

Impact of salbutamol on muscle metabolism assessed by ^{31}P NMR spectroscopy

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The potential ergogenic effects of oral salbutamol intake were demonstrated for decades but the underlying mechanisms remain to elucidate. We hypothesized that improved exercise performance after acute oral salbutamol administration is associated with changes in muscle metabolism. Twelve healthy, nonasthmatic, moderately trained, male subjects were recruited to compare in a double-blind crossover randomized study, an oral dose of salbutamol (4 mg) and a placebo. After treatment administration, subjects performed repetitive plantar flexions to exhaustion in a 3T magnet. Continuous ^{31}P nuclear magnetic resonance spectroscopy assessment of the calf muscles was performed at rest, during exercise, and during recovery. No significant difference between treatments was detected in metabolite concentration at rest ($P > 0.05$). Creatine phosphate and inorganic

phosphate changes during and immediately after exercise were similar between treatments ($P > 0.05$). Intramuscular pH (pHi) was significantly higher at rest, at submaximal exercise but not at exhaustion with salbutamol (pHi at 50% of exercise duration, $6.8 \pm 0.1/6.9 \pm 0.1$ for placebo and salbutamol, respectively, $P < 0.05$). The maximal power ($28 \pm 7 \text{ W}/23 \pm 7 \text{ W}$; $P = 0.001$) and total work ($1702 \pm 442 \text{ J}/1381 \pm 432 \text{ J}$; $P = 0.003$) performed during plantar flexions were significantly increased with salbutamol. Salbutamol induced significant improvement in calf muscle endurance with similar metabolic responses during exercise, except slight differences in pHi. Other mechanisms than changes in muscle metabolism may be responsible for the ergogenic effect of salbutamol administration.

Selective β_2 -agonists, such as salbutamol, are in widespread use for patients with asthma or chronic obstructive pulmonary diseases, but also commonly use for the management of exercise-induced asthma in endurance sport competition. A number of previous reports have consistently described a larger prevalence of airway dysfunctions such as bronchial hyperresponsiveness and inflammation in endurance sportsmen compared with sedentary controls (Weiler et al., 1998; Verges et al., 2005). Nevertheless, because of the suspicion of ergogenic effects, concerns have been raised regarding the possible performance improvement associated with salbutamol administration and especially its potential effect on skeletal muscle function.

Although substantial effects of oral β_2 -agonists administration in human have been demonstrated on muscle strength (Martineau et al., 1992) and exercise performance (Collomp et al., 2000a,b, 2005; Le Panse et al., 2005, 2006), the underlying mechanisms, especially regarding muscle metabolism (Collomp et al.,

2000a,b; Sanchez et al., 2012), are not fully determined. In animal models, β_2 -agonists were shown to increase muscle contractility (Cairns & Dulhunty, 1993a), to reduce muscle fatigue (Cairns & Dulhunty, 1993b) and to improve force recovery (Derom et al., 1997). Collomp et al. (2000a,b) have observed during submaximal whole-body exercise an increase in blood lactate, glucose, and free fatty acids concentrations after oral ingestion of salbutamol. These authors have also reported following oral salbutamol administration increased insulin levels and lactate removal rate that may be linked to improved endurance during submaximal exercise through an enhancement of the overall contribution to energy production of both aerobic and anaerobic metabolisms (Collomp et al., 2000a,b). Salbutamol appears however to have no effect on gas exchange (oxygen consumption, respiratory exchange ratio) during whole-body submaximal and maximal exercise (Arlettaz et al., 2009; Elers et al., 2012). These contrasting results regarding the effects of β_2 -agonists on metabolism are based on blood sampling and indirect calorimetry that cannot precisely and specifically assess

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changes in muscle metabolism. ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMRS) is a noninvasive method to assess muscle metabolism *in vivo*, both at rest and during exercise, by focusing on changes in concentrations of high-energy phosphate metabolites such as adenosine triphosphate (ATP), creatine phosphate (PCr), and inorganic phosphate (Pi). This technique represents a powerful tool to investigate at a cellular level the muscle metabolic pathways and the effect of interventions such as drug administration (Wary et al., 1999; Kurosawa et al., 2003).

Therefore, this study aimed to assess changes in muscle metabolism potentially responsible for performance improvements following acute oral ingestion of salbutamol in moderately trained subjects. We hypothesized that oral doses of salbutamol may modify muscle oxidative capacities and maximal glycolytic ATP synthesis with (a) identical submaximal intensities increased oxidative activity linked to smaller PCr depletion and smaller decline of intramuscular pH (pHi), (b) exhaustion enhanced phosphorous metabolites degradation inducing higher Pi/PCr ratio and smaller pHi, and (c) increase of mitochondrial function as reflected by faster PCr repletion immediately after exercise compared with placebo condition. To test these hypotheses, we used a local incremental concentric plantar flexion task until exhaustion by using ^{31}P NMRS during and after exercise. This kind of localized muscle exercise avoids large cardiorespiratory stimulation and permits to evaluate the impact of salbutamol specifically on the muscle energetic status.

Material and methods

Subjects

Twelve healthy, nonasthmatics, male subjects (age: 28 ± 6 years; height: 179 ± 7 cm; weight: 73 ± 6 kg) provided informed consent to participate in the present study. The subjects were moderately trained (mean strength and endurance training = 6 ± 2 h/week) and had no history of atopy, asthma, or other cardiorespiratory disorders. They all had normal lung function [forced vital capacity (FVC), $113 \pm 20\%$ of theoretical values; forced expiratory volume in 1 s, $103 \pm 11\%$ of theoretical values; forced expiratory flow at 25–75% of FVC, $109 \pm 23\%$ of theoretical values]. They were not allowed to eat or drink any caffeinated products and to perform any physical exercise on the 48 h before the day of the test. They had to keep their training activity constant during the whole testing period. Subjects took a high-carbohydrate meal (i.e., at least 800 kcal with 60% carbohydrates) at least 2 h before each test to load their energetic stores. The experimental procedures and potential risks associated with participation in the study were explained, and informed consent was obtained from each subject. The study was approved by the local ethics committee and performed according to the Declaration of Helsinki.

Study design

After a preliminary session including a maximal incremental cycling test to determine training status, a prospective double-blind, randomized, two-way crossover design was used to compare an acute oral administration of salbutamol (4 mg) and a placebo

(with exactly the same film-coated tablets, provided and controlled by an independent pharmacologic department), administered before a standardized exercise protocol in the magnet. Each test session was performed at least 96 h apart, within a maximum period of 3 weeks. To ensure blinding, the technician who gave treatments in a random order was not involved in the other parts of the protocol. Measurements started 40 min after treatment administration and the whole test (from resting measurement to recovery assessment) lasted for ~ 30 min.

Maximal incremental cycling test

Before performing the two experimental sessions, subjects performed a maximal incremental exercise test (80 W initial power and 25 W/min increment until subject exhaustion) on a computer-controlled electrically braked cycle ergometer (Ergometrics 800, Ergoline, Bitz, Germany) with electrocardiogram, breath-by-breath ventilation and gas analysis (Medisoft, Dinant, Belgium) for the determination of maximal aerobic power and maximal oxygen uptake (Medisoft). A fingertip blood sample was obtained 3 min after exhaustion and was analyzed for lactate concentration (NOVA+, Nova Biomedical Corporation, Waltham, Massachusetts, USA).

Plantar flexion protocol

Participants were first familiarized with plantar flexions in the confinement of a whole-body magnetic resonance imaging system. Subjects were requested to perform repetitive plantar flexions of the right leg at a frequency of 0.5 Hz (1-s contraction, 1-s relaxation, provided by a rhythmic soundtrack) in the magnet. The initial exercise workload was 3 daN and the intensity was incremented by 1 daN every min until volitional exhaustion. The pedaling rate was kept constant at 30 rev/min during the whole exercise period. The test ended when the subject could not maintain the frequency or produce the required power output for three consecutive flexions (task failure). During the 2 min before the start of exercise, during plantar flexions as well as over the 4 min immediately following task failure, ^{31}P NMRS acquisitions were continuously conducted (Fig. 1).

Muscular contractions were automatically monitored and recorded by a pneumatic custom-made nonmagnetic ergometer in order to calculate work output. The ergometer treadle returned automatically to the resting position (i.e., the ankle flexed at 90°).

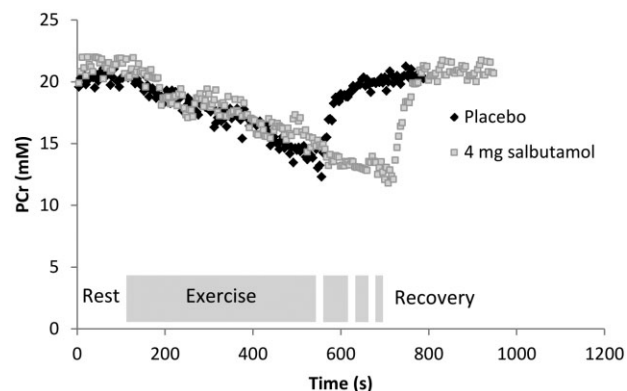


Fig. 1. Calf muscle creatine phosphate (PCr) concentration (in mM, i.e., mmol/L of cell water) during the maximal incremental plantar flexion test from rest until exhaustion and during the recovery period. Data from one representative subject following 4 mg of salbutamol and placebo.

The plantar flexor muscles performed concentric contractions to an ankle angle of 120°. The knee angle was fixed at 135° (full extension = 180°).

Muscle metabolism assessed by ³¹P NMR spectroscopy

³¹P NMRS was performed by using a clinical 3.0 T system (Philips Achieva TX, Best, the Netherlands) operating at 51.8 MHz for ³¹P. All data were acquired with a transmit/receive surface coil (14 cm) placed under the calf muscle at its maximal diameter. The centering of the leg was confirmed by a T1-weighted ¹H localizing images obtained in the axial plane and the coil was repositioned if not properly centered on the gastrocnemius muscle. Shimming on the proton signal from tissue water optimized magnetic field homogeneity and the ³¹P NMRS signal was also optimized by prescan transmitter gain adjustment. A 500-μs hard pulse was used for signal excitation. The spectral width was 2500 Hz and a single free induction decay (FID) was acquired every 4 s (2 NEX) to improve the signal to noise ratio of the profile and to provide high-resolution data for kinetic analysis. As a result, the data were expressed with a time resolution of 4 s. To facilitate the post-data analysis, an offset of frequency was used to center the PCr peak at 0 ppm.

Data analysis

Data were processed using jMRUI® software (Naressi et al., 2001). Each FID consisted of 2048 complex points and was processed with 8.5 Hz exponential line broadening prior to zero filling and Fourier transformation. All spectra were manually phased using zero and first-order phase corrections. There were no phase variations during all the experimental protocol. The levels of PCr determined from the intensity of that peak were normalized to ATP concentration (measured from the three ATP peaks of the ³¹P spectra). Muscle intracellular pH (pHi) was calculated from the chemical shift difference (δ) of the Pi peak relative to the PCr peak using the following equation:

$$pH_i = 6.75 + \log\left(\frac{(\delta - 3.27)}{(5.69 - \delta)}\right) \quad (\text{Taylor et al., 1983}) \quad (1)$$

The free cytosolic ADP concentration ([ADP]) was calculated from the Pi/PCr ratio by using a reformulation of the creatine kinase reaction:

$$[ADP] = \frac{[ATP] \cdot \left(\frac{[TCr]}{[PCr]} - 1\right)}{[H^+] \cdot K_{CK}} \quad (2)$$

In this equation, [ATP] was taken as 8.2 mM, K_{CK} is the creatine kinase equilibrium constant taken as $1.66 \times 10^9 \text{ M}^{-1}$ and assuming that phosphocreatine represents 85% of creatine content (TCr) (Kemp et al., 2001). In addition, the cytosolic $[Mg^{2+}]$ was calculated from the chemical shift of the β-ATP measured from the resonance of the PCr and this information was used to correct the calculated pH for changes in $[Mg^{2+}]$ (Iotti et al., 2000). The signal to noise ratios was controlled at rest to allow PCr levels to be determined with a temporal resolution of 4 s during the protocol (Taylor et al., 1983). Changes in PCr during recovery immediately after task failure were fit using a monoexponential function (Matlab, MathWorks, Natick, Massachusetts, USA) to the unaveraged data set (temporal resolution of 4 s):

$$PCr(t) = PCr_b + PCr_r \left[1 - e^{-(t/\tau)}\right] \quad (3)$$

where PCr_b is the baseline value, PCr_r is the difference between the baseline and the recovery value, t is time and τ is the time

constant. The pH was introduced into the formula to correct for the dependence of PCr recovery on cytosolic pH (Iotti et al., 1993).

The time course of decline in PCr during exercise was determined from a nonlinear regression using an exponential function:

$$PCr(t) = PCr_b - PCr_d \left[1 - e^{-(t - TD_p/\tau)}\right] \quad (4)$$

where PCr_b refers to the baseline value, PCr_d is the difference between baseline and PCr depletion at the end of the exercise, TD_p is the time delay before the onset of changes and τ is the time constant. Changes in pH and in the concentration of phosphorus metabolites during the recovery period were used to calculate proton efflux rates as previously described (Kemp et al., 1993; Trenell et al., 2006).

Statistical analysis

The number of subjects was determined considering previous reports on the metabolic effects of salbutamol (Collomp et al., 2000a,b) and the reproducibility of assessment of metabolic variables *in vivo* using MRS ³¹P (Layec et al., 2009) to provide 80% power with an α-level of 0.05. The average of the last four spectra at rest, at 25%, 50%, and 75% of the individual total number of plantar flexions performed during the placebo condition (i.e., at similar individual submaximal power outputs) and at task failure (i.e., maximal exercise) was used to compare metabolite concentration kinetics during the exercise protocols following salbutamol and placebo administration. Data were tested with a one (i.e., treatment for PCr repletion, exercise duration, total work and maximal power output) or two (i.e., time × treatment, for metabolic measurements during the plantar flexions) repeated-measures analyses of variance (ANOVAs) with Tukey–Kramer multiple-comparison test for post-hoc comparisons by using a statistical software (GraphPad Software Inc., San Diego, California, USA). Data were considered significantly different when $P \leq 0.05$. The results are presented as mean ± SD throughout the manuscript.

Results

Maximal cycling test responses confirmed the moderately trained status of the subjects. Maximal power output and maximal oxygen consumption during the cycling test were $315 \pm 38 \text{ W}$ and $47 \pm 15 \text{ mL/min/kg}$, respectively.

Because salbutamol can induce some cardiac function disturbances, subjects were carefully supervised during the 2 h following oral absorption and monitored continuously during the test in the magnet (Invivo Precess®, Gainesville, Florida, USA). No adverse effects were observed during or following oral administration of salbutamol in all the subjects.

At rest, PCr concentration (19.1 ± 2.6 vs $20.6 \pm 2.9 \text{ mM}$, for salbutamol and placebo, respectively), Pi/PCr ratio (0.23 ± 0.08 vs 0.21 ± 0.07), and ADP concentration (12.4 ± 0.5 vs $10.37 \pm 0.6 \text{ μM}$) were not different between treatments (all $P > 0.05$). However, pHi was significantly higher with 4 mg of salbutamol compared with placebo (7.1 ± 0.16 vs 6.9 ± 0.14 ; $P < 0.05$).

Changes in metabolic variables recorded by ³¹P NMRS during and after plantar flexions are presented

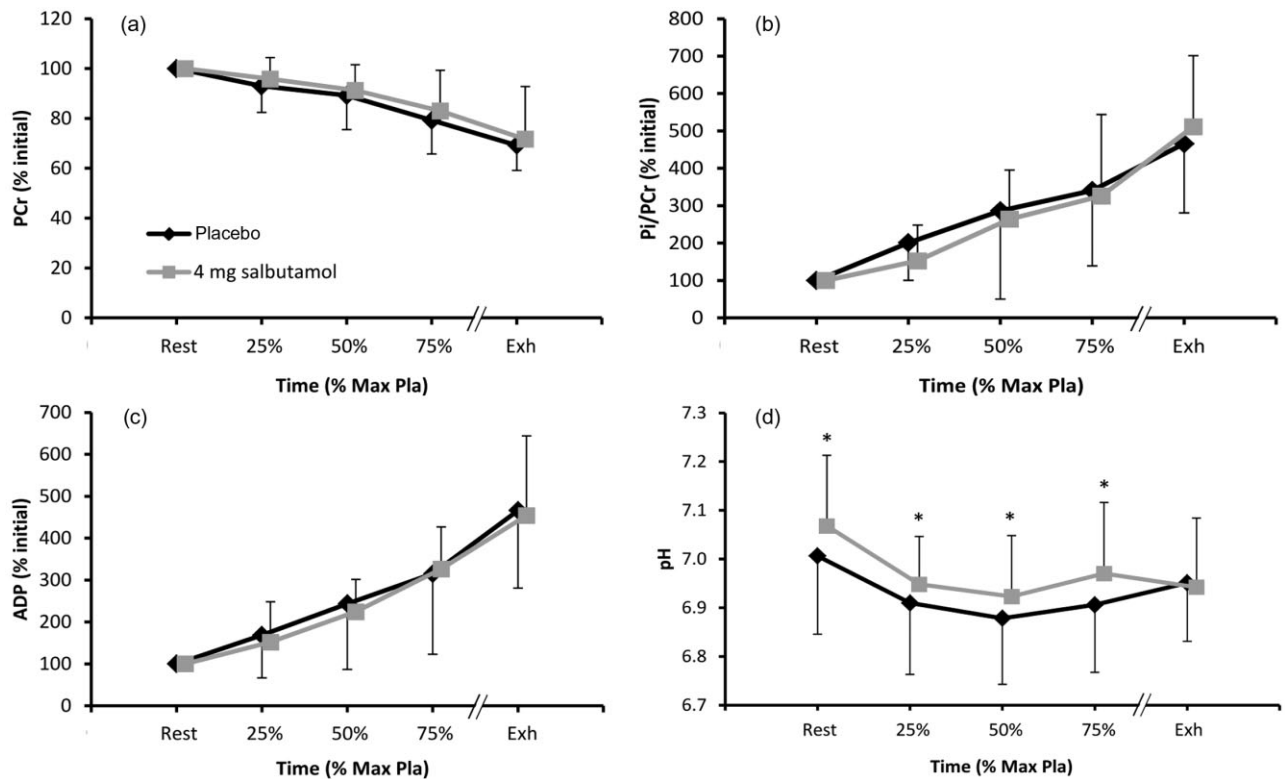


Fig. 2. Metabolic parameters (a: PCr concentration normalized to total ATP concentration; b: Pi/PCr ratio; c: muscle ADP concentration; d: intramuscular pH) measured in the calf muscle before, during (at 25%, 50%, and 75% of the maximum exercise duration with placebo, % Max Pla), and at the end (Exh) of the maximal incremental plantar flexion test. *Significant difference between salbutamol and placebo. Significant time effects were observed for all variables.

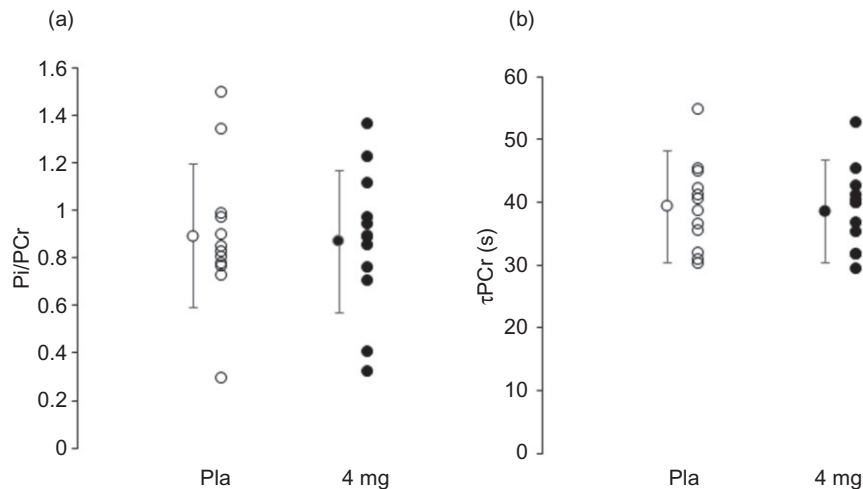


Fig. 3. Individual data points and mean values of metabolic parameters (a: Pi/PCr ratio; b: rate constant of PCr resynthesis, τ_{PCr}) at the end of the maximal incremental plantar flexion test.

in Figs 2 and 3. The PCr concentration was reduced during exercise by $\sim 35\%$ with no difference between treatments (ANOVA treatment main effect, $P = 0.90$; $f = 0.31$). The initial rate of PCr depletion was not significantly modified by treatment (13.8 ± 1.2 and 14.1 ± 1.6 mM/min with placebo and 4 mg of salbutamol, respectively; $P = 0.44$; $f = 0.47$).

The Pi/PCr ratio increased until exhaustion (from 0.21 ± 0.06 to 0.89 ± 0.30 and from 0.22 ± 0.11 to

0.86 ± 0.31 for placebo and 4 mg salbutamol, respectively, $P < 0.05$) with no difference between treatments (ANOVA treatment main effect, $P = 0.64$; $f = 0.26$). Similarly, the muscle ADP concentration was not significantly altered by salbutamol (ANOVA treatment main effect, $P = 0.71$; $f = 0.16$).

The intramuscular pH kinetic during the test was significantly different between treatments (ANOVA treatment main effect, $P = 0.019$, treatment \times time inter-

action, $P = 0.012$). The pH was significantly higher with 4 mg of salbutamol compared with placebo at submaximal exercise intensities (all $P < 0.05$). At exhaustion, however, no significant difference in pH was observed between the two treatments. The proton efflux rate calculated from the post-exercise pH and PCr recovery was not significantly different with placebo compared with 4 mg of salbutamol (4.2 ± 1.1 and 4.1 ± 0.9 mM/min).

The recovery of PCr concentration measured immediately after the end of exercise was well fitted with a monoexponential function ($R = 0.97 \pm 0.02$). The PCr recovery time constant is presented with individual data points in Fig. 3(b) and was not significantly different between the two treatments ($\tau_{\text{PCr}} = 39.7 \pm 8.9$ s and 38.5 ± 8.2 s with placebo and 4 mg of salbutamol, respectively; $P = 0.80$; $f = 0.28$).

Exercise duration was 10.4 ± 2.2 min with placebo and 11.7 ± 1.9 min with 4 mg of salbutamol ($P < 0.05$). The total work performed during plantar flexions was significantly higher with 4 mg of salbutamol compared with placebo (1702 ± 442 vs 1381 ± 432 J; $P < 0.001$). The maximal power output was significantly enhanced by $\sim 23\%$ with 4 mg of salbutamol compared with placebo (28 ± 7 vs 23 ± 7 W; $P < 0.001$).

Discussion

The present study aimed to clarify the mechanisms underlying the ergogenic effects of oral β_2 -agonist administration by focusing on muscle metabolism. This is the first *in vivo* evaluation of human muscle metabolism following salbutamol administration, at rest, and during exercise, by using ^{31}P NMRS. Oral administration of 4 mg of salbutamol induced a significant improvement in exercise performance but modified neither high-energy phosphate metabolite concentrations at rest nor their kinetics during and after exercise. Muscle intracellular pH was slightly but significantly higher with salbutamol at rest and during submaximal exercise while similar values were measured at exhaustion compared with placebo.

Salbutamol is a β_2 -adrenergic receptor agonist, which accounts for its pronounced bronchodilatory, vascular, uterine, and metabolic effects (Price & Clissold, 1989). Several studies also mentioned that salbutamol may induce a significant increase in blood glucose concentrations and may possibly modify carbohydrates availability and utilization (Collomp et al., 2000a,b; Le Panse et al., 2007; Sanchez et al., 2012). It has been shown that β_2 -agonists can induce a stimulation of hepatic glucose production and glucose release (Clutter et al., 1980), a stimulation of glycogenolysis and glycolysis with increased lactate and pyruvate release from tissues such as muscle (Rizza et al., 1980), a stimulation of lipolysis with increased glycerol and fatty acid release and lipid oxidation (Kendall et al., 1991; Schifflers et al., 1999).

Some studies reported no significant difference in total energy expenditure and substrate oxidation (based on gas exchange measurement during whole-body exercise) following salbutamol administration, but the substrate oxidation balance might be modified during and after exercise (Collomp et al., 2000a,b, 2005; Arlettaz et al., 2009). Moreover, an acute therapeutic oral intake of salbutamol seems to improve performance during a supramaximal exercise together with significant modifications in both blood insulin and glucose concentrations as well as significantly enhanced blood lactate concentration during the recovery period (24).

Despite these previous studies suggesting some potential effects of salbutamol on metabolism, the present results indicate no significant effect of salbutamol on high-energy phosphate metabolite kinetic during exercise. Similar initial rate of PCr depletion suggests that the larger amount of work performed with salbutamol is probably not due to reduced ATP cost (Layec et al., 2012, 2014). Similarly, this increased work may not be due to larger amount of ATP produced since the amplitudes of PCr depletion and ADP increase for any workload were similar with salbutamol and placebo. An increased mitochondrial capacity with salbutamol seemed also unlikely since PCr recovery was unchanged immediately after exercise. Increased work with salbutamol might also arise from an increase in glycolytic ATP synthesis. This should have been associated with lower pH with salbutamol, which was not the case in this study. Enhanced glycolytic ATP synthesis without reduced pH may have occurred if cellular H^+ efflux was enhanced (Layec et al., 2013). However, since salbutamol did not modify pH recovery immediately after exercise, this hypothesis is also unlikely. Slight differences in pH were however observed at rest and submaximal intensities with higher pH with salbutamol. The effect of salbutamol on maximal workload might arise from its vasodilatory action since peripheral vasodilation in human muscles can be initiated by β_2 -receptors (Dawes et al., 1997). Whether salbutamol may increase muscle perfusion and whether this effect may influence pH and maximal work capacity remains to be investigated.

In accordance with previous study (van Baak et al., 2000), maximal exercise performance was enhanced following acute oral salbutamol ingestion with a $> 20\%$ increase in maximal power output. Since at exhaustion similar changes in metabolite concentrations were observed with salbutamol and placebo, these results may be interpreted as a better metabolic efficiency with salbutamol, i.e., with similar metabolic responses greater mechanical work could be achieved. The present results are in line with our previous study showing that a supra-therapeutic dose of inhaled salbutamol increases isolated quadriceps endurance without any effect on peripheral neuromuscular fatigue (assessed by evoked muscle responses during artificial muscle stimulation) both at submaximal exercise and at exhaustion (Decorte et al.,

2013). Finally, based on both the results of the present study and our previous study (Decorte et al., 2013), one can speculate that the ergogenic effect of β 2-agonists may underlie on mechanisms outside the muscles, within the central nervous system for instance (Elias et al., 2004; Rauls et al., 2005).

Some limitations of the present study should be acknowledged. First, ^{31}P signal was acquired from all plantar flexor muscles simultaneously, without voxel-based localization for instance distinguishing the signal from distinct muscle groups. Although the gastrocnemius and soleus muscles may show different metabolic phenotypes and responses to exercise, the present results indicate that, within the same experimental setup and the same subjects, acute oral salbutamol administration does not modify the overall plantar flexors metabolism. Furthermore, although exercise-induced changes in Pi/PCr ratio, pH, and post-exercise PCr repletion are valuable indexes of mitochondrial oxidative capacity and glycolytic activity, one should emphasize that these parameters do not provide an exhaustive evaluation of all metabolic pathways during exercise. Because of the relatively small size of the study, we cannot completely exclude a risk of a type II error, which would occult a possible effect of salbutamol. At last, while moderately trained subjects were investigated during a maximal incremental exercise in the present study, whether the effect of salbutamol administration may differ depending on training status and the type of exercise protocol (e.g., submaximal constant-load exercise) remains to be elucidated.

In conclusion, this study showed that an acute oral dose of salbutamol did not induce significant changes in phosphorous compounds during exercise but provided significant ergogenic effects regarding muscle endurance. Therefore, changes in muscle metabolism are unlikely to explain the improved exercise performance

following acute oral salbutamol administration and alternative mechanisms should be considered.

Perspectives

In this study, using a noninvasive method to investigate muscle metabolism, we presented an update on the impact of an acute oral dose of salbutamol on muscle function. While salbutamol had no significant effect on plantar flexors phosphorus compounds (which indirectly determine muscle metabolism) during an incremental and localized fatiguing task, it increased significantly endurance performance. Although some studies suggested that oral acute administration of β 2-agonist may improve muscle contractility and metabolism (Arlettaz et al., 2009; Crivelli et al., 2011), the present findings together with our previous results focusing on neuromuscular fatigue (Decorte et al., 2013) suggest that the ergogenic effect of β 2-agonists may arise from mechanisms beyond the muscle. Based on the potential central nervous system effect of β 2-agonists as shown by the stimulant effect of salbutamol for instance (Barrot et al., 2009), one could hypothesize that salbutamol-induced changes in cerebral responses to exercise may underlie performance improvement.

Key words: Muscle, metabolism, β 2-agonists, performance, fatigue, doping.

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