Effect of inhaled terbutaline on substrate utilization and 300-kcal time trial performance

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1Respiratory Research Unit, Bispebjerg University Hospital, Copenhagen, Denmark; 2Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark; 3Norwegian Doping Control Laboratory, Oslo University Hospital, Oslo, Norway; and 4School of Pharmacy, University of Oslo, Oslo, Norway

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Kalsen A, Hostrup M, Karlsson S, Hemmersbach P, Bangsbo J, Backer V. Effect of inhaled terbutaline on substrate utilization and 300-kcal time trial performance. J Appl Physiol 117: 1180–1187, 2014. First published September 25, 2014; doi:10.1152/japplphysiol.00635.2014.—In a randomized, double-blind crossover design, we investigated the effect of the beta2-agonist terbutaline (TER) on endurance performance and substrate utilization in nine moderately trained men [maximum oxygen uptake (VO2 max) 58.9 ± 3.1 ml·min⁻¹·kg⁻¹]. Subjects performed 60 min of submaximal exercise (65–70% of VO2 max) immediately followed by a 300-kcal time trial with inhalation of either 15 mg of TER or placebo (PLA). Pulmonary gas exchange was measured during the submaximal exercise, and muscle biopsies were collected before and after the exercise bouts. Time trial performance was not different between TER and PLA (1,072 ± 145 vs. 1,054 ± 125 s). During the submaximal exercise, respiratory exchange ratio, glycogen breakdown (TER 266 ± 32, PLA 195 ± 28 mmol/kg dw), and muscle lactate accumulation (TER 20.3 ± 1.6, PLA 13.2 ± 1.2 mmol/kg dw) were higher (P < 0.05) with TER than PLA. There was no difference between TER and PLA in net muscle glycogen utilization or lactate accumulation during the time trial. Intramyocellular triacylglycerol content did not change with treatment or exercise. Pyruvate dehydrogenase-E1α phosphorylation at Ser203 and Ser306 was lower (P < 0.05) before submaximal exercise with TER than PLA, with no difference after the submaximal exercise and the time trial. Before submaximal exercise, acetyl-CoA carboxylase 2 (ACC2) phosphorylation at Ser210 was higher (P < 0.05) with TER than PLA. There was no difference in phosphorylation of alpha 5′-AMP-activated protein kinase (αAMPK) at Thr172 between treatments. The present study suggests that beta2-agonists do not enhance 300-kcal time trial performance, but they increase carbohydrate metabolism in skeletal muscles during submaximal exercise independent of AMPK and ACC phosphorylation, and that this effect diminishes as drug exposure time, exercise duration, and intensity are increased.

terbutaline; substrate utilization; exercise performance

BETA2-AGONISTS ARE FREQUENTLY USED in the treatment of chronic obstructive pulmonary disease, asthma, and exercise-induced bronchoconstriction, but due to their potential ergogenic effects (7, 20, 34) these substances may also be misused by athletes with the intent to improve performance. Acute ingestion of 4–6 mg of salbutamol has been shown to improve endurance performance (6, 39). The mechanisms behind this improved endurance performance have been suggested to involve an increased energy production from carbohydrate metabolism (6, 7, 9), but little knowledge exists about the effects of selective beta2-adrenergic stimulation on substrate oxidation and utilization in skeletal muscles in relation to endurance performance.

Through a cAMP-dependent pathway (28), beta2-agonists may alter substrate utilization in skeletal muscles by various mechanisms. Thus beta2-adrenergic stimulation by epinephrine has been shown to activate hormone-sensitive lipase, glycogen phosphorylase (24), phosphofructokinase (1), and pyruvate dehydrogenase (PDH) (40) in skeletal muscles. Furthermore, beta2-adrenergic stimulation increases release of glucose from the liver (30) and free fatty acids (FFA) from adipose tissue (18), thereby increasing the availability of glucose and FFA to the skeletal muscles.

In resting skeletal muscles, beta2-adrenergic stimulation increases energy expenditure and fat oxidation with no changes in carbohydrate oxidation (18, 37). In contrast, epinephrine infusion has been shown to increase carbohydrate oxidation and glycogen utilization during submaximal steady-state exercise (13, 40), whereas other studies show no changes in substrate utilization (5, 24, 42). Aside from a study showing no effect of epinephrine at 34% of VO2 max (43), there is limited knowledge about the effect of beta2-adrenergic stimulation on intramyocellular triacylglycerol (IMTG) utilization. Most studies have focused on the acute effect of epinephrine (targeting both α- and β-adrenoceptors) on substrate oxidation and utilization during submaximal nonstrenuous exercise (5, 13, 24, 40–43), whereas the acute effect of selective beta2-receptor stimulation on substrate utilization during strenuous endurance exercise has been poorly investigated.

An important enzyme that regulates skeletal muscle metabolism and substrate utilization during exercise is the intracellular energy sensor 5′-AMP-activated protein kinase (AMPK), which is activated by increases in ratios of AMP/ATP and ADP/ATP. AMPK is regulated by covalent modification of Thr172 of the catalytic α subunit by liver kinase B1 and Ca2+/calmodulin-dependent protein kinase kinase-β (21), where the latter has been shown to be activated by cAMP in C2C12 myotubes (33) and primary rat adipocytes (32). One of the downstream targets of AMPK, acetyl-CoA carboxylase 2 (ACC2), may play a role in regulation of fatty acid oxidation by catalyzing the carboxylation of acetyl-CoA to malonyl-CoA (16). The influence of beta2-adrenergic stimulation on AMPK and ACC2 phosphorylation in relation to substrate utilization during exercise has not been examined.

Thus the aim of the present study was to investigate the effects of the beta2-agonist terbutaline (TER) on performance, substrate utilization, and AMPK/ACC2 phosphorylation during strenuous endurance exercise.
Venous blood sample

Breath-by-breath measurements were collected throughout exercise at after the time trial using a 5-mm Bergström needle with suction. Biopsies were collected before and after the submaximal exercise and VO2 max followed by a 300-kcal time trial. The time trial was performed 60 min of submaximal cycle exercise at 65–70% of V˙O2 max. Breath-by-breath measurements were interrupted for 2 min to make the subjects measure measurements were analyzed. Halfway through the test breath-by-breath measurements were interrupted for 2 min to make the subjects forced exertory volume in 1 s; FVC, forced vital capacity.

MATERIALS AND METHODS

Subjects

Nine healthy, moderately trained, nonasthmatic male subjects were included in the study (Table 1). None of the subjects had previously used β2-agonists. The subjects were fully informed about potential risks and discomforts related to the experimental procedures. Written informed consent was obtained from all subjects prior to the study. The study was performed in accordance with the Helsinki II declaration and with approval from the local scientific ethics committee of Copenhagen (H-4-2012-110).

Experimental Protocol

The study was carried out with a double-blinded, randomized crossover design. It consisted of one screening visit and two identical study visits with inhalation of either TER or placebo (PLA) (Fig. 1). The screening visit consisted of lung function measurements and collection of anthropometric data. Furthermore, an incremental test on a cycle ergometer (839E; Monark, Vansbro, Sweden) was performed an incremental cycle test with gas exchange measurements. Pulmonary oxygen uptake (V˙O2) was measured using a breath-by-breath gas analyzing system (JAEGER MasterScreen CPX; Viasys Healthcare, Hoechberg, Germany). The system was calibrated with a 3-liter syringe and with gases of known O2 and CO2 concentrations. Throughout the test, subjects were told to keep a cadence between 70 and 90 revolutions per minute (rpm). The test ended when the pedaling frequency fell below 60 rpm for more than 5 s. VO2 max was defined as the highest value averaged over a 30-s period. A respiratory exchange ratio (RER) above 1.15 and no further increase in oxygen uptake despite an increased power output were used as criteria for VO2 max achievement.

Submaximal exercise at 65–70% of VO2 max and 300-kcal time trial. During submaximal exercise, the same absolute load was used for both study visits and was aimed to elicit an intensity corresponding to 65–70% of VO2 max. Breath-by-breath measurements were collected and RER, VO2, pulmonary ventilation (VE), and respiratory rate (RR) were averaged in periods of 8 to 10 min. Measurements from the first 2 min of exercise were discarded to make sure that only steady-state measurements were analyzed. Halfway through the test breath-by-breath measurements were interrupted for 2 min to make the subjects drink 500 ml of tap water.

Following submaximal exercise at 65–70% of VO2 max subjects were instructed to complete a 300-kcal time trial on a cycle ergometer as fast as possible. Starting load was chosen by the subjects, and they were free to change the load throughout the test. All subjects had previously completed self-paced time trials on cycle ergometers, but none had completed a 300-kcal time trial. During the familiarization trial at the screening visit, subjects were informed about the estimated duration of the test, and the starting load was chosen to elicit ~85% of VO2 max. Subjects were not provided with any information about pacing strategies, but were supervised during the familiarization time trial to ensure that proper pacing strategies were used. On the basis of

### Table 1. Subject characteristics

<table>
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<tr>
<th>Characteristic</th>
<th>Value</th>
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<td>Age, yr</td>
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<td>Height, cm</td>
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<tr>
<td>Weight, kg</td>
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<td>VO2 max, liter/min</td>
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<td>VO2 max, ml·min⁻¹·kg⁻¹</td>
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<td>FEV1, liter</td>
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<td>FVC, liter</td>
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<td>Training volume, h/wk</td>
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</table>

Values are means ± SE. FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.
the familiarization trial, subjects were instructed to select a pacing strategy and to use the same strategy on both study visits. The coefficient of variation between the time trial at the screening visit and the PLA visit was <3%.

Blood analysis. Blood samples were collected in heparinized syringes and analyzed immediately for lactate and glucose with an ABL 800 FLEX blood gas analyzer (Radiometer, Copenhagen, Denmark).

Serum concentrations of TER were determined after submaximal exercise (80 min after drug administration). Briefly, blood samples were collected in vacutainers with clot activator and spun at 3,000 rpm for 15 min, after which serum samples were collected and stored at −80°C. Serum TER was analyzed by liquid chromatography–tandem mass spectrometry as previously described (11) and performed by the World Anti-Doping Agency-accredited Norwegian Doping Control Laboratory at Oslo University Hospital, in Oslo, Norway.

Muscle analysis. Muscle biopsies were immediately frozen in liquid nitrogen and stored at −80°C. Biopsies were freeze-dried and dissected free of visible blood, connective tissue, and adipose fat under a stereo microscope. Dissection was carried out at 18°C with humidity 30%.

Glycogen, lactate, and glucose-6-phosphate (G-6-P). Muscle tissue [2 mg dry weight (dw)] was extracted in 1 N HCl and hydrolyzed at 100°C for 3 h. Glycogen content was determined by the hexokinase method (26).

Muscle tissue (2 mg dw) was extracted in a solution of 0.6 M perchloric acid and 1 mM EDTA, neutralized to pH 7.0 with 2.2 M KHCO3, and stored at −80°C until analyzed fluorometrically for lactate and G-6-P (26).

Intramyocellular triacylglycerol. Approximately 2 mg of dw muscle tissue was analyzed for IMTG as previously described (23, 38).

SDS-PAGE and Western blotting. Muscle tissue (2 mg dw) was homogenized on ice in a fresh buffer (10% glycerol, 20 mM Na-pyrophosphate, 150 mM NaCl, 50 mM HEPES, 1% Nonidet P-40, 20 mM β-glycerophosphate, 10 mM Na2F, 2 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 1 mM EGTA, 10 μM aprotinin, 10 μM leupeptin, and 3 mM benzamidine) with a Polytron 3100 (Kinematica) for 30 s. Samples were rotated end-over-end for 1 h at 4°C before being centrifuged for 30 min at 17,500 × g at 4°C. Lysate was collected and the supernatant and protein concentrations were determined by ELISA using BSA standards (Pierce Reagents). The lysates were diluted to appropriate protein concentrations in a ×6 sample buffer (0.5 M Tris-base, dithiothreitol, SDS, glycerol, and bromphenol blue), and for each sample, 16 μg of protein was loaded into different wells of 4–15% precasted Criterion TGX stain-free gels (Bio-Rad Laboratories). Samples from the same subject were loaded next to each other on the same gel. Gel electrophoresis was carried out for 90–120 min at 60 mA and a maximum of 150 V per gel followed by semidy transfer of proteins to polyvinylidene difluoride membranes (Immobilon Transfer Membrane; Millipore, Billerica, MA) at 70 mA and a maximum of 25 V per gel for 120 min. Membranes were incubated overnight with 30–40 ml of primary antibody diluted in 2% nonfat milk before being washed briefly in Tris-buffered saline (Tween) and incubated with secondary antibody (diluted in 2% nonfat milk) for 1 h at room temperature. Primary antibodies used were pAMPK Thr172 (2531; Cell Signaling, Danvers, MA), AMPK α2 (2757; Cell Signaling), pACC2 Ser21 (07–303; Millipore), ACC2 [P0397 (horseradish peroxidase-conjugated streptavidin that binds to the biotin moiety of ACC); Dako, Glostrup, Denmark], pPDE-E1α Ser203 (ABS204; Millipore), pDH-E1α Ser194 (Millipore), and pDH-E1α (sc-377092; Santa Cruz Biotechnology, Santa Cruz, CA).

Horseradish peroxidase (HRP)-conjugated antibodies (P0447 and P0448, both from Dako) were used as secondary antibodies. After primary and secondary antibody treatment, membranes were incubated in chemiluminescent substrate (Immobilon Western Chemilum HRP Substrate; Millipore, Denmark) and the signal was detected on a Chemi Doc MP (Bio-Rad Laboratories, Hercules, CA). Band intensities were quantified using Image Lab (v. 4.0; Bio-Rad).

Drugs

TER (Bricanyl, 0.5 mg/dose) and PLA were provided by Asta Zeneca (London, UK) and administered by inhalation from identical turbohalers. Following inhalation, TER peaks in serum after 30 to 60 min and remains elevated for 1 to 2 h before slowly decreasing (11). The terminal half-life of TER is ~17 h (31). Before study visits, subjects were instructed about the inhalation technique and informed about the side effects of TER. Drug administration was carried out by a person who was not involved in other parts of the study. Randomization procedures were performed using Excel software (Microsoft, Redmond, WA) and randomization was confirmed in serum samples analyzed by the Norwegian Anti-doping Laboratory at Oslo University Hospital. Seven out of nine subjects reported tremor and tachycardia after inhaling 15 mg of TER. However, the subjects reported that side effects were markedly reduced (n = 3) or completely gone (n = 4) during the submaximal exercise and the time trial. Subjects did not report any differences in perceived exertion between treatments during the time trial.

Statistics

SPSS version 18.0 (IBM, Armonk, NY) was used for statistical analysis. Sample size was determined for a crossover design for the primary outcome, time trial performance, as previously described (10). Power was set to 0.8, the significance level was set to 0.05, and effect size was based on previous observations of increased endurance performance following administration of beta2-agonists (6, 39). Sample size calculations revealed that eight subjects were necessary to obtain a power of 0.8. With the risk of one dropout, nine subjects were included. All data were normally distributed (Shapiro-Wilk test). Differences in time trial performance and serum TER were analyzed with a paired t-test. Glycogen, IMTG, muscle lactate, G-6-P, phosphorylation data, venous blood, and breath-by-breath measurements were analyzed with a repeated measures ANOVA with two within factors (treatment and sampling point). In case of a significant ANOVA, the Bonferroni correction was used as a post hoc test. Significance level was 0.05.

RESULTS

The concentration of serum TER was 23.4 ± 0.7 ng/ml 80 min after administration.

Endurance Performance

There was no difference in time trial performance between the two treatments (PLA 1,054 ± 125 s, TER 1,072 ± 145 s, Fig. 2). Performance was improved for three subjects and reduced for five subjects, with one subject completing the time trial in the same time with both treatments (Fig. 2).

Pulmonary Measurements During Exercise at 65–70% of $\dot{V}O_{2\text{max}}$

RER was higher (P < 0.05) for TER than PLA during the first 20 min of exercise, whereas RER tended to be higher (P < 0.05) only for TER compared with PLA during the last 40 min of exercise (Fig. 3). VE and RR were higher (P < 0.05) for TER than PLA for all sampling points, whereas no difference in $\dot{V}O_{2}$ was observed at any sampling point between TER and PLA. There was no difference in tidal volumes between treatments for any sampling points.

Muscle Glycogen and IMTG

Before exercise at 65–70% of $\dot{V}O_{2\text{max}}$ there was no difference in glycogen content between TER and PLA (434 ± 67 vs. 429 ± 67 mg/kg dw muscle). The concentration of serum TER was 23.4 ± 0.7 ng/ml 80 min after administration.
Muscle glycogen before and after exercise at 65–70% of VO₂ max was lower (P < 0.05) for TER compared with PLA after exercise at 65–70% of VO₂ max (197 ± 72 vs. 239 ± 77 mmol/kg dw) and after the time trial (77 ± 31 vs. 144 ± 52 mmol/kg dw). The net rate of muscle glycogen utilization was higher (P < 0.05) with TER (4.43 ± 0.53 mmol·kg dw⁻¹·min⁻¹) compared with PLA (3.25 ± 0.47 mmol·kg dw⁻¹·min⁻¹) during exercise at 65–70% of VO₂ max (Fig. 4A), but no difference between treatments was observed between after exercise at 65–70% of VO₂ max and after the time trial (Table 2).

Muscle Lactate and G-6-P

Muscle lactate before and after exercise at 65–70% of VO₂ max was higher (P < 0.05) with TER compared with PLA with no difference between TER and PLA after the time trial. Before exercise at 65–70% of VO₂ max, muscle G-6-P was higher for TER than PLA, whereas no difference was observed between treatments after exercise at 65–70% of VO₂ max and after the time trial.

Muscle Protein Phosphorylation

Phosphorylation state and representative blots are shown in Fig. 5. Before exercise at 65–70% of VO₂ max, PDH-E1 Ser293 and Ser300 phosphorylation were lower (P < 0.05) for TER than PLA, but no difference was observed between TER and PLA after the submaximal exercise and after the time trial. There was no difference in AMPK Thr172 phosphorylation between TER and PLA at any sampling point. Before exercise at 65–70% of VO₂ max, ACC2 Ser221 phosphorylation was higher (P < 0.05) for TER compared with PLA, with no difference between treatments after exercise at 65–70% of VO₂ max and after the time trial.

Venous Glucose and Lactate

Plasma glucose was higher (P < 0.05) for TER compared with PLA at all sampling points except from 5 min after the 300-kcal time trial. Furthermore, plasma lactate was higher (P < 0.05) for TER than PLA at all sampling points (Table 3).

DISCUSSION

The present study is the first to investigate the muscular effects of selective beta2-adrenergic stimulation on substrate utilization during exercise.

**Fig. 2.** Performance in a 300-kcal time trial performed 30 s after 60 min of cycle exercise at 65–70% of VO₂ max after administration of either TER or PLA. Values are means ± SE.

**Fig. 3.** Respiratory exchange ratio (A), oxygen uptake (B), ventilation (C), and respiratory rate (D) during 60 min of cycle exercise at 65–70% of VO₂ max after administration of either TER or PLA. Values are averaged over the time periods shown and presented as means ± SE. *Different from PLA (P < 0.05).
utilization during strenuous endurance exercise. The main findings were that beta_2-adrenergic stimulation with TER increased carbohydrate oxidation (RER), muscle glycogen utilization, and muscle lactate accumulation during 60 min of exercise at 65–70% of VO_2_max, and that glycogen utilization, lactate accumulation, and performance were unaltered during a 300-kcal time trial performed 30 s after the exercise at 65–70% of VO_2_max. Furthermore, our findings suggest that beta_2-adrenergic effects on carbohydrate metabolism of skeletal muscles are independent of AMPK and ACC phosphorylation.

In the present study, we observed a higher RER with TER during the first 20 min of exercise at 65–70% of VO_2_max, indicating a higher carbohydrate oxidation. This observation contrasts with the finding by Arlettaz and coworkers (2) who observed no effect of 4 mg of oral salbutamol on substrate oxidation during the first 40 min of a 60-min cycle exercise bout at 60% of VO_2_max. Because the present study was carried out at a higher intensity, the different findings may be attributed to differences in exercise intensity. However, the lower PDH-E1α phosphorylation (on Ser^{293} and Ser^{306}) before exercise with TER indicates an increased entry of carbohydrate-derived substrate into the citric acid cycle (25, 35) and hence does not support that the beta_2-adrenergic effect on carbohydrate oxidation should disappear with reduced intensity. A more plausible explanation for the different findings may be that the dose administered by Arlettaz and coworkers was too low to alter carbohydrate oxidation, which is supported by the finding that venous glucose and lactate, in contrast to the present study, were not higher following administration of 4 mg of salbutamol.

The higher carbohydrate oxidation observed with administration of TER during exercise at 65–70% of VO_2_max was associated with higher utilization of muscle glycogen with no difference in IMTG utilization. Although the present study is the first to investigate the effect of selective beta_2-adrenergic stimulation on glycogen utilization, other studies have shown increased (13, 40) or unchanged (24, 42) utilization of muscle glycogen during submaximal exercise following infusion of epinephrine. The effect of beta_2-adrenergic stimulation on IMTG utilization during exercise has been poorly investigated, but in line with the present study, a study by West and coworkers (43) found no effect of epinephrine infusion on IMTG utilization during 90 min of exercise at 34% of VO_2_max.

Increased glycogen utilization and carbohydrate oxidation with TER during exercise at 65–70% of VO_2_max were associated with a higher accumulation of muscle and blood lactate, which is in agreement with observations following epinephrine infusion (13). On the other hand, we observed no difference in glycogen utilization and lactate accumulation during the time trial between treatments. An explanation for the different findings may be that the higher intensity during the time trial elevated epinephrine, increased intracellular Ca^{2+} release from the sarcoplasmic reticulum, and increased concentrations of intramuscular allosteric activators such as AMP and inosine 5’-monophosphate, which fully activated glycogen phosphorylase with no further effect of TER (36). Another explanation could be that the lower glycogen content observed with TER before the time trial counteracted the effect of TER on glycogen breakdown. This is supported by Hespel and Richter (17), who found that lowered glycogen reduced the activity of glycogen phosphorylase in rats.

The unchanged performance during the time trial with TER fits well with the observation of unchanged muscle metabolism. In accordance, previous studies also failed to find effects of inhaled beta_2-agonists on endurance performance (12, 22, 34), whereas Collomp et al. (6) and van Baak et al. (39) showed improved endurance performance following oral administration of beta_2-agonists. The contrasting findings be-

Table 2. Muscle lactate and glucose-6-phosphate before and after 60 min of cycle exercise and after a 300-kcal time trial after administration of either terbutaline or placebo

<table>
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<th>Before Cycle Exercise*</th>
<th>After Cycle Exercise*</th>
<th>After Time Trial†</th>
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<tr>
<td></td>
<td>PLA</td>
<td>TER</td>
<td>PLA</td>
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<tr>
<td>Lactate</td>
<td>11.2 ± 1.1</td>
<td>14.3 ± 1.2‡</td>
<td>13.2 ± 1.2</td>
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<tr>
<td>G-6-P</td>
<td>2.8 ± 0.3</td>
<td>5.6 ± 0.6‡</td>
<td>2.5 ± 0.4</td>
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Values are means ± SE. G-6-P, glucose-6-phosphate in mmol/kg dry weight; PLA, placebo; TER, terbutaline. *Cycle exercise was performed at 65–70% of VO_2max. †The 300-kcal time trial was performed 30 s after exercise at 65–70% of VO_2max. ‡Different from PLA at same sampling point (P < 0.05).
Blood samples were drawn after 0, 15, 30, 45, and 60 min of cycle exercise at 65–70% of $\dot{V}O_2_{\text{max}}$ and after completion of 100, 200, and 300 kcal during the

Table 3. Venous glucose and lactate during 60 min of cycle exercise and during a 300-kcal time trial after administration of either terbutaline or placebo

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<th>0</th>
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<th>30</th>
<th>45</th>
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<th>200 kcal</th>
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<td>Glucose*</td>
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<tr>
<td>PLA</td>
<td>5.4 ± 0.1</td>
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<td>TER</td>
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<td>8.3 ± 0.2‡</td>
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<td>PLA</td>
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<td>10.5 ± 0.8‡</td>
<td>12.0 ± 0.7‡</td>
<td>13.0 ± 0.8‡</td>
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Values are means ± SE. Cycle exercise was performed at 65–70% of $\dot{V}O_2_{\text{max}}$. The 300-kcal time trial was performed 30 s after exercise at 65–70% of $\dot{V}O_2_{\text{max}}$ and after completion of 100, 200, and 300 kcal during the 300-kcal time trial. *Glucose and lactate were measured in mmol/liter. †A blood sample was drawn 5 min after the 300-kcal time trial (p 5). ‡Different from PLA at same sampling point ($P < 0.05$).

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between the present study and the studies by Collomp et al. (6) and van Baak et al. (39) cannot be attributed to insufficient systemic drug concentrations because the present study showed markedly higher serum concentrations compared with 10 mg of oral TER (11). Instead, differences in exercise protocol may explain the different findings. In the present study, subjects completed 60 min of exercise at 65–70% of $\dot{V}O_2_{\text{max}}$ followed by a 300-kcal time trial, whereas in the studies by Collomp et al. (6) and van Baak et al. (39), exercise was performed to exhaustion at 80–85% and 70% of $\dot{V}O_2_{\text{max}}$, respectively, with a shorter exercise duration (~30 and 55 min, respectively) than in the present study (~80 min). Muscle glycogen content becomes increasingly important for performance as exercise duration increases beyond 30 min (8). Therefore, in the present study, lower muscle glycogen content before the time trial after administration of TER may have exerted a negative effect on performance, whereas performance in the studies by Collomp et al. (6) and van Baak et al. (39) may not have been limited by

Fig. 5. Protein phosphorylation before and after 60 min of CE at 65–70% of $\dot{V}O_2_{\text{max}}$ and after a 300-kcal TT performed 30 s after exercise at 65–70% of $\dot{V}O_2_{\text{max}}$ after administration of either TER or PLA. A: Pyruvate dehydrogenase-E1α (PDH-E1α) phosphorylation at Ser293. B: PDH-E1α phosphorylation at Ser300. C: AMP-activated protein kinase (AMPK) phosphorylation at Thr172. D: acetyl-CoA carboxylase 2 (ACC2) phosphorylation at Ser221. Phosphorylation state is expressed relative to total protein content. Values are means ± SE. *Different from PLA at same sampling point ($P < 0.05$).
glycogen availability to the same extent as in the present study. Based on the present findings it is likely that beta2-agonists impair performance in long-lasting endurance events due to premature depletion of muscle glycogen.

ACC2 Ser\textsuperscript{221} phosphorylation was higher with TER before exercise at 65–70% of VO\textsubscript{2 max}, but no difference was observed between TER and PLA after exercise. If ACC has an important role in regulation of fat oxidation, a higher ACC2 Ser\textsuperscript{221} phosphorylation would be expected to increase fat oxidation. This was not the case in the present study because RER measurements revealed a higher carbohydrate oxidation and lower fat oxidation during exercise after administration of TER. Furthermore, the present study found no effect of TER on AMPK Thr\textsuperscript{172} phosphorylation, demonstrating that AMPK is not important in beta2-adrenergic regulation of substrate utilization and oxidation during exercise.

Before exercise at 65–70% of VO\textsubscript{2 max}, phosphorylation of PDH-E1/\textalpha Ser\textsuperscript{293} and Ser\textsuperscript{300} were lower with beta2-adrenergic stimulation, but this effect was ablated after exercise at 65–70% of VO\textsubscript{2 max}. This may explain that RER was higher with TER in the first but not during the last period of exercise at 65–70% of VO\textsubscript{2 max} and that muscle G-6-P was the same after exercise. Other factors may explain why there was no effect of TER on carbohydrate metabolism during the last phase of exercise at 65–70% of VO\textsubscript{2 max}. Beta2-receptor desensitization and sequestration occur rapidly upon agonist binding, and increase with the duration of agonist exposure, resulting in reduced intracellular concentrations of cAMP (27). Therefore, the effect of TER may have diminished in a time-dependent manner due to gradually reduced cAMP concentrations in the skeletal muscles. Another possibility is that the effect of cAMP on glycogenolysis was reduced with prolonged exposure to elevated cAMP, which is supported by observations of a reduced glycogenolytic rate after prolonged epinephrine infusion despite an unchanged percentage of glycogen phosphorylase in the active form (4).

The subjects in the present study were moderately trained, and it is unclear whether the results also apply to elite athletes or untrained subjects. It seems plausible, however, that the physiological response evoked by beta2-agonists is dependent on training status because beta2-receptors are widely distributed in tissues affected by exercise training. Thus training-induced alterations in enzymes involved in metabolic pathways affected by beta2-agonists are likely to affect the physiological responses mediated by beta2-agonists. Moreover, beta2-receptor density is dependent on training status (3) and fiber type composition (29), which further suggests a differentiated response to beta2-agonists in subjects with different training status. Nevertheless, studies in elite athletes, in line with the present study, also failed to find any effect on endurance performance following administration of high doses of beta2-agonists (12, 19, 22). The current literature thus does not indicate that elite athletes respond differently to beta2-agonists than moderately trained subjects, but studies specifically investigating the relationship between training status and beta2-agonist responsiveness are needed to elucidate this question.

The present study has some limitations. The total muscle anaerobic energy production could not be determined because muscle ATP, phosphocreatine, and lactate release were not measured. Another issue is that indirect calorimetry is influenced by formation of lactate because the associated increase in hydrogen ions may displace CO\textsubscript{2} from bicarbonate stores (14, 15). Thus RER may have been higher than the respiratory quotient during part of the submaximal exercise after TER treatment due to the larger accumulation of lactate, but the difference is probably minor (14). The higher RER during the first 20 min of submaximal exercise in the TER trial probably reflects a larger carbohydrate oxidation, which is supported by the finding of a lower phosphorylation of PDH before exercise with TER.

In summary, the present study showed that beta2-adrenergic stimulation with TER increased carbohydrate oxidation, muscle glycogen utilization, and lactate accumulation during 60 min of exercise at 65–70% of VO\textsubscript{2 max} with no difference in glycogen utilization, lactate accumulation, and performance during a 300-kcal time trial performed 30 s after exercise at 65–70% of VO\textsubscript{2 max}. In addition, AMPK Thr\textsuperscript{172} and ACC Ser\textsuperscript{221} phosphorylation data from the present study suggest that AMPK and ACC are not important in beta2-adrenergic regulation of substrate oxidation and utilization during submaximal exercise.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.K., M.H., S.K., J.B., and V.B. conception and design of research; A.K., M.H., S.K., J.B., and V.B. performed experiments; A.K., M.H., and P.H. analyzed data; A.K., M.H., J.B., and V.B. interpreted results of experiments; A.K. prepared figures; A.K. drafted manuscript; A.K., M.H., S.K., P.H., J.B., and V.B. edited and revised manuscript; A.K., M.H., S.K., P.H., J.B., and V.B. approved final version of manuscript.

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