Mechanisms underlying enhancements in muscle force and power output during maximal cycle ergometer exercise induced by chronic β2-adrenergic stimulation in men

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Hostrup M, Kalsen A, Onslev J, Jessen S, Haase C, Habib S, Ørtenblad N, Backer V, Bangsbo J. Mechanisms underlying enhancements in muscle force and power output during maximal cycle ergometer exercise induced by chronic β2-adrenergic stimulation in men. J Appl Physiol 119: 475–486, 2015. First published July 9, 2015; doi:10.1152/japplphysiol.00319.2015.—The study was a randomized placebo-controlled trial investigating mechanisms by which chronic β2-adrenergic stimulation enhances muscle force and power output during maximal cycle ergometer exercise in young men. Eighteen trained men were assigned to an experimental group (oral terbutaline 5 mg/30 kg body weight (bw) twice daily (TER); n = 9] for a 4-wk intervention. No changes were observed with the intervention in PLA. Isometric muscle force of the quadriceps increased (P ≤ 0.01) by 97 ± 29 N (means ± SE) with the intervention in TER compared with PLA. Maximal oxygen consumption (V̇O2max) and mean power output during 30 s of maximal cycling increased (P ≤ 0.01) by 32 ± 8 and 25 ± 9 W, respectively, with the intervention in TER compared with PLA. Maximal oxygen consumption (V̇O2max) and time to fatigue during incremental cycling did not change with the intervention. Lean body mass increased by 1.95 ± 0.8 kg (P ≤ 0.05) with the intervention in TER compared with PLA. Change in single fiber cross-sectional area of myosin heavy chain (MHC) I (1,205 ± 558 µm2; P ≤ 0.01) and MHC II fibers (1,277 ± 595 µm2; P ≤ 0.05) of the vastus lateralis muscle was higher for TER than PLA with the intervention, whereas no changes were observed in MHC isoform distribution. Expression of muscle proteins involved in growth, ion handling, lactate production, and clearance increased (P ≤ 0.05) with the intervention in TER compared with PLA, with no change in oxidative enzymes. Our observations suggest that muscle hypertrophy is the primary mechanism underlying enhancements in muscle force and peak power during maximal cycling induced by chronic β2-adrenergic stimulation in humans.

β2-adrenoceptor agonists (β2-agonists) are among the most commonly used drugs worldwide given the high prevalence of asthma, exercise-induced bronchoconstriction, and chronic obstructive pulmonary disease (24, 46, 62). While their main therapeutic application is to induce bronchodilation, β2-agonists elicit several extrapulmonary effects (34, 44). β2-Adrenoceptors are abundant throughout the human body and are widely expressed in various tissues (44). Chronic administration of oral β2-agonists has been shown to enhance muscle force and power output during maximal cycling in humans (10, 12, 39, 40, 45, 69), which based on observations in animals, have been speculated to be attributed to hypertrophy or changes in fiber type composition of skeletal muscle (21, 23, 51, 63, 65, 81). However, limited mechanistic insight exists in humans that can explain enhancements in muscle force and power output during maximal cycling. While it is well described that chronic β2-adrenergic stimulation induces muscle hypertrophy in animals (23, 63, 67), no such effect has been observed in healthy humans (10, 39, 40, 45). This discrepancy may be related to methodology, since human studies relied on measurements of changes in body composition, whereas animal studies determined changes in the cross-sectional area (CSA) and specific weight of skeletal muscle (9, 21, 66, 81). Another explanation could relate to dosing regimen, as the doses used in animal studies were markedly higher than those administered to humans (44, 59). The hypertrophic response to β2-agonists in animals is as such dependent on the dose, route of administration, type of agonist used, and duration of treatment (14, 50, 67). Most human studies that have investigated effects of chronic β2-agonists used oral doses of salbutamol (12, 39, 40, 45), whereas limited data exist of other β2-agonists. In a recent study, acute oral administration of terbutaline was shown to elicit a significant systemic response in trained men (32). Thus it may be that high-dose terbutaline possesses a hypertrophic effect when administered chronically to humans. Moreover, while several animal studies have examined the molecular mechanisms by which chronic β2-adrenergic stimulation induces hypertrophy of skeletal muscle (37, 52, 54, 55, 75), no such studies exist in humans. Attenuation of myostatin has been highlighted as an important factor by which β2-agonists elicit their hypertrophic effects (8, 33, 54, 55). However, studies suggest that other factors may be involved (1, 37). One of these factors could be follistatin, which was recently shown to be associated with hypertrophy in rodents following treatment with the β2-agonist formoterol (8). The β2-adrenergic hypertrophic response has also been shown to vary between slow- and fast-twitch muscles of animals (21, 66, 81). To our knowledge, no studies in humans have investigated the effect of chronic administration of β2-agonists on the expression of muscle myostatin and follistatin or whether muscle fiber type-specific differences exist in the hypertrophic response to β2-agonists.
Animal studies have provided evidence that chronic β2-adrenergic stimulation leads to a slow-to-fast fiber-type transition of the myosin heavy chain (MHC) isoforms, thus shifting the contractile properties of skeletal muscle (76, 81, 82). The shift in MHC isoforms, along with gains in muscle mass, is associated with increased peak twitch and tetanic force (21, 82), increased maximal shortening velocity (21) and shortened time-to-peak tension (82). In humans, however, no studies have investigated whether chronic β2-adrenergic stimulation affects MHC distribution, peak twitch force, and time-to-peak twitch force of skeletal muscle. Moreover, it has been suggested that the enhanced muscle force observed with chronic administration of β2-agonists in humans may be related to central effects (45). β2-Adrenoceptors are abundant in the brain and β2-agonists have been shown to have antidepressant properties possibly through the serotonergic system (61). It has as such been speculated that β2-agonists alters the degree of central activation during a maximal muscle contraction (45). It is therefore of interest to investigate whether chronic β2-adrenergic stimulation affects the degree of voluntary activation during a superimposed maximal muscle contraction.

Chronic β2-adrenergic stimulation has also been shown to modify oxidative and glycolytic properties of skeletal muscle in animals (54, 75). Thus studies in rodents have shown that oxidative capacity and capillary density are repressed following chronic treatment with the β2-agonist clenbuterol (28, 78), which may be associated with compromised fatigue resilience during muscle activity (22, 66). In humans, on the other hand, chronic treatment with β2-agonists was shown not to affect maximal oxygen consumption (VO_{2max}) and time to fatigue during incremental cycling in endurance athletes (30) and to prolong time to fatigue during submaximal cycling in trained men (16), suggesting that oxidative capacity remains unaltered or improved in humans. Moreover, chronic administration of β2-agonists enhances power output during 30 s of maximal cycling in humans (30, 40, 69), which may be related to increased expression of glycolytic enzymes of skeletal muscle. However, no studies have investigated whether chronic administration of β2-agonists affects expression of oxidative and glycolytic enzymes in skeletal muscle of humans.

Thus the aim of the present study was to investigate underlying mechanisms by which chronic β2-adrenergic stimulation enhances muscle force and power output during 30 s of maximal cycling in humans. Our hypothesis was that enhancements in muscle force and power output during maximal cycling induced by chronic β2-adrenergic stimulation are primarily explained by skeletal muscle hypertrophy. A secondary aim was to examine whether muscle fiber type-specific differences exist in the hypertrophic response to chronic β2-adrenergic stimulation in humans and how a potential hypertrophic response related to expression of muscle myostatin and follistatin.

MATERIALS AND METHODS

Subjects. Twenty healthy trained men were initially screened and included in the study. Before inclusion, subjects received oral and written information about the aims and contents of the study and possible risks involved including side effects of the study drug (terbutaline). Each subject gave his oral and written informed consent. A physician screened each subject for unknown cardiopulmonary disease with lung and heart stethoscopy and electrocardiography (ECG). Upon inclusion, subjects were randomly allocated to two groups, either an experimental group [terbutaline (TER)] or a control group [placebo (PLA)]. Two subjects did not complete the study (one from each group) due to inadequate time in their schedule. The subjects who completed the study were 25 ± 1 (TER) and 25 ± 1 (PLA) yr and 183 ± 2 (TER) and 178 ± 2 (PLA) cm in height and had a VO_{2max} of 55 ± 2 (TER) and 57 ± 2 (PLA) ml·min^{-1}·kg^{-1}. Subjects were recreationally active, engaging in running, biking, and light resistance training with a training volume of 4-8 h/wk. Subjects were told to refrain from competitive events for the entire study and not to change their daily physical activities and nutritional habits, which were recorded. The study was performed in accordance with the Helsinki II Declaration and was approved by the Ethics Committee of Copenhagen, Denmark (H-1-2012-090).

Study design. The study was designed as a randomized placebo-controlled trial with an experimental group (TER) and a control group (PLA). In TER, β2-agonist terbutaline (Bricanyl Retard; AstraZeneca, London, UK) in oral doses of 5 mg/30 kg body weight (bw) twice daily for 28 ± 1 days. In PLA, subjects received oral placebo tablets (lactose monohydrate/starch; PLA) for 28 ± 1 day. Study drugs were delivered to the subjects in bottles. The duration of treatment was based on previous studies showing that 3–4 wk of treatment with β2-agonists is effective to enhance muscle force and power output during 30 s of maximal cycling in humans (40, 45, 69). Since a previous study found no acute effect of 8 mg oral terbutaline on muscle strength and performance (70), we chose a higher dose that has been shown to elicit a significant systemic response (32). Lactose-monohydrate/starch tablets were used as placebo. Study drugs were delivered by the regional pharmacy of Copenhagen. Both subjects and investigators were blinded to the treatments. Randomization was conducted in SPSS (IBM, Armonk, NY) by a technician who did not take part in any of the experimental procedures. Drug compliance of each subject was assessed by counting the number of unused tablets in the bottle after the intervention, comparing with the number of tablets in the bottle before the intervention. The number of tablets in the bottle before the intervention was random.

Experimental protocol. Before the start of the intervention, subjects met at the laboratory for two visits. At the first visit, subjects’ body composition was measured by dual X-ray absorbance (DXA). After DXA, a biopsy was taken from the vastus lateralis muscle of the right thigh under local anesthesia (20 mg/ml Xylcon; AstraZeneca). Muscle biopsies were quickly frozen in liquid nitrogen and stored at −80°C for later analysis. Subjects then warmed up for 10 min on a bike ergometer (Monark E839, Stockholm, Sweden), after which they performed an incremental exercise test starting at 150 W increasing by 30 W every min until time of fatigue. During the incremental test, subjects’ pulmonary gas exchange was measured breath-by-breath with a gas analyzer system (Oxycon Pro, CareFusion, San Diego, CA) for determination of VO_{2max}. Furthermore, subjects were accustomed to the experimental procedures conducted during the second visit. At the second visit, subjects’ contractile properties of the quadriceps muscle were measured during maximal voluntary isometric contractions (MVC) with electrical percutaneous muscle stimulations delivered on top of the plateau of each MVC as well as 1 s following relaxation. Before measurements, subjects performed three submaximal contractions of a 3-s duration. Subjects then performed three MVCs of a 3- to 4-s duration, each interspersed by 90 s of recovery. MVC measurements were preceded by a 15 min warm-up at 150 W for 10 min and 200 W for 5 min on a bike ergometer. After MVC measurements, subjects completed 30 s of maximal cycling on a bike ergometer (Monark E839). During the test, subjects pedaled against a brake force of 0.90 N/kg bw for 30 s as fast as possible. Subjects were instructed to remain seated throughout the entire test. Cadence and power output were recorded by a computer with a sampling frequency of 1 Hz. After the test, peak power was calculated as the highest power achieved for five consecutive seconds and mean power as the average power output during the 30 s of maximal cycling.
After the second visit, subjects received study drugs for a 4-wk intake. Subjects were instructed to ingest the study drugs twice daily during a meal (morning and afternoon). After the 4-wk period and 36–72 h of washout (terminal half-life of terbutaline: ~4–6 h), subjects reported to the laboratory again for two separate visits that were similar to those conducted before the drug intervention.

Subjects were told to abstain from alcohol, caffeine, nicotine, and strenuous exercise for 48 h before each laboratory visit.

**Dual-energy X-ray absorptiometry.** Subjects’ whole-body composition was determined by DXA (Lunar DPX-IQ, Version 4.7 E; Lunar, Madison, WI). Subjects were placed in the scanner in supine position undressed and euhydration. To reduce intradyad variation, two scans at medium speed were performed according to the manufacturer’s guidelines using medium research analysis software. The scanner was calibrated before scan, using daily calibration procedures (Lunar “System Quality Assurance”). All scans were conducted by the same hospital technician.

**Contractile properties of the quadriceps muscle.** For measurements of contractile properties of the quadriceps muscle, subjects were positioned on a chair with an adjustable chair back with thighs being parallel to the floor and right leg fixed in a knee joint angle of 90° of flexion. To ensure that subjects remained in the same position during each measurement, three Velcro straps were tied around the chest, hips, and thighs. Subjects’ body position was recorded and used for each measurement before and after the intervention. Isometric contractions of the quadriceps, and thighs. Subjects’ body position was recorded and used for each measurement, three Velcro straps were tied around the chest, hips, and thighs. Subjects’ body position was recorded and used for each measurement before and after the intervention. Isometric contraction force was recorded using a strain gauge (Tedea-Huntleigh) positioned on a chair with an adjustable chair back with thighs being parallel to the floor and right leg fixed in a knee joint angle of 90° of flexion. To ensure that subjects remained in the same position during each measurement, three Velcro straps were tied around the chest, hips, and thighs. Subjects’ body position was recorded and used for each measurement before and after the intervention. Isometric contraction force was recorded using a strain gauge (Tedea-Huntleigh) strapped around the right ankle just above the malleoli. Strain gauge signal was fed to an amplifier that was connected to a computer. Data were recorded at 1 kHz in LabChart 7 (ADInstruments).

During and immediately after each MVC, superimposed percutaneous electrical muscle stimulation (58) were delivered to the vastus lateralis muscle and rectus femoris muscle by two self-adhesive traction force was recorded using a strain gauge (Tedea-Huntleigh) for each measurement before and after the intervention. Isometric contraction force was recorded using a strain gauge (Tedea-Huntleigh) strapped around the right ankle just above the malleoli. Strain gauge signal was fed to an amplifier that was connected to a computer. Data were recorded at 1 kHz in LabChart 7 (ADInstruments).

During and immediately after each MVC, superimposed percutaneous electrical muscle stimulations were recorded using a strain gauge (Tedea-Huntleigh) for each measurement before and after the intervention. Isometric contraction force was recorded using a strain gauge (Tedea-Huntleigh) strapped around the right ankle just above the malleoli. Strain gauge signal was fed to an amplifier that was connected to a computer. Data were recorded at 1 kHz in LabChart 7 (ADInstruments).

During and immediately after each MVC, superimposed percutaneous electrical muscle stimulations (58) were delivered to the vastus lateralis muscle and rectus femoris muscle by two self-adhesive electrodes (PALS Platinum 5 × 9 cm; Axelgaard Manufacturing, Lystrup, Denmark). Electrodes were placed on the skin 25% distal from spina iliaca anterior superior and 25% proximal from patella covering the vastus lateralis muscle and rectus femoris muscle. Muscle stimulations were produced by a constant current stimulator (Stimulator model DS7AH; Digitimer, Hertfordshire, UK) in rectangular pulses of 1 ms. After placement of electrodes, stimulation intensity was progressively increased until a plateau in peak twitch force was observed, until maximal stimulator output (999 mA) was achieved, or if subjects felt pain. Thus a stimulation intensity of 999 mA, despite no plateau in peak twitch force, was considered the arbitrary maximum. With this stimulation intensity, we were able to activate 38 ± 1% of peak MVC, being similar to that observed by Verges et al. (80) using electrical and magnetic femoral nerve stimulation at 100 Hz. The same stimulation intensity was used for the same subject throughout the intervention. To determine degree of voluntary activation level, a single stimulation was delivered on top of the plateau of each MVC (48). A single stimulation was delivered 1 s following relaxation of each MVC to determine potentiated peak twitch force (18, 57). During MVC measurements, subjects received verbal encouragement with no visual feedback.

The following parameters were determined: MVC (N): highest force during a MVC; peak twitch force (N): highest force during a potentiated single stimulation 1 s following relaxation from a MVC; half-relaxation time (ms): time from peak twitch force until force reached half of peak twitch force; and time-to-peak twitch force (ms): time from single stimulation until peak twitch force was reached.

Degree of voluntary activation level (VA) was calculated from the single twitches as described by Bachasson et al. (3) using the following equation:

\[
VA = \left[1 - \left(\frac{T_{w_s}}{T_{wp}}\right)\right] \times 100
\]

where \(T_{w_s}\) is the superimposed twitch delivered on top of the plateau of the MVC and \(T_{wp}\) is the potentiated twitch delivered following relaxation after a MVC. A correction was applied to the equation if the superimposed stimulation was delivered slightly before or after the peak MVC (77).

**MHC composition in mixed muscle homogenate.** MHC composition was determined from homogenate using gel electrophoresis (53). Muscle homogenate (80 μl) was mixed with 200 μl of sample buffer (10% glycerol, 5% 2-mercaptoethanol and 2.3% SDS, 62.5 mM Tris, and 0.2% bromophenol blue at pH 6.8), boiled in water at 100°C for 3 min and loaded (10–40 μl) on a SDS-PAGE gel [6% polyacrylamide (100:1 acrylamid: bis-acrylamid), 30% glycerol, 67.5 mM Tris-base, 0.4% SDS, and 0.1 M glycine]. Gels were run at 80 V for at least 42 h at 4°C and MHC bands were made visible by staining with Coomassie. The gels were scanned (Lino-scan 1400 scanner, Heidelberg, Germany) and MHC bands were quantified densitometrically (Phoretix 1D, nonlinear, Newcastle, UK) as an average of the three loaded protein amounts.

**Single fiber analysis.** In total, 720 segments of single fibers (20 from each subject’s PRE and POST biopsies) were dissected free of any connective tissue under a microscope by pulling lengthwise using sharp needles and forceps. Each of the dissected fibers were then put in 0.5-ml Eppendorf plastic tubes and stored at −80°C until determination of fiber size.

Single fiber CSA was determined as described by Frontera and Larsson (25). Single fibers were incubated for 5 min in a fresh relaxing buffer containing the following (in mM): 4 Mg-adenosine triphosphate, 1 free Mg2+, 20 imidazole, 7 ethyleneglycoltetraacetic acid (EGTA), 14.5 creatine phosphate, sufficient KCl to adjust the ionic strength to 180 mM, and a pH of 7.0. The concentration of free Ca2+ was 10−9 M. Individual single fibers were then placed under a microscope at ×500 magnification. The microscope was connected to a camera (model VCC-2997; Sanyo) and interfaced to a computer, which allowed the single fibers to be viewed during the measurements using the software Tema95 (v. 1.04; CheckVision, Hadsund, Denmark). Fiber diameter was measured at four different segments of each of the individual single fibers. Digitalized images of each measurement were stored on an external drive. Fiber CSA was calculated from the average diameter under the assumption that the fiber forms a cylindrical shape, without correction for swelling (5). After measurements, the fiber was frozen in liquid nitrogen and stored at −80°C for later Western blotting.

**Western blotting of single fibers.** Before SDS-PAGE, 20 μl of 6× Laemmli buffer (0.7 ml of 0.5 M Tris base, 3 ml glycerol, 0.93 g DTT, 1 g SDS, and 1.2 mg bromophenol blue) diluted (1:3, vol:vol) in double distilled H2O (ddH2O) was added to each single fiber and incubated for 1 h at room temperature. Five microliters (~0.25 mg/ml protein) of lysate were used for determination of expression of MHC I, MHC II, and sarcoplasmic reticulum Ca2+ ATPase isoform I (SERCA1) in each single fiber.

Individual single fiber lysates were loaded in 26-well TGX Criterion gels (4–15% Tris-HCl; Bio-Rad Laboratories, Hercules, CA). Proteins were separated by SDS-PAGE (60 mA per gel and a maximum of 250 V) for ~60 min. After SDS-PAGE, proteins were visualized using stain-free technology (ChemiDoc MP Imaging System; Bio-Rad Laboratories). The visualized stain-free signals were used to obtain an indication of the amount of protein in each lane and used to normalize for loading between the fibers. Afterwards, gels were transferred (TransBlot Turbo; Bio-Rad Laboratories) to a polyvinylidene difluoride (PVDF) membrane (Transfer Pack; Bio-Rad Laboratories) for 7.5–10 min at 2.5 mA per gel and 20–25 V. Membranes were blocked in Tris-buffered saline including 0.1% Tween-20 (TBST) with 2% milk for 30 min and then incubated in primary antibodies (MHC I: no. A4.840; Developmental Studies Hybridoma Bank, University of Iowa; MHC II: no. A4.74; Developmental Studies Hybridoma Bank; SERCA1: no. MA3-912, Thermo Fisher Scientific), overnight at 5°C. After two washes in TBST, membranes were incubated for 1 h in horseradish peroxidase-conjugated secondary antibody (DAKO, Copenhagen, Denmark) diluted 1:5000 in TBST with 2% milk. Membranes were then washed in...
TBST (3 times, for 15 min each), after which bands were visualized using chemiluminescent detection (Super Signal West Femtomaximum Sensitivity Substrate; ThermoScientific, Rockford, IL) and images were collected on the ChemiDoc MP Imaging System. Band intensities were quantified using Image Lab (Image Lab v. 4.0; Bio-Rad Laboratories).

Data treatment of single fibers. Single fibers were divided according to their expression of either MHC I (n = 354) or MHC II (n = 266). Fibers were excluded from further analysis if they coexpressed MHC I and MHC II (n = 32), if no signal was detected (n = 46), or if the individual fiber was too short (<1 mm) (n = 22). Thus 620 single fibers were included in the data analysis.

Western blotting of mixed muscle lysate. We also determined specific expression of various proteins in mixed muscle homogenate. Two milligrams of dry weight muscle tissue were dissected free from connective tissue 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 NaF, 2 mM PMSE, 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 as the supernatant and protein concentrations were determined by the biuret method and with a fresh sample buffer.

RESULTS

Side effects, drug compliance, and habitual training volume.

Side effects were reported by seven subjects in TER and by three in PLA. Side effects in TER included tremor (n = 7), tachycardia (n = 4), restlessness (n = 3), and headache (n = 1) during the first days of treatment that surpassed within the first week of treatment. In PLA, side effects included tachycardia (n = 2), nausea (n = 1), and restlessness (n = 1). Drug compliance was 92 ± 2 and 94 ± 2% in TER and PLA, respectively. Subjects’ habitual training volume did not change with the intervention, being 4.9 ± 0.4 and 4.8 ± 0.3 h/wk, before and after the intervention in TER, respectively, and 5.1 ± 0.3 and 5.1 ± 0.3 h/wk in PLA, respectively.

Contractile properties of the quadriceps muscle. MVC before and after the intervention in TER and PLA are presented in Fig. 1. No differences were observed in the contractile properties of quadriceps muscle between the groups before the intervention. Significant interactions (treatment × time) were observed for MVC (P ≤ 0.01) and peak twitch force (P ≤ 0.01) between TER and PLA with the intervention. TER increased MVC by 97 ± 29 N and peak twitch force by 67 ± 14 N compared with PLA (Fig. 1, A and B). Degree of voluntary activation level, time-to-peak twitch force, and half-relaxation time did not change with the intervention in either group (Table 1).

Power output during 30 s of maximal cycling and V̇O2max and time to fatigue during incremental cycling. Peak and mean power during 30 s of maximal cycling as well as V̇O2max and time to fatigue during incremental cycling in TER and PLA before and after the intervention are presented in Table 2. No differences were observed between the groups before the intervention. Significant interactions (treatment × time) were observed for peak power (P ≤ 0.01) and mean power (P ≤ 0.01) between TER and PLA with the intervention. Peak power and mean power increased by 32 ± 8 and 25 ± 9 W,
respectively, with the intervention in TER compared with PLA. No changes were observed in $V_{\text{O}_{2\text{max}}}$ and time to fatigue during incremental cycling with the intervention in either group (Table 2).

Body composition. Body composition in TER and PLA before and after the intervention and within-group change scores are presented in Table 3. No differences were observed in body composition between the groups before the intervention. Significant interactions (treatment $\times$ time) were observed for lean body mass ($P \leq 0.05$) and fat mass ($P \leq 0.05$) between TER and PLA with the intervention. TER increased lean body mass by $1.95 \pm 0.8$ kg and reduced fat mass by $0.97 \pm 0.44$ kg compared with PLA with the intervention. Change in lean body mass with the intervention is presented in Fig. 2. Body mass and bone mineral density did not change with the intervention in either group.

MHC isoform distribution and single fiber CSA. MHC isoform distribution and single fiber CSA in TER and PLA before and after the intervention are presented in Table 4. MHC isoform distribution and single fiber CSA did not differ between the groups before the intervention. MHC isoform distribution did not change with the intervention in either group, whereas significant interactions (treatment $\times$ time) were observed for single fiber CSA of MHC I ($P \leq 0.05$) and MHC II ($P \leq 0.05$) fibers between TER and PLA with the intervention. Single fiber CSA increased by $1,205 \pm 558$ $\mu$m$^2$ in MHC I fibers and by $1,277 \pm 595$ $\mu$m$^2$ in MHC II fibers with the intervention in TER compared with PLA. Change in single fiber CSA of MHC I and MHC II fibers with the intervention is presented in Fig. 2.

Muscle $Na^+/K^+$-ATPase subunits, $Ca^{2+}$ handling proteins, and monocarboxylate transporter 1. Changes in expression of muscle proteins involved in ion handling and lactate clearance in TER and PLA before and after the intervention are presented in Fig. 3. Significant interactions (treatment $\times$ time) were observed in expression of $Na^+/K^+$-ATPase subunits $\alpha_2$ and $\beta_1$ and $FXYD1$ between TER and PLA with the intervention. In TER, expression of $Na^+/K^+$-ATPase subunits $\alpha_2$ and $\beta_1$ and $FXYD1$ was higher ($P \leq 0.05$) after the intervention compared with before, whereas no change was observed in PLA (Fig. 3, B–D). No changes were observed in expression of $Na^+/K^+$-ATPase subunit $\alpha_1$ with the intervention in either group (Fig. 3A).

Significant interactions (treatment $\times$ time) were observed in expression of DHPR, SERCAI and monocarboxylate transporter 1 (MCT1) between TER and PLA with the intervention. In TER, expression of DHPR ($P \leq 0.01$), SERCAI ($P \leq 0.05$), and MCT1 ($P \leq 0.01$) was higher after the intervention compared with before, whereas no change was observed in PLA (Fig. 3, E–I). Expression of RyR1 and SERCAII did not change with the intervention in either group (Fig. 3, F and H).

Muscle glycolytic and oxidative enzymes. Changes in expression of muscle glycolytic and oxidative enzymes in TER and PLA before and after the intervention are presented in Fig. 4. Significant interactions (treatment $\times$ time) were observed in expression of PDH and LDH between TER and PLA with the intervention. In TER, expression of PDH ($P \leq 0.05$) and LDH ($P \leq 0.01$) was higher after the intervention compared with before, whereas no changes were observed in PLA (Fig. 4, B and C). No changes were observed in expression of CS, PFK, HAD, COX4, and OXPHOS complex III-V with the intervention in either group (Fig. 4, A–I).

Muscle follistatin and myostatin. Changes in expression of muscle follistatin and myostatin in TER and PLA before and after the intervention are presented in Fig. 5. A significant interaction (treatment $\times$ time) was observed in expression of follistatin between TER and PLA with the intervention. In TER, expression of follistatin was higher ($P \leq 0.01$) after the intervention compared with before, whereas no changes were observed in PLA (fig. 5A). Expression of myostatin did not change with the intervention in either group (Fig. 5B).

Predictors of change in MVC and power output during 30 s of maximal cycling with the intervention in TER. Multiple linear regression $[F(4, 4) = 7.1, \ P \leq 0.05, \ R^2 = 0.88, \ P \leq 0.01]$ from PRE to POST. HAD, $P \leq 0.01$)

| Table 1. Contractile properties of the quadriceps muscle PRE and POST 4 wk of oral administration of either TER or PLA in trained men |
|------------------|------------------|------------------|------------------|
|                  | PRE (n = 9)      | POST (n = 9)     |
| Degree of voluntary activation level, % | 92.7 ± 1.9 | 94.1 ± 1.4 | 91.1 ± 1.5 | 90.4 ± 1.8 |
| Time-to-peak twitch force, ms     | 73.2 ± 1.4 | 69.0 ± 1.7 | 74.7 ± 2.3 | 74.6 ± 2.7 |
| Half-relaxation time, ms          | 26.0 ± 2.2 | 27.6 ± 4.0 | 25.6 ± 4.3 | 23.8 ± 1.8 |

Data are presented as means ± SE. PRE, before; POST, after; TER, terbutaline; PLA, placebo.

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Fig. 1. Maximal voluntary contraction (A) and peak twitch force (B) of the quadriceps muscle before (PRE) and after (POST) 4 wk of oral administration of either terbutaline [TER; 5 mg/30 kg body weight (bw) twice daily; n = 9] or placebo (PLA; n = 9). Data are presented as means ± SE. *Significant ($P \leq 0.01$) interaction (treatment $\times$ time) between TER and PLA with the intervention. **Different ($P \leq 0.01$) from PRE (within-group).
The novel findings of the present study were that enhancements in muscle force and power output during 30 s of maximal cycling induced by chronic β2-adrenergic stimulation in humans primarily are explained by skeletal muscle hypertrophy. In addition, that change in amount of MHC IIa isoforms and in expression of proteins involved in lactate production (LDH), Ca2+ uptake (SERCAI), and oxidative phosphorylation (OXPHOS complex V) of skeletal muscle mediated by chronic β2-adrenergic stimulation were complimentary mechanisms for enhancements in power output during 30 s of maximal cycling.

In the present study, chronic β2-adrenergic stimulation with terbutaline increased isometric muscle force of the quadriceps (MVC and peak twitch force) and power output during 30 s of maximal cycling. Although several studies have described that treatment with β2-agonists increases muscle force and power output during maximal cycling in humans (10, 30, 40, 45, 69), the present study is first to elucidate the underlying mechanisms. Our observations suggest that the increased muscle force induced by chronic β2-adrenergic stimulation can be explained by skeletal muscle hypertrophy rather than changes in fiber-type composition or degree of voluntary activation level of skeletal muscle. This was also supported by multiple regression analysis, in which increase in MVC primarily was explained by hypertrophy of MHC I and MHC II fibers in response to terbutaline. Observations in animals also indicate that hypertrophy is the determining factor for the increased muscle force associated with chronic β2-adrenergic stimulation rather than fiber-type transitions of the MHC isoforms, as treatment with β2-agonists increases absolute tension while specific force, i.e., muscle force per CSA, remains unchanged (21, 26, 66). For enhancements in power output during 30 s of maximal cycling induced by terbutaline in the present study, regression analysis showed that change in leg lean body mass could predict 66% (Pearson’s correlation: $R^2 = 0.66$, $P \leq 0.01$) of change in peak power output. Notably, with inclusion of change in amount of MHC IIa isoforms and expression of LDH, along with change in leg lean body mass, in a multiple linear regression, 89% of change in peak power output could be predicted with the intervention in TER. Thus the present observations indicate that muscle hypertrophy is the primary mechanism by which chronic treatment with β2-agonists enhances peak power during 30 s of maximal cycling, with change in amount of MHC IIa isoforms and LDH expression as complimentary mechanisms. For change in mean power, 88%

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### Table 2. Peak power and mean power during 30 s of maximal cycling as well as $\dot{V}O_{2max}$ and time of fatigue during incremental cycling PRE and POST 4 wk of oral administration of either TER or PLA in trained men

<table>
<thead>
<tr>
<th></th>
<th>TER (n = 9)</th>
<th>POST</th>
<th>PLA (n = 9)</th>
<th>POST</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 s of maximal cycling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak power,* W</td>
<td>863 ± 17</td>
<td>888 ± 17‡</td>
<td>909 ± 31</td>
<td>902 ± 28</td>
</tr>
<tr>
<td>Mean power,* W</td>
<td>646 ± 14</td>
<td>664 ± 15†</td>
<td>693 ± 21</td>
<td>686 ± 17</td>
</tr>
<tr>
<td>Incremental cycling</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_O_{2max}$, l/min</td>
<td>4.3 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Time of fatigue, s</td>
<td>406 ± 20</td>
<td>419 ± 23</td>
<td>441 ± 24</td>
<td>487 ± 15</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. $V_O_{2max}$, maximal oxygen consumption. *Significant ($P \leq 0.01$) interaction (treatment × time) between TER and PLA with the intervention. †Different ($P \leq 0.05$) from PRE (within-group). §Different ($P \leq 0.01$) from PRE (within-group).

### Table 3. Body composition PRE and POST 4 wk of oral administration of either TER or PLA in trained men

<table>
<thead>
<tr>
<th></th>
<th>TER (n = 9)</th>
<th>Δ-Change</th>
<th>PLA (n = 9)</th>
<th>Δ-Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, kg</td>
<td>77.6 ± 1.7</td>
<td>0.3 ± 0.7</td>
<td>76.9 ± 2.8</td>
<td>-0.7 ± 0.4</td>
</tr>
<tr>
<td>Lean body mass,* kg</td>
<td>64.1 ± 1.3</td>
<td>(Fig. 2)</td>
<td>65.9 ± 2.2</td>
<td>65.7 ± 2.0</td>
</tr>
<tr>
<td>Fat mass,* kg</td>
<td>9.7 ± 1.0</td>
<td>-1.4 ± 0.6§</td>
<td>7.6 ± 0.9</td>
<td>-0.4 ± 0.6</td>
</tr>
<tr>
<td>Bone mineral density, g/cm³</td>
<td>1.36 ± 0.02</td>
<td>0.01 ± 0.00</td>
<td>1.26 ± 0.02</td>
<td>1.27 ± 0.02</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. *Significant ($P \leq 0.05$) interaction (treatment × time) between TER and PLA with the intervention. †Different ($P \leq 0.05$) from PRE (within-group). §Different ($P \leq 0.01$) from PRE (within-group). §Different ($P \leq 0.01$) from PLA (between-groups).

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could be explained by change in leg lean body mass, MHC Iia, SERCAI, and OXPHOS complex V in multiple regression analysis with the intervention in TER. While it has been speculated that muscle fiber-type composition and training status may influence the chronic response to β2-agonists in humans (30, 59, 69), we observed no indication that baseline characteristics in MHC distribution, habitual training volume, or VO2max of the included subjects affected response to terbutaline for MVC and power output during maximal cycling. This observation is also consistent with that observed by Le Panse et al. (39), in which 3 wk of oral administration of salbutamol enhanced peak power output during 30 s of maximal cycling to a similar extent in trained and untrained subjects (40).

The observation that muscle hypertrophy is the primary mechanism by which β2-agonists enhance peak power output during maximal cycling is not surprising, since resistance training also enhances peak power output during maximal exercise (38, 68). Thus a larger muscle mass may allow a higher force development during each stroke of maximal cycling. In agreement, Sanchez et al. (69) observed that the enhanced power output during 30 s of maximal cycling induced by oral salbutamol was attributed to a 14% higher force at given velocities (70–200 rpm). For the complimentary mechanisms of mean power output observed in the present study, the increased expression of SERCAI induced by chronic treatment of terbutaline may have augmented Ca2+ uptake function of the sarcoplasmic reticulum. A higher content of SERCAI could potentially accelerate Ca2+ reuptake and hence muscle relaxation between each stroke during maximal cycling. As such, Seebacher et al. (71) observed that SERCA activity is important in maintaining Ca2+ availability of the sarcoplasmic reticulum for subsequent release during maximal muscle activity. Thus the enhancing effects of chronic administration of β2-agonists on maximal cycling may be attributed to both increased force production and accelerated relaxation respectively mediated by hypertrophy and augmented Ca2+ handling function of skeletal muscle.

In rodents, chronic clenbuterol treatment has been shown to compromise muscle fatigue resilience (22, 66, 73), which may be attributed to depressed oxidative capacity (13, 28, 43) and reduced capillary density (78). In the present study, however, we observed no effect of terbutaline on VO2max and time to fatigue during incremental exercise, which is consistent with that observed in endurance athletes after 2-wk treatment of oral salbutamol (30). Collomp et al. (16) even observed that 3-wk treatment of oral salbutamol increased time to fatigue during submaximal exercise in trained men. Our muscle protein data also showed no indication of depressed oxidative capacity, since no changes were observed in CS, HAD, COX4, and OXPHOS complexes III-V with terbutaline. In fact, we observed that terbutaline increased muscle expression of PDH as well as LDH and MCT1, which is essential for lactate production and clearance during exercise. Likewise, muscle expression of Na+K+-ATPase subunits α2 and β1 and FXYD1 was higher after terbutaline treatment in the present study. Since maximal muscle activity is associated with large ion shifts of K+ and Na+ that influence excitation-contraction coupling (47), the higher content of Na+/K+-ATPase subunits α2 and β1 and FXYD1 observed with terbutaline in the present study...

Table 4. MHC isoform distribution and CSA of MHC I and MHC II fibers PRE and POST 4 wk of oral administration of either TER or PLA in trained men

<table>
<thead>
<tr>
<th>Fiber CSA</th>
<th>MHC I fibers, μm² (n = 354)</th>
<th>MHC II fibers, μm² (n = 266)</th>
<th>MHC isoforms</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE</td>
<td>7,567 ± 185</td>
<td>7,936 ± 187</td>
<td>MHC I, %</td>
</tr>
<tr>
<td></td>
<td>8,700 ± 190†</td>
<td>8,955 ± 183†</td>
<td>55 ± 5</td>
</tr>
<tr>
<td>POST</td>
<td>8,170 ± 197</td>
<td>8,611 ± 193</td>
<td>52 ± 5</td>
</tr>
<tr>
<td></td>
<td>8,098 ± 199</td>
<td>8,353 ± 194</td>
<td>56 ± 4</td>
</tr>
<tr>
<td>PLA (n = 9)</td>
<td>8,170 ± 197</td>
<td>8,611 ± 193</td>
<td>43 ± 4</td>
</tr>
<tr>
<td></td>
<td>8,098 ± 199</td>
<td>8,353 ± 194</td>
<td>44 ± 4</td>
</tr>
<tr>
<td></td>
<td>53 ± 3</td>
<td>44 ± 2</td>
<td>1 ± 1</td>
</tr>
<tr>
<td></td>
<td>3 ± 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. MHC, myosin heavy chain; CSA, cross-sectional area. *Significant (P ≤ 0.05) interaction (treatment × time) between TER and PLA with the intervention. †Different (P ≤ 0.05) from PRE (within-group). ‡Different (P ≤ 0.01) from PRE (within-group).
could help counteract contraction-induced accumulation of extracellular K⁺ and thus prevent loss of force development caused by membrane inexcitability (15). Taken together, it does therefore not appear that chronic β₂-adrenergic stimulation compromises oxidative capacity and fatigue resilience in humans. Our observations rather support the proposition made by Pearen et al. (54) that the responses to β₂-adrenergic stimulation in skeletal muscles in some ways are comparable to those observed with exercise training (38, 47, 68). The discrepancies between the detrimental effects of chronic β₂-adrenergic stimulation on muscle performance in animal studies compared with that observed in humans could be related to the markedly higher doses administered to animals. While clenbuterol compromises fatigue resilience in animals, low-dose administration of formoterol was found to have no effect on muscle fatigability in mice despite of marked hypertrophy (26).

To our knowledge the present study is first to show a hypertrophic effect of β₂-adrenergic stimulation in skeletal muscle fibers of humans. Hypertrophy was not only evident in single fibers of the vastus lateralis muscle but also by a 1.7-kg gain in lean body mass with the intervention in TER. Although these observations are comparable to those observed in animals (34, 44), previous human studies did not find hypertrophic effects of chronic administration of β₂-agonists (10, 12, 39, 40, 45). However, in these studies, only whole body composition was measured, whereas animal studies along with the present determined CSA of skeletal muscle fibers (9, 21, 66, 81). Studies in humans have shown that the relative hypertrophic response to resistance training and protein supplementation is
higher when measured as CSA of muscle fibers compared with lean body mass \( (17, 38, 42, 79) \). In accordance, we observed that the increase in lean body mass induced by terbutaline treatment was \( 3\% \) when measured by DXA-scan, whereas the increase observed in CSA of muscle fibers was \( 13–15\% \). Discrepancies of the studies in humans could also be attributed to differences in dosing regimen or the \( \beta_2 \)-agonist used, since animal studies have shown that the hypertrophic response is

Fig. 4. Expression of glycolytic and oxidative enzymes before (PRE) and after (POST) 4 wk of oral administration of either TER (5 mg/30 kg bw twice daily; \( n = 9 \)) or PLA (\( n = 9 \)). A: phosphofructokinase (PFK). B: pyruvate dehydrogenase (PDH). C: lactate dehydrogenase. D: citrate synthase (CS). E: hydroxyacyl CoA dehydrogenase (HAD). F: cytochrome \( c \) oxidase 4 (COX4). G: OXPHOS complex III. H: OXPHOS complex IV. I: OXPHOS complex V. Representative blots from same membrane and subjects are presented. Data are presented as means \( \pm \) SD. Values are relative to PRE. *Significant (\( P \leq 0.05 \)) interaction (treatment \( \times \) time) between TER and PLA with the intervention. **Different (\( P \leq 0.01 \)) from PRE (within-group).

Fig. 5. Expression of follistatin and myostatin before (PRE) and after (POST) 4 wk of oral administration of either TER (5 mg/30 kg bw twice daily; \( n = 9 \)) or PLA (\( n = 9 \)). A: follistatin. B: myostatin. Representative blots from same membrane and subjects are presented. Data are presented as means \( \pm \) SD. Values are relative to PRE. *Significant (\( P \leq 0.01 \)) interaction (treatment \( \times \) time) between TER and PLA with the intervention. **Different (\( P \leq 0.01 \)) from PRE (within-group).
related to dose, route of administration, duration, and type of β2-agonist used (14, 50, 64, 67). This is also supported by acute administration studies of β2-agonists in humans, in which supratherapeutic administration elicits a systemic response that enhances exercise performance and muscle force (31, 35, 69), whereas therapeutic low-dose acute administration has no ergogenic effect on these parameters (59). Thus the choice of a supratherapeutic dose of terbutaline known to elicit a significant systemic response (32) may explain why a hypertrophic response was observed in the present study. Moreover, most of the human studies that have investigated chronic effects of β2-agonists on lean body mass administered salbutamol (10, 12, 39, 40, 45), whereas the present study, to our knowledge, is first to investigate the effect of terbutaline on hypertrophy in humans. Other factors that may explain discrepancies could also relate to differences in muscle fiber-type composition and training status of the included subjects, all of which have been speculated to affect the response to β2-agonists in humans (30, 59, 69). However, while slow- and fast-twitch fibers have been shown to respond differently to β2-adrenergic stimulation in some animal studies (21, 43, 66, 81), we observed no differences in the hypertrophic response to terbutaline between MHC I and MHC II fibers of the vastus lateralis muscle. In addition, baseline characteristics in MHC-distribution of the vastus lateralis muscle, habitual training volume, and VO2max of the included subjects could not predict any change in CSA of MHC I and MHC II fibers and lean body mass with the intervention in a multiple linear regression of the present study. Thus our observations suggest that fiber-type composition and training status have limited influence on the hypertrophic response to chronic β2-adrenergic stimulation in humans.

The gain in muscle mass elicited by terbutaline treatment in the present study was associated with a higher muscle expression of follistatin. This observation adds to that observed by Busquets et al. (8) where formoterol increased gene expression of follistatin, which was associated with skeletal muscle hypertrophy in tumor-bearing rats. In contrast to that observed in cell cultures and animals (8, 33, 54), however, we observed no change in the expression of myostatin with terbutaline. This discrepancy may be explained by the duration of treatment. Indeed, Abo et al. (1) observed that expression of myostatin was higher after 21 days of clenbuterol treatment with no differences in the first week of treatment, suggesting that myostatin functions as a negative regulator in the latter stages of β2-agonist treatment. Nonetheless, while the present observations suggest that follistatin may play a role in β2-adrenergic mediated hypertrophy, the mechanisms are complex and possibly involve an interplay of several factors that directly or indirectly attenuate the negative regulatory action of the myostatin system on growth (8, 54, 55, 72, 75).

Interestingly, we observed a reduction in fat mass with terbutaline, which is in agreement with observations in other species (9, 51). The fat-reducing effect of chronic β2-adrenergic stimulation could be attributed to different factors. Foremost, studies in both humans and animals have shown that acute β2-adrenergic stimulation increases thermogenesis (2) and administration of β2-agonists elevates energy expenditure and oxidation of fat and carbohydrates in humans (27, 31, 36, 41). In accordance, animal studies have also shown that β2-adrenoceptor knockout models develop obesity (4). Lastly, the increase in lean body mass associated with terbutaline treatment in the present study may have elevated the basal metabolic rate. Nonetheless, previous studies found no effect of chronic administration of oral salbutamol on fat mass in humans (40, 45), which may be related to differences in dosing regimen as discussed above.

In conclusion, the present study demonstrated that muscle hypertrophy is the primary mechanism by which chronic administration of β2-agonists enhances muscle force and power output during maximal cycling in humans. Furthermore, the study showed that the hypertrophic response to chronic β2-adrenergic stimulation with terbutaline is similar in slow- and fast-twitch muscle fibers of humans and that the hypertrophic response is associated with increased expression of muscle follistatin. Lastly, our study showed that chronic administration of terbutaline does not compromise VO2max, incremental cycling performance, or muscle expression of oxidative enzymes. Given the increased muscle mass, muscle force, and power output during maximal cycling along with the higher expression of proteins involved in ion handling as well as in lactate production and clearance, our observations indicate that the response to β2-adrenergic stimulation in skeletal muscle is comparable to that observed with resistance training and speed endurance training (38, 47, 68).

Although produced for treatment of bronchoconstriction (74), β2-agonists have been used to improve feed efficiency and meat quality in livestock (49) and bodybuilders have misused β2-agonists as pharmacological supplement in their training (20, 56, 60). Consequently, antidoping restrictions have been introduced towards β2-agonists in competitive sports. The muscle accretion associated with β2-agonists has also led to the suggestion that these drugs might be applicable in treatment of muscle-wasting disorders and in prevention of strength loss incurred from spaceflight (10, 11, 12, 34, 44). Although the present study supports that β2-agonists can be used to increase muscle mass and force, administration of high doses of β2-agonists may be associated with side effects. In rats, high-dose treatment with clenbuterol has been shown to induce apoptosis in heart and soleus muscle (7). The deleterious effects of chronic treatment with β2-agonists, however, are dose dependent and are as such only evident in very high doses that are not applied to humans (6). Therefore, it seems unlikely that therapeutic treatment would affect the heart deleteriously in humans. Given the performance-enhancing nature of acute (19, 30, 35) and chronic β2-adrenergic stimulation on muscle mass, muscle force (45), and power output (30, 39, 69) during maximal exercise, it seems logical that systemic use of β2-agonists should remain on the list of prohibited substances in competitive sports.

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**DISCLOSURES**

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


