YCL (by 10%) and MCL (by 9%) than in respective control groups (P<0.05). The

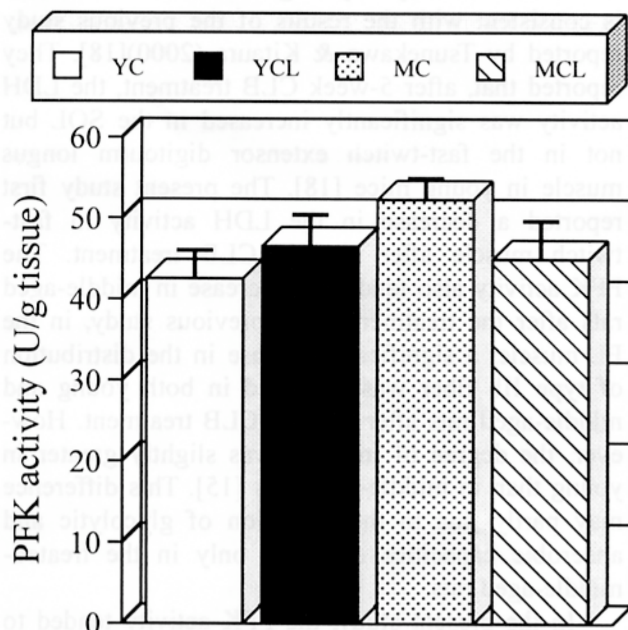

Figure 1
Changes in phosphofructokinase (PFK) activity after chronic clenbuterol treatment in plantaris muscle. YC, young non-treated control group; YCL, young treated group; MC, middle-aged non-treated control group; MCL, middle-aged treated goup.

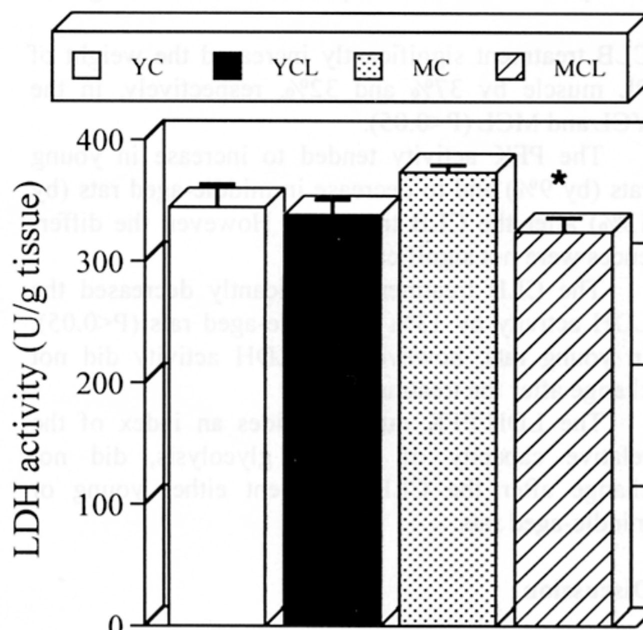

Figure 2
Changes in lactate dehydrogenase (LDH) activity after chronic clenbuterol treatment in plantaris muscle. Groups are the same as in Fig. 1. *, significantly different from age-matched control group at P<0.05.

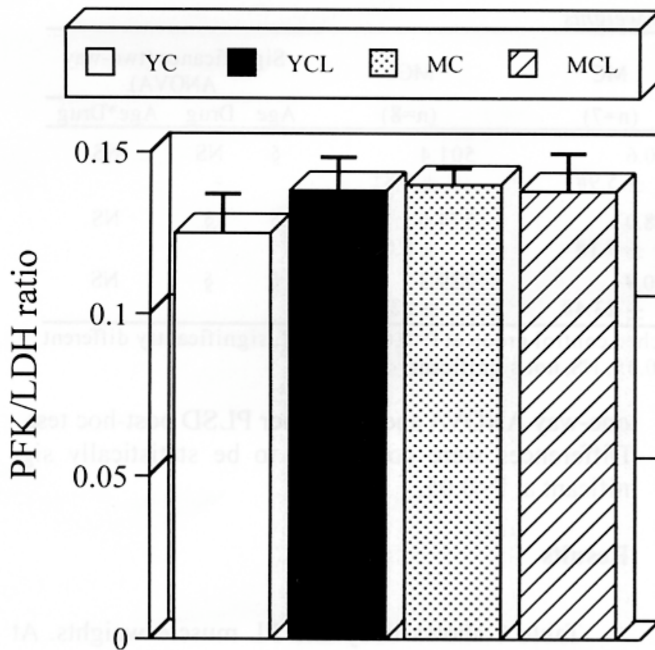

Figure 3
Effect of clenbuterol treatment on the PFK/LDH ratio in plantaris muscle. Groups are the same as in Fig. 1.

CLB treatment significantly increased the weight of PL muscle by 37% and 32%, respectively, in the YCL and MCL (P<0.05).

The PFK activity tended to increase in young rats (by 9%) and to decrease in middle-aged rats (by 13%) after the CLB treatment. However, the differences were not significant.

The CLB treatment significantly decreased the LDH activity by 13% in middle-aged rats (P<0.05). In young rats, however, the LDH activity did not change after the treatment.

The LDH/PFK ratio, provides an index of the relative capacity of aerobic glycolysis, did not change after the CLB treatment either young or middle-aged rats.

Discussion

The results of this study show that the intermittent CLB feeding for 32 days decreased the lactate dehydrogenase (LDH) activity in fast-twitch plantaris (PL) muscle in middle-aged but not in young rats. As reported by previous investigators [4,7,10,12], the present CLB treatment caused hypertrophy in the PL muscle identified by muscle weights (Table 1).

In the present study, the experimental groups were fed a diet containing 2 mg/kg-food clenbuterol hydrochloride. Cartana & Stock (1995) observed that the administration of a diet containing the same amount of CLB for 3 days caused a significant increase in the weights of hind-limb muscles in male Wistar rats [3]. When CLB is administered, a marked response is obtained within 1-2 weeks [1,14]. After several weeks of continuous treatment, the response is attenuated, presumably by a downregulation of the adrenoreceptors [17]. In the present study, the experimental groups were fed the CLB diet continuously for 1 week, followed by an intermittent protocol (2-day-on / 2-day-off) for following 25 days. The intermittent administration could prevent an attenuation of the response occurred while continuous treatment [11].

The major finding of this study was that, in middle-aged but not in young rats, the LDH activity was significantly decreased in fast-twitch muscle after the CLB treatment (P<0.05; Fig. 2). No change in the LDH activity in young rats after the treatment is consistent with the results of the previous study reported by Tsunekawa & Kitaura (2000)[18]. They reported that, after 5-week CLB treatment, the LDH activity was significantly increased in the SOL but not in the fast-twitch extensor digitourm longus muscle in young mice [18]. The present study first reported a decrease in the LDH activity in fast-twitch muscle after chronic CLB treatment. The PFK activity also tended to decrease in middle-aged rats after the treatment. In a previous study, in the PL muscle, a significant increase in the distribution of type IIb fiber was observed in both young and middle-aged rats after chronic CLB treatment. However, the degree of increase was slightly greater in young than in middle-aged rats [15]. This difference may partly due to the reduction of glycolytic and anaerobic capacities observed only in the treated-middle-aged rats.

In the present study, the PFK activity tended to increase, but not significant, in young rats after the CLB treatment. This result may consistent with the results of previous study showing that the CLB treatment for 14 days significantly increased the PFK activity in the slow-twitch SOL muscle, but not in the fast-twitch PL muscle [5].

The LDH/PFK ratio provides an index of the relative capacity of aerobic glycolysis. In the present study, the LDH/PFK ratio remained constant after the CLB treatment in both young and middle-aged rats (Fig. 3). Although, in the PL, the relative capacity of aerobic glycolysis remained constant after the treatment, a previous study showed significant decrease in the succinate dehydrogenase (SDH) activity of type IIa and IIb fibers in both young and middle-aged rats [15]. Further, the activity of ß-hydroxyacyl-CoA dehydrogenase, key enzyme of ß-oxidation, was significantly decreased after the treatment in the PL [16]. Taken together, total aerobic capacity of the PL muscle may decrease after chronic CLB treatment. The reductions in mitochondrial oxidative enzyme activity after clenbuterol treatment may be secondary to selectively increased myofibrillar protein and the transformation of slow to fast-twitch fibers [15].

In conclusion, the present study has shown that a decrease in lactate dehydrogenase activity in fast-twitch muscle of middle-aged rats treated with CLB intermittently for 32 days. The muscle hypertrophy caused by the CLB treatment without improving glycolytic and anaerobic capacity may attenuate the anaerobic performance in fast-twitch skeletal muscle.

References

1. Agbenyega ET and Wareham AC (1990) Effect of clenbuterol on normal and denervated muscle growth and contractility. Muscle & Nerve 13, 199-203.
2. Bass A, Brdiczka, Eyer P, Hofer S and Pette D (1969) Metabolic differentiation of distinct muscle types at the level of enzymatic organization. European J Biochem 10: 198-206.
3. Cartana J and Stock MJ (1995) Effects of clenbuterol and salbutamol on tissue rubidium uptake in vivo. Metabolism 44, 119-125.
4. Choo JJ, Horan MA, Little RA and Rothwell NJ (1992) Anabolic effects of clenbuterol on skeletal muscle are mediated by $ß_2$-adrenoceptor activation. Am J Physiol 263 (Endocrinol Metab 26), E50-E56.
5. Dodd SL, Powers SK, Vrabas IS, Criswell D, Stetson S and Hussain R (1996) Effects of clenbuterol on contractile and biochemical properties of skeletal muscle. Med Sci Sports Exerc 28, 669-676.
6. Dupont-Versteegden, EE (1996) Exercise and clenbuterol as strategies to decrease the progression of muscular dystrophy in mdx mice. J Appl Physiol 80, 734-741.
7. Hayes A and Williams DA (1994) Long-term clenbuterol administration alters the isometric contractile properties of skeletal muscle from normal and dystrophin-deficient mdx mice. Clin Exper Pharmacol Physiol 21, 757-765.
8. Henriksson J, Chi MMY, Hintz CZ, Young DA, Kaiser KK, Salmons S and Lowry OH (1986) Chronic stimulation of mammalian muscles: changes in enzymes of six metabolic pathways. Am J Physiol 251 (Cell Physiol 20), C614-C632.
9. Ingalls CP, Barnes WS and Smith SB (1996) Interaction between clenbuterol and run training: effects on exercise performance and MLC isoform content. J Appl Physiol 80, 795-801.
10. Maltin CA, Delday MI, Hay SM and Baillie GS (1992) Denervation increases clenbuterol sensitivity in muscle from young rats. Muscle and Nerve 14, 188-192.
11. McElligott MA, Barreto AJr and Chaung LY (1989) Effect of continuous and intermittent clenbuterol feeding on rat growth rate and muscle. Comp Biochem Physiol 92C, 135-138.
12. Moore NG, Pegg GG and Sillence MN (1994) Anabolic effects of the $ß_2$-adrenoceptor agonist salmeterol are dependent on route of administration. Am J Physiol 267 (Endocrinol Metab 30), E475-E484.
13. Passonneau, JV and Lowry OH (1993) Enzymatic analysis: A practical guide. Humana Press, New Jersey.
14. Smith SB, Davis SK, Wilson JJ, Stone RT, Wu FY, Garcia DK, Lunt DK and Schiavetta AM (1995) Bovine fast-twitch myosin light chain-1: cloning and mRNA amount in muscle of cattle treated with clenbuterol. Am J Physiol 268 (Endocrinol Metab 31), E858-E865.
15. Suzuki J (2001) Effects of chronic $ß_2$ adrenergic agonist clenbuterol administration on skeletal muscle capillarity in young and middle-aged rats. J Winter Sports 3: 1-12.
16. Suzuki J (2001) Effects of chronic $ß_2$ adrenergic agonist clenbuterol administration on 3-hydroxyacyl-CoA dehydrogenase activity in rat skeletal muscles. J Winter Sports 3: 13-18.
17. Torgan CE, Etgen GJJr, Brozinick JTJr, Wilcox RE and Ivy JL (1993) Interaction of aerobic exercise training and clenbuterol: effects on insulin-resistant muscle. J Appl Physiol 75, 1471-1476.

18. Tsunekawa N and Kitaura T (2000) Effects of drinking administration of clenbuterol on SOL and EDL of mice. Jpn J Phys Fitness Sports Med 49, 149-156.