




# Muscle hypertrophic effect of inhaled beta<sub>2</sub>-agonist is associated with augmented insulin-stimulated whole-body glucose disposal in young men

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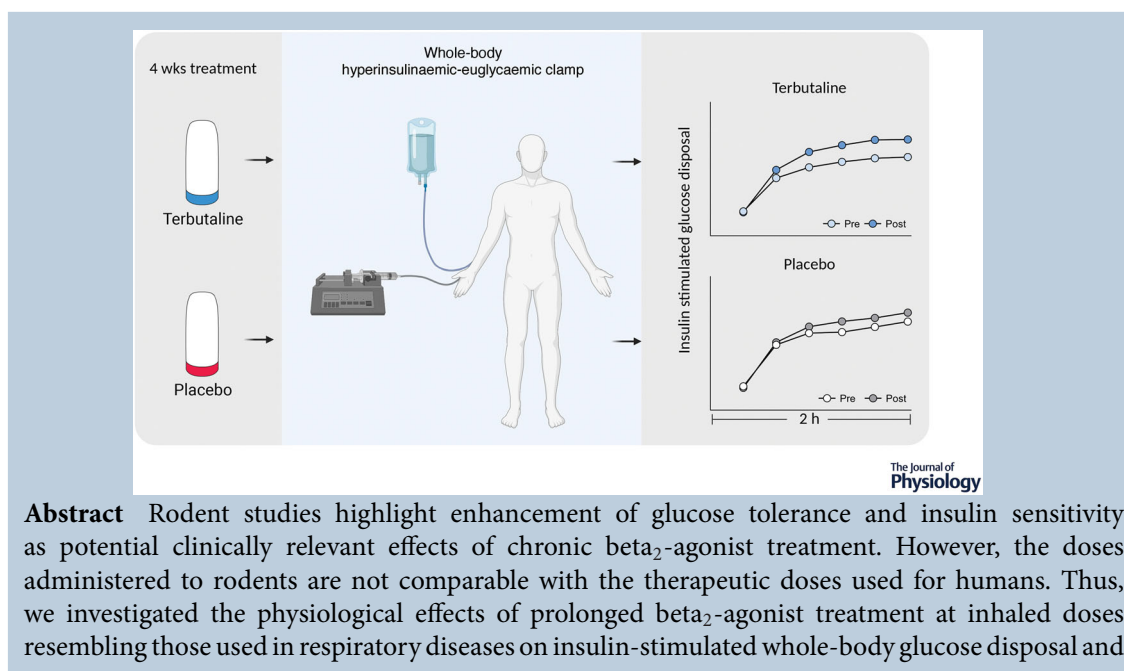
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**Søren Jessen** is a postdoctoral researcher at the Department of Nutrition, Exercise and Sports (NEXS), University of Copenhagen, Denmark, whose research interests include muscle physiology and exercise performance. A special area of interest is the effects of pharmacological compounds on skeletal muscle metabolism, hypertrophy, and exercise performance in humans. **Morten Hostrup**, PhD, is Associate Professor of human integrative physiology at NEXS. His research focuses on the health- and performance-related benefits of exercise, performance optimization strategies and ergogenic substances. An extensive amount of his research has been devoted to beta<sub>2</sub>-agonist pharmacology, with an emphasis on its effects in human skeletal muscle.



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putative mechanisms in skeletal muscle and adipose tissue of healthy men. Utilizing a randomized placebo-controlled parallel-group design, we assigned 21 healthy men to 4 weeks daily inhalation of terbutaline (TER;  $4 \text{ mg} \times \text{day}^{-1}$ ,  $n = 13$ ) or placebo (PLA,  $n = 8$ ). Before and after treatments, we assessed subjects' whole-body insulin-stimulated glucose disposal and body composition, and collected vastus lateralis muscle and abdominal adipose tissue biopsies. Glucose infusion rate increased by 27% (95% CI: 80 to 238  $\text{mg} \times \text{min}^{-1}$ ,  $P = 0.001$ ) in TER, whereas no significant changes occurred in PLA (95% CI:  $-37$  to 195  $\text{mg} \times \text{min}^{-1}$ ,  $P = 0.154$ ). GLUT4 content in muscle or adipose tissue did not change, nor did hexokinase II content or markers of mitochondrial volume in muscle. Change in lean mass was associated with change in glucose infusion rate in TER ( $r = 0.59$ ,  $P = 0.03$ ). Beta<sub>2</sub>-agonist treatment in close-to-therapeutic doses may augment whole-body insulin-stimulated glucose disposal in healthy young men and part of the change is likely to be explained by muscle hypertrophy. These findings highlight the therapeutic potential of beta<sub>2</sub>-agonists for improving insulin sensitivity.

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**Abstract figure legend** In this study, we show that selective beta<sub>2</sub>-agonist, terbutaline, in close-to-therapeutic doses, may increase insulin-stimulated whole-body glucose disposal with 4 weeks of treatment in humans, and that at least part of this increase is attributed to skeletal muscle hypertrophy. This finding highlights the therapeutic potential of beta<sub>2</sub>-agonists in enhancing the capacity for glucose disposal and whole-body insulin sensitivity in humans.

### Key points

- While studies in rodents have highlighted beta<sub>2</sub>-agonists as a means to augment insulin sensitivity, these studies utilized beta<sub>2</sub>-agonists at doses inapplicable to humans.
- Herein we show that a 4-week treatment period with daily therapeutic inhalation of beta<sub>2</sub>-agonist increases insulin-stimulated whole-body glucose disposal in young healthy lean men.
- This effect was associated with an increase of lean mass but not with changes in GLUT4 and hexokinase II or basal glycogen content in skeletal muscle nor GLUT4 content in abdominal adipose tissue.
- These findings suggest that the enhanced insulin-stimulated whole-body glucose disposal induced by a period of beta<sub>2</sub>-agonist treatment in humans, at least in part, is attributed to muscle hypertrophy.
- Our observations extend findings in rodents and highlight the therapeutic potential of beta<sub>2</sub>-agonists to enhance the capacity for glucose disposal and whole-body insulin sensitivity, providing important knowledge with potential application in insulin resistance.

## Introduction

Inhaled beta<sub>2</sub>-agonists are first-line drugs in obstructive respiratory diseases (Simpson *et al.* 2016; Tesse *et al.* 2018). While the airways are the main therapeutic target, inhaled beta<sub>2</sub>-agonists have a high systemic bioavailability of around 20–30% and elicit several systemic effects (Ward *et al.* 2000; Dyreborg *et al.* 2016). In particular, beta<sub>2</sub>-agonists exert effects in skeletal muscle and adipose tissue where beta<sub>2</sub>-adrenergic receptors are abundant (Elfellah *et al.* 1989; Deng *et al.* 1996; Blondin *et al.* 2020). These effects include muscle growth (Hostrup *et al.* 2015; Jessen *et al.* 2018), slow-to-fast twitch

muscle fibre type transition (Hostrup *et al.* 2018a), lipolysis (Onslev *et al.* 2019), and alterations of numerous proteins involved in cellular metabolism (Hostrup *et al.* 2018a,b). This has made the beta<sub>2</sub>-adrenergic receptor an emerging area in pharmaceutical drug development, with ongoing pre-clinical (Koziczak-Holbro *et al.* 2019; Skagen *et al.* 2021) and clinical trials aiming to assess the applicability of selective beta<sub>2</sub>-agonists in lifestyle diseases such as obesity and type 2 diabetes, as well as muscle atrophic conditions (patent no: WO2005072714A1, ClinicalTrials.gov Identifier: NCT03005717.23-25).

Because chronic treatment with beta<sub>2</sub>-agonists enhances insulin sensitivity and glucose tolerance in

rodents (Torgan *et al.* 1993; Pan *et al.* 2001; Sato *et al.* 2014; Kalinovich *et al.* 2020), there has been speculation about the potential applicability of beta<sub>2</sub>-agonists in treatment of insulin resistance (Pan *et al.* 2001; Kalinovich *et al.* 2020). However, the treatment regimens showing effects on insulin sensitivity in rodents are not comparable with the therapeutic doses used for humans. For example, daily intraperitoneal or oral doses of clenbuterol at 25  $\mu\text{g} \times \text{kg}_{\text{bw}}^{-1}$  or 105  $\mu\text{g}$ , respectively, for 4–5 days improve glucose tolerance in rodents (Kalinovich *et al.* 2020; Meister *et al.* 2022), but are also associated with adverse cardiac hypertrophy, even in doses as low as 10  $\mu\text{g} \times \text{kg}_{\text{bw}}^{-1}$  (Burniston *et al.* 2006). Such doses exceed those considered safe for humans (Kamalakkannan *et al.* 2008; Koeberl *et al.* 2018) and larger mammals (Sleeper *et al.* 2002), and the adverse cardiac effects are one of the main reasons why clenbuterol is seldomly prescribed to humans and why it is not marketed in several countries (Spiller *et al.* 2013; Milano *et al.* 2018). But given the compelling findings related to glucose tolerance in rodents, it is relevant to assess the physiological effect of a commonly prescribed beta<sub>2</sub>-agonist at clinically relevant doses on insulin-stimulated glucose disposal in humans.

To our knowledge, only one study (Scheidegger *et al.* 1984) has investigated the effect of chronic beta<sub>2</sub>-agonist treatment on insulin-stimulated glucose uptake in humans. In that study, oral treatment with terbutaline (15 mg  $\times$  day<sup>-1</sup>) for 1–2 weeks enhanced insulin-stimulated whole-body glucose disposal by 29% in young healthy lean men. However, the study utilized a non-placebo controlled open-label design (Scheidegger *et al.* 1984), and the subjects ingested 5 mg oral terbutaline ~13 h before the hyperinsulinaemic-euglycaemic clamp. Given the slow absorption of oral terbutaline, which reaches peak systemic concentrations after 10–18 h after repeated daily dosing (Borgström *et al.* 1989), this precludes inferences on whether the enhanced insulin-stimulated whole-body glucose disposal was due to adaptation from chronic treatment or due to an acute effect of terbutaline on glucose uptake and metabolism (Onslev *et al.* 2019). Indeed, Scheidegger *et al.* observed a ~8% higher basal metabolic rate during the post-treatment clamp with terbutaline compared to control, which suggests some residual effect of terbutaline on metabolism. Hence, data are needed to assess whether chronic treatment of beta<sub>2</sub>-agonists at more clinically relevant inhaled doses, and with a sufficient wash-out period before post-intervention assessment, affects glucose disposal during insulin stimulation in humans.

Given that skeletal muscle is the primary site for insulin-stimulated glucose disposal (DeFronzo *et al.* 1981), the muscle hypertrophic actions of beta<sub>2</sub>-agonists may be a mechanism underlying the greater capacity for glucose disposal and augmented insulin sensitivity observed in rodents (Torgan *et al.* 1993; Jacob *et al.* 1999;

Castle *et al.* 2001; Pan *et al.* 2001; Sato *et al.* 2014). Although human data on the effect of beta<sub>2</sub>-agonists on insulin sensitivity are lacking, studies show that a few weeks of treatment with beta<sub>2</sub>-agonist at high inhaled or oral doses increases muscle mass in young healthy trained men (Holgate *et al.* 1980; Hostrup *et al.* 2015; Jessen *et al.* 2018). Furthermore, data in rodents suggest that beta<sub>2</sub>-agonists may alter the regulation and expression of proteins involved in glucose transport and metabolism in both skeletal muscle (Pan *et al.* 2001) and adipocytes (Granneman *et al.* 2005), such as GLUT4 (Sato *et al.* 2014) and hexokinase II (Jones & Dohm, 1997). Thus, beta<sub>2</sub>-agonists may potentially enhance the capacity for glucose disposal via other mechanisms than hypertrophy of skeletal muscle but this hypothesis warrants further investigation in humans.

Herein we investigated the effect of daily inhaled treatment with the selective beta<sub>2</sub>-agonist terbutaline on insulin-stimulated whole-body glucose disposal, along with putative physiological mechanisms in skeletal muscle and adipose tissue. As a proof of concept approach, we examined these effects in healthy young men who were not insulin resistant or suffering from other chronic diseases. We hypothesized that daily inhalation of beta<sub>2</sub>-agonist for 4 weeks would increase insulin-stimulated whole-body glucose disposal, which would be associated with an increase in lean mass.

## Methods

### Study design

The study was part of a larger study (Jessen *et al.* 2018) and was designed as a randomized, placebo-controlled, double-blinded parallel study (Fig. 1). The present study included a subgroup that underwent assessment of insulin-stimulated whole-body glucose disposal and body composition, and had muscle and adipose tissue biopsies taken. In this subgroup analysis, the main outcome measure was insulin-stimulated whole-body glucose disposal estimated by glucose infusion rate during the final 20 min of a 2 h hyperinsulinaemic-euglycaemic clamp. The study adhered to the 2013 Helsinki Declaration and was approved by the ethics committee of Copenhagen, Denmark (H-4-2014-002), and registered at Clinicaltrials.gov (NCT02557581). Subjects provided oral and written informed consent before inclusion in the study.

### Subjects and eligibility criteria

Subjects were recruited via online posters and flyers. Before inclusion, subjects met for an assessment of eligibility criteria. The criteria were: healthy

men, 18–36 years of age, non-smoker, activity level  $2\text{--}5\text{ h} \times \text{week}^{-1}$ , maximum oxygen uptake ( $\dot{V}_{O_{2\max}}$ )  $40\text{--}60\text{ ml} \times \text{kg}^{-1} \times \text{min}^{-1}$ , lean mass  $55\text{--}65\text{ kg}$  or lean mass index  $14\text{--}22\text{ kg} \times \text{m}^{-2}$ , no use of beta<sub>2</sub>-agonists or other prescription medicine, and no allergy towards terbutaline. Withdrawal criteria were unacceptable side effects, complications related to the study, or non-compliance. Subjects' body composition was assessed by dual-energy X-ray absorptiometry (Lunar DPX IQ, Version 4.7 E, Lunar Corporation, Madison, WI, USA) (DXA) followed by incremental cycling to exhaustion on a bike ergometer for assessment of  $\dot{V}_{O_{2\max}}$  by indirect calorimetry (Monark LC4, Monark Exercise AB, Vansbro, Sweden) as previously described (Jessen *et al.* 2018).

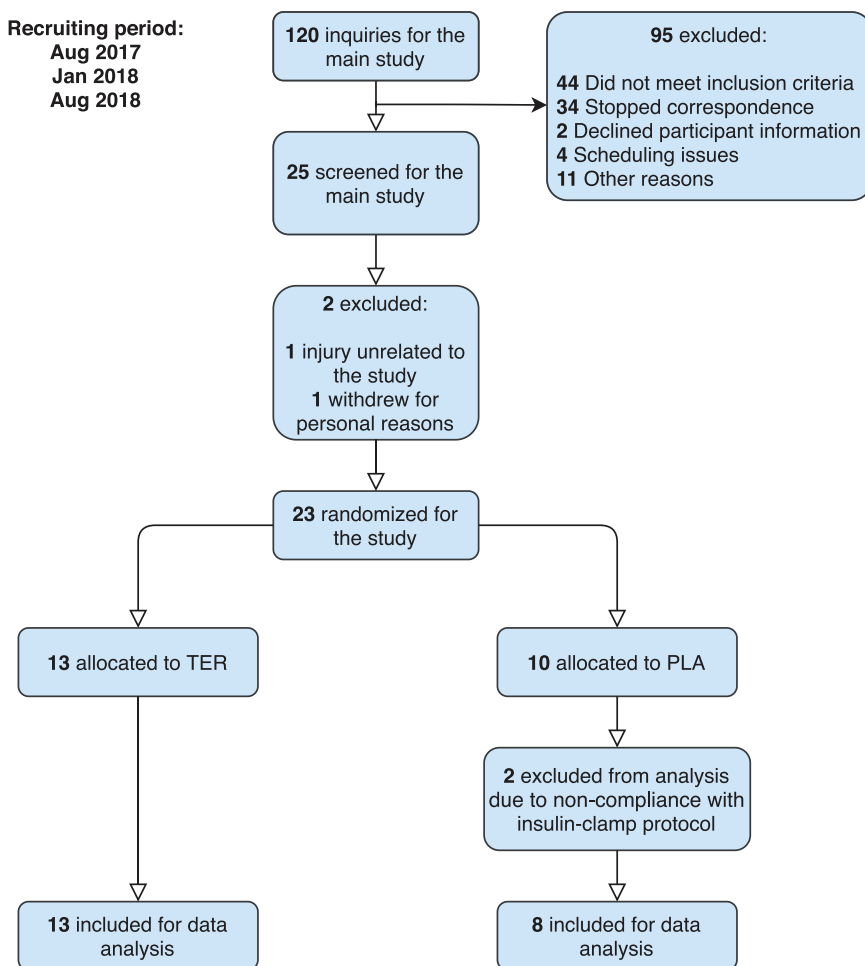
### Randomization

Subjects were block-randomized to either an experimental group receiving terbutaline (TER) or placebo (PLA), stratified for  $\dot{V}_{O_{2\max}}$  and lean mass. Subjects were assigned (1.3:1) in favour of active treatment as we expected a higher drop-out rate due to side effects in TER and a larger

within-group variability due to individual differences in beta<sub>2</sub>-agonist response.

### Intervention

Subjects in TER were instructed to inhale eight doses of  $0.5\text{ mg} \times \text{dose}^{-1}$  terbutaline ( $4\text{ mg} \times \text{day}^{-1}$ ), while subjects in PLA were instructed to inhale eight doses of placebo once daily for a 4-week treatment period. During the intervention, inhalations were supervised daily via online monitoring (Skype or Facetime) by the investigators, hence ensuring 100% drug compliance. Subjects and investigators were blinded during the intervention and unblinding occurred after collection of all data. Terbutaline Turbuhalers (Bricanyl, Turbuhaler,  $0.5\text{ mg} \times \text{dose}^{-1}$ , AstraZeneca, Cambridge, UK) were delivered by the regional pharmacy of Copenhagen, Denmark, while identical-looking placebo Turbuhalers were kindly delivered by AstraZeneca (AstraZeneca, Cambridge, UK). The elimination half-life of inhaled terbutaline is normally around 4–6 h (Krogh *et al.* 2017) and has a duration of action around 6 h (Sears & Lötvall, 2005).



**Figure 1. Flow chart of subject allocation in the study**

[Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



### Pre and post-intervention assessment

Before and 48–72 h after the final day of the 4-week treatment period (to ensure washout of the experimental drug), subjects reported to the laboratory for assessment of insulin-stimulated whole-body glucose disposal during a hyperinsulinaemic-euglycaemic clamp in a fasting state, having abstained from vigorous physical activity and alcohol for 48 h and caffeine and nicotine for 24 h (Fig. 2). Before the clamp, subjects ingested four to five potassium tablets to prevent hypokalaemia (Kaleorid, 750 mg KCl, Karo Pharma, Sweden) and had a catheter placed in the antecubital vein for infusion of glucose and insulin. A second catheter was placed in the contralateral dorsal hand vein, which was arterialized with a heating pad to ensure an O<sub>2</sub>-saturation > 92%. The clamp was initiated with a 1 min priming dose of  $53.5 \text{ pmol} \times \text{kg}_{\text{bw}}^{-1}$  insulin ( $100 \text{ IU} \times \text{ml}^{-1}$ , Novo Nordisk, Copenhagen, Denmark) and continued for 120 min with a constant insulin infusion ( $8 \text{ pmol} \times \text{kg}_{\text{bw}}^{-1} \times \text{min}^{-1}$ ). Arterialized venous blood samples were collected before the clamp and every 5 min during the clamp for assessment of glucose concentration. Furthermore, blood samples were collected before and during the clamp for determination of plasma insulin. Glucose was infused during the clamp from a 20% glucose solution (Fresenius Kabi,  $200 \text{ mg} \times \text{ml}^{-1}$ ) to maintain euglycaemia at  $\sim 5 \text{ mM}$ .

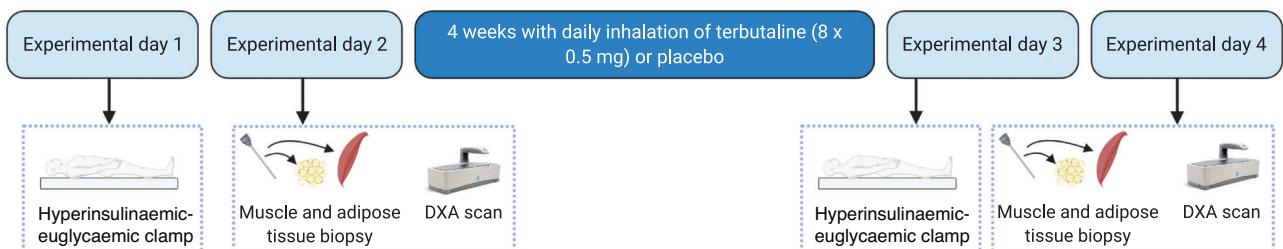
On a separate day in a fasting state before and after the intervention, body composition was measured during a whole-body DXA scan using the mean of two separate scans. Adipose tissue compartments were assessed with automated software estimating adipose tissue deposition in the pelvic (gynoid) and abdominal area (android) as defined by standard region of interest settings. After the scan, resting vastus lateralis muscle and abdominal subcutaneous adipose biopsies were obtained under local anaesthesia (2 ml lidocaine without noradrenaline (epinephrine), Xylocaine  $20 \text{ mg} \times \text{ml}^{-1}$ , AstraZeneca, Cambridge, UK) using the Bergström biopsy needle procedure (Bergström, 1975). After sampling, muscle and adipose biopsies were cleaned to remove visible blood, connective tissue, and fat (muscle samples) and immediately frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until analysis. Muscle biopsies were assessed for content of

GLUT4, hexokinase II, citrate synthase (CS), cytochrome *c* oxidase subunit 4 (COXIV), as well as for glycogen (only muscle). Adipose tissue biopsies were assessed for content of GLUT4. Subjects were instructed to replicate nutritional intake and activity level before study visits.

### Immunoblotting

Protein content was determined in muscle and subcutaneous adipose lysates through SDS-PAGE and western blot analysis. First, freeze-dried and dissected muscle and adipose sample were homogenized for  $2 \times 30 \text{ s}$  at 29 Hz (Qiagen Tissuelyser II, Retsch GmbH, Haan, Germany) in fresh cold buffer for the muscle samples (10% glycerol, 20 mM sodium pyrophosphate, 150 mM NaCl, 50 mM Hepes (pH 7.5), 1% NP-40, 20 mM  $\beta$ -glycerophosphate, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 10 mM NaF-poison, 2 mM PMSE, 1 mM EDTA (pH 8.0), 1 mM EGTA (pH 8.0),  $10 \mu\text{g} \times \text{ml}^{-1}$  aprotinin,  $10 \mu\text{g} \times \text{ml}^{-1}$  leupeptin, 3 mM benzamidine) and the adipose samples (10% glycerol, 20 mM sodium pyrophosphate, 150 mM NaCl, 50 mM Hepes, 1% NP-40, 20 mM  $\beta$ -glycerophosphate, 10 mM NaF-poison, 1 mM EDTA, 1 mM EGTA,  $20 \mu\text{g} \times \text{ml}^{-1}$  aprotinin,  $10 \mu\text{g} \times \text{ml}^{-1}$  leupeptin, 2 mM Na<sub>3</sub>VO<sub>4</sub>, and 3 mM benzamidine, pH 7.5). Sodium dodecyl sulfate (SDS) was added to the adipose samples immediately after homogenization to obtain a final concentration of 2% SDS. After homogenization, both muscle and adipose samples were rotated end over end for 1 h at  $4^\circ\text{C}$ . Muscle and adipose samples were then centrifuged for 20 min at 13,000 rpm at  $4^\circ\text{C}$ . The supernatant in the muscle samples was collected for further analysis. Total protein concentration for each sample was determined in triplicate using a BSA standard kit (Thermo Scientific, Waltham, MA, USA) for muscle and adipose lysates. Samples were mixed with  $6 \times$  Laemmli buffer (7 ml of 0.5 M Tris-base, 3 ml glycerol, 0.93 g DTT, 1 g SDS, and 1.2 mg Bromophenol Blue) to reach equal protein concentration before protein expression was determined by western blotting.

Equal amounts of protein were loaded in separate wells on 4–15% (hexokinase II, CS, COXIV) and 7.5% (GLUT4) pre-cast Criterion gels (Bio-Rad Laboratories, Hercules,



**Figure 2. Experimental overview of the study**

DXA: dual-energy X-ray absorptiometry. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

CA, USA). All samples from the same subject were loaded on the same gel in adjacent wells and individual samples were normalized to the average intensity of two human standards in each gel, to allow between-gel comparisons. Proteins were separated according to their molecular weight via SDS-PAGE and proteins were semi-dry blotted to a polyvinylidene difluoride membrane (MilliporeSigma, Burlington, MA, USA). The membranes were blocked in 2% skimmed milk in Tris-buffered saline (TBS) including 0.1% Tween-20 (TBST) before incubation overnight at 4°C in primary antibody (GLUT4; Thermo Fischer Scientific, PA1-1065; hexokinase; Cell Signalling Technology, 2867, CS: Abcam, ab96600; COXIV; Cell Signalling Technology, 4844). Afterwards, membranes were washed in TBST and incubated in horseradish peroxidase-conjugated secondary antibody at room temperature for 1 h. Membranes were then washed in TBST for 3 × 15 min. Membrane staining was visualized by incubation with a chemiluminescent horseradish peroxidase substrate (MilliporeSigma, Burlington, MA, USA) and recorded with a digital camera (ChemiDoc MP Imaging System, Bio-Rad Laboratories). Densitometry quantification of the western blot band intensity was done using Image Lab version 4.0 (Bio-Rad Laboratories) and determined as the total band intensity adjusted for background intensity. Representative blots are shown in Fig. 6. Due to imaging issues for GLUT4 determination in adipose tissue samples, six samples were excluded from analysis (TER,  $n = 8$ ; PLA,  $n = 7$ ).

### Muscle glycogen content

Approximately 2 mg dry weight muscle tissue was extracted in 1 N HCl and hydrolysed at 100°C for 3 h. Glycogen was determined by the hexokinase method as previously described (Lowry & Passonneau, 1972).

### Plasma glucose and insulin concentrations

Blood samples were collected in heparinized syringes during the hyperinsulinaemic-euglycaemic clamp for determination of arterialized venous plasma glucose concentrations using a blood gas analyser (ABL 800 FLEX, Radiometer, Copenhagen, Denmark). Further arterialized venous blood samples were drawn in EDTA tubes and centrifuged at 4°C and 3000 rpm for 5 min, after which plasma was collected and stored at -80°C until analysis for insulin. Plasma insulin was determined using an enzyme immunoassay ELISA kit (Alpco; Salem, New Hampshire, USA), and plates were read on a Multiskan FC plate reader (Thermo Fischer Scientific) according to manufacturer's instructions.

### Statistics

Statistical analyses were performed in SPSS version 26 (IBM, Armonk, US). Data were normally distributed based on Q-Q plots and the Shapiro Wilk's test, and are presented as means ± SD, with effect sizes presented with 95% confidence intervals (CI) for physiological outcomes. To estimate within- and between-group changes with the intervention, a linear mixed model was used with group and time as fixed factors, and subjects as a random factor and baseline value of lean mass as a time-invariant covariate, as it may influence the effect of beta<sub>2</sub>-agonists (Cheymol, 2000; Jessen *et al.* 2018; Hostrup *et al.* 2018b). In case of repeated measures, the Benjamini-Hochberg procedure was applied to adjust *P* values. Pearson's correlation coefficient was used to estimate associations for the primary outcome measure. Sample size was determined based on the predicted effect of terbutaline on lean mass (Jessen *et al.* 2018). The significance level was set at  $P \leq 0.05$ .

### Results

#### Subjects

Of the 67 subjects included in the main study (Jessen *et al.* 2018), 23 subjects (all from the non-exercise intervention arm of Jessen *et al.* 2018) participated in this sub-study consisting of the hyperinsulinaemic-euglycaemic clamp and sampling of muscle and adipose tissue biopsies. Of these 23 subjects, 21 completed all measurements and were included for analysis (Fig. 1). Characteristics of the subjects who completed the sub-study are presented in Table 1.

#### Hyperinsulinaemic-euglycaemic clamp

During the hyperinsulinaemic-euglycaemic clamp, arterialized venous plasma insulin concentrations increased from basal levels of  $35 \pm 11 \text{ pmol} \times \text{l}^{-1}$  to levels of  $599 \pm 139 \text{ pmol} \times \text{l}^{-1}$  after 30 min during the clamp and maintained a steady-state with no within- or between-group differences (Fig. 3A). Euglycaemia was maintained throughout the clamp in both groups before and after the intervention with a CV < 6% and with no within- or between-group differences (Fig. 4A-C).

Glucose infusion rate during the final 20 min of the 2 h hyperinsulinaemic-euglycaemic clamp increased by 27% with the intervention in TER (95% CI: 80 to  $238 \text{ mg} \times \text{min}^{-1}$ ,  $P = 0.001$ ) but was not significantly different ( $P = 0.153$ ) from the change in PLA (95% CI: -37 to  $195 \text{ mg} \times \text{min}^{-1}$ ,  $P = 0.154$ ) (Fig. 5). *Post hoc* analysis revealed that glucose infusion rate was higher ( $P < 0.001$ ) 40-120 min into the clamp after the intervention than before in TER, whereas no within-group

**Table 1. Characteristics of healthy lean men receiving either terbutaline (TER) or placebo (PLA)**

	TER (n = 13)	PLA (n = 8)	Between-group differences P value
Age, years	24.5 ± 2.4	23.8 ± 3.0	0.723
Height, cm	182 ± 6	184 ± 6	0.367
Weight, kg	76.3 ± 5.7	80.1 ± 5.7	0.180
BMI, kg × m <sup>-2</sup>	23.1 ± 0.8	23.4 ± 1.4	0.241
Fat%	17.1 ± 3.3	17.8 ± 4.0	0.703
Fat mass, kg	12.7 ± 2.7	13.8 ± 3.7	0.471
Lean mass, kg	61.1 ± 4.3	63.8 ± 3.6	0.246
Android fat mass, kg	0.8 ± 0.3	0.9 ± 0.3	0.250
Gynoid fat mass, kg	2.0 ± 0.5	2.2 ± 0.5	0.539
$\dot{V}O_{2max}$ , ml × kg <sup>-1</sup> × min <sup>-1</sup>	51.7 ± 4.9	50.9 ± 6.4	0.779

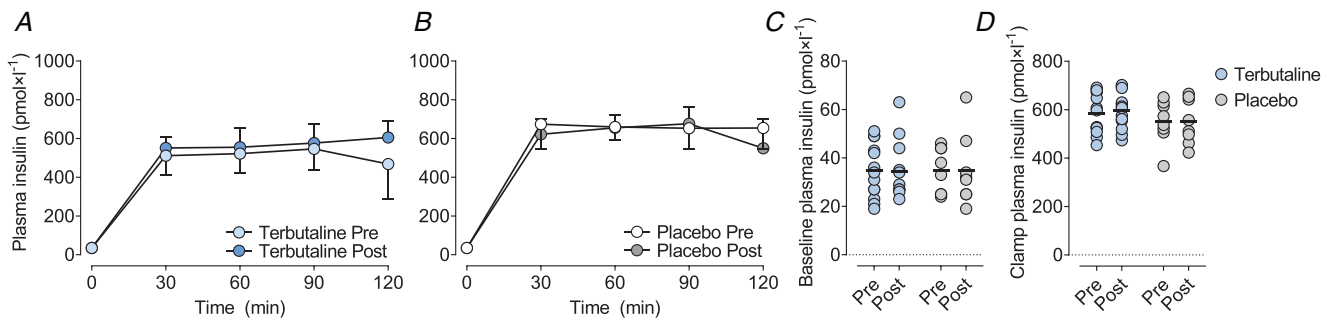
Data are presented as means ± SD. Fat%; body fat percentage; BMI: body mass index;  $\dot{V}O_{2max}$ : maximal oxygen uptake.

changes were observed at specific sampling times in PLA (Fig. 5).

**Proteins regulating glucose transport.** No within- or between-group changes were observed with the intervention for muscle content of GLUT4, hexokinase II, CS, COXIV, and adipose tissue content of GLUT4 (Fig. 6).

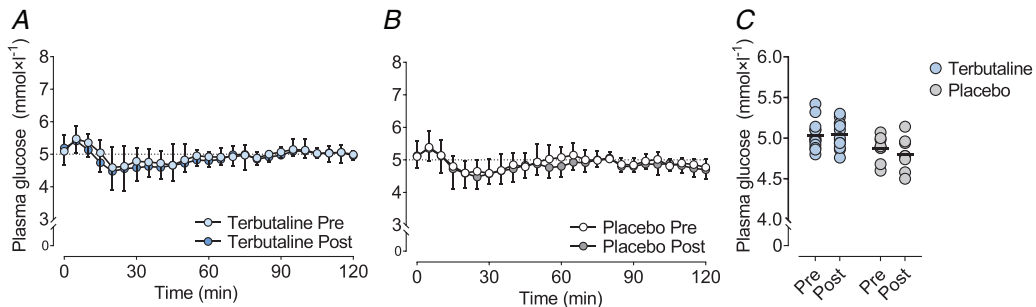
**Muscle glycogen content.** Basal muscle glycogen content did not change with the intervention in either group, being 434 ± 73 and 412 ± 77 mmol × kgdw<sup>-1</sup> before and after, respectively, the intervention in TER (*P* = 0.363) and 405 ± 48 and 350 ± 47 mmol × kgdw<sup>-1</sup> in PLA (*P* = 0.089).

**Body composition.** Lean mass increased by 1.1 kg with the intervention in TER (95% CI: 0.6 to 1.6 kg, *P* < 0.001; Jessen *et al.* 2018), being higher (*P* = 0.001) than the change in PLA (95% CI: -0.9 to 0.3 kg, *P* = 0.366), while fat mass declined by 0.5 kg with the intervention in TER (95% CI: -1.0 to -0.1 kg, *P* = 0.013) but not



**Figure 3. Plasma insulin concentrations**

Mean plasma insulin concentrations during a 2-h hyperinsulinaemic-euglycaemic clamp in healthy lean subjects (A and B), individual baseline plasma insulin concentrations (C), and plasma insulin concentrations during the hyperinsulinaemic-euglycaemic clamp (D) before (Pre) and after (Post) 4 weeks of either terbutaline (inhalation, 4 mg × day<sup>-1</sup>; TER, n = 13; A) or placebo (PLA, n = 8; B) treatment. Data are presented as means ± SD. Horizontal bars in C and D represent means. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



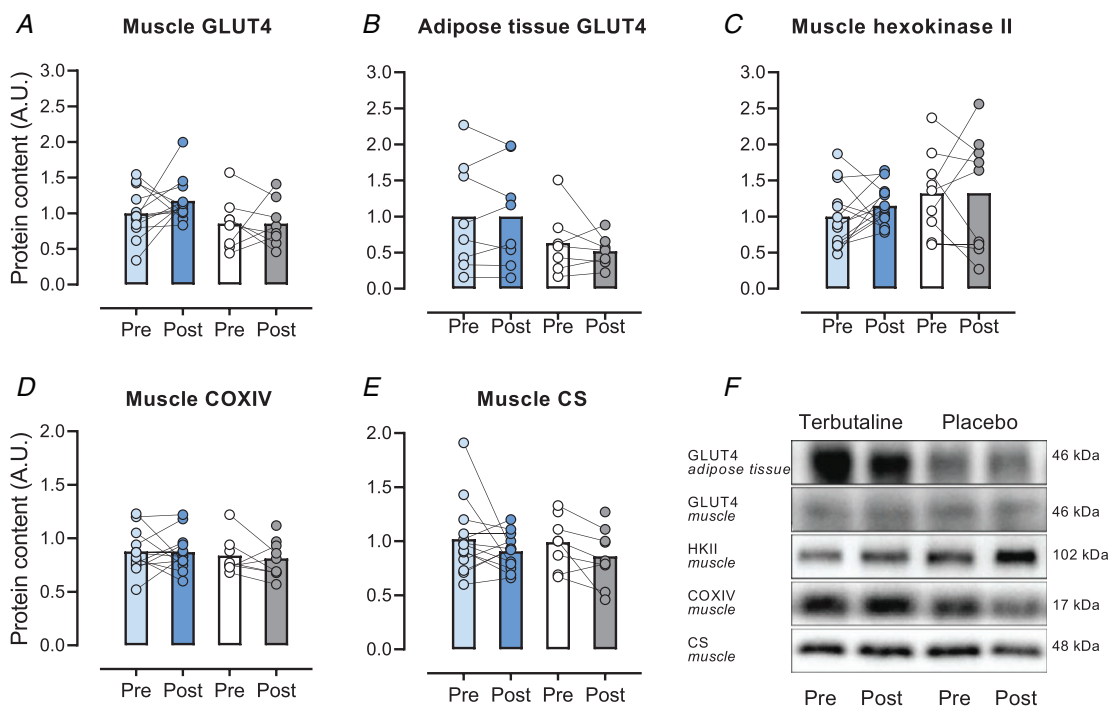
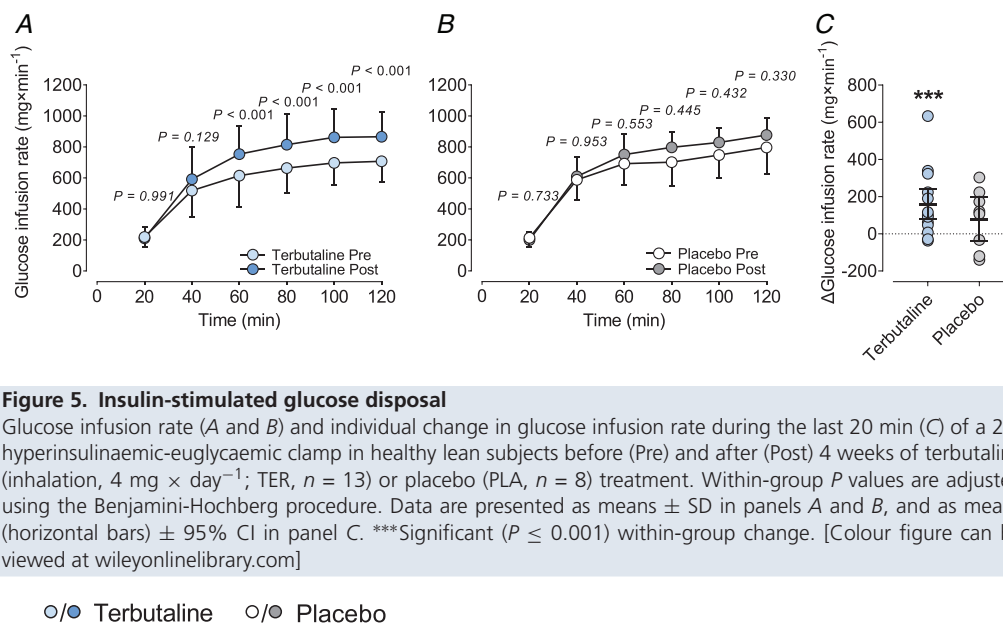
**Figure 4. Plasma glucose concentrations**

Mean plasma glucose concentrations (A and B) and individual plasma glucose concentrations during the last 20 min of a 2-h hyperinsulinaemic-euglycaemic clamp (C) in healthy lean subjects, before (Pre) and after (Post) 4 weeks of terbutaline (inhalation, 4 mg × day<sup>-1</sup>; TER, n = 13) or placebo (PLA, n = 8) treatment. Data are presented as means ± SD in panels A and B. Horizontal bars in C represent means. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

different from the change in PLA (95% CI:  $-1.0$  to  $0.0$  kg,  $P = 0.065$ ). Gynoid fat mass declined by  $0.1$  kg with the intervention in TER (95% CI:  $-0.2$  to  $0.0$  kg,  $P = 0.004$ ) but was not different from the change in PLA (95% CI:  $-0.2$  to  $0.0$  kg,  $P = 0.109$ ). No within- or between-group

changes were observed with the intervention for android fat mass.

**Predictors of change in insulin sensitivity.** Change in lean mass was the only significant independent predictor of the





**Table 2. Predictors for the change in glucose infusion rate (GIR) during the last 20 min of the 2-h hyperinsulinaemic-euglycaemic clamp in healthy lean men receiving either terbutaline (TER) or placebo (PLA)**

Parameter	TER (n = 13)		PLA (n = 8)		All (n = 21)	
	Pearson's <i>r</i>	<i>P</i> value	Pearson's <i>r</i>	<i>P</i> value	Pearson's <i>r</i>	<i>P</i> value
ΔLean mass	0.59	<b>0.04*</b>	0.44	0.28	0.55	<b>0.01**</b>
ΔFat mass	0.19	0.54	0.40	0.32	0.26	0.25
ΔFat%	0.05	0.86	0.30	0.47	0.09	0.67
ΔAndroid fat mass	0.07	0.81	0.40	0.32	0.22	0.33
ΔGynoid fat mass	-0.19	0.51	0.46	0.25	0.10	0.66
ΔGLUT4 (muscle)	0.36	0.22	0.40	0.32	0.40	0.07
ΔGLUT4 (adipose)	0.18	0.66	0.22	0.62	0.23	0.40
ΔHexokinase II	0.08	0.78	0.58	0.13	0.30	0.17
ΔCitrate synthase	0.44	0.14	0.29	0.49	0.39	0.08
ΔCOXIV	0.43	0.15	0.30	0.47	0.39	0.08
ΔGlycogen	0.31	0.29	0.08	0.84	0.29	0.19

Significant (\**P* < 0.05, \*\**P* < 0.01) correlation between change in glucose infusion rate (GIR). COXIV: cytochrome c oxidase subunit 4.

change in glucose infusion rate (final 20 min of the clamp) in TER (Pearson's *r* = 0.59, 95% CI: 0.05 to 0.86; *P* = 0.03, Fig. 7) but was not different in PLA (Pearson's *r* = 0.44, 95% CI: -0.38 to 0.87; *P* = 0.28) (Table 2).

## Discussion

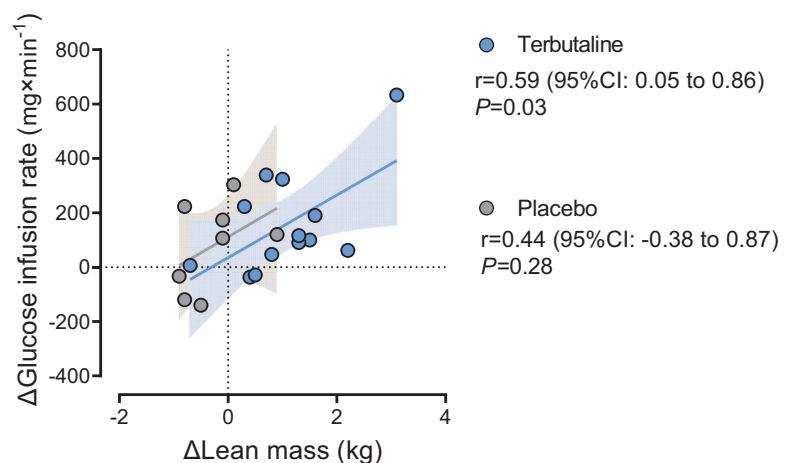
The novel finding of the present study was that daily inhalation of the selective beta<sub>2</sub>-agonist terbutaline for 4 weeks increased insulin-stimulated whole-body glucose disposal in healthy lean young men, which was associated with an increase in lean mass, while no changes were observed in GLUT4 and hexokinase II or basal glycogen content in skeletal muscle nor GLUT4 content in abdominal adipose tissue.

The observation that daily treatment with inhaled beta<sub>2</sub>-agonist enhanced insulin-stimulated whole-body

glucose disposal in lean young men extends findings in rodents highlighting a therapeutic potential of chronic beta<sub>2</sub>-agonist treatment in enhancing the whole-body insulin sensitivity (Torgan *et al.* 1993; Jacob *et al.* 1999; Castle *et al.* 2001; Pan *et al.* 2001; Sato *et al.* 2014). We administered inhaled doses (4 mg terbutaline) resembling those used for people with obstructive pulmonary disease. Even with such a dose, glucose infusion rate during the 2 h hyperinsulinaemic-euglycaemic clamp was improved by 27% after the 4-week treatment period with terbutaline, with 11 of the 13 subjects who received terbutaline experiencing an increase. This effect is on par with the 29% increase in glucose infusion rate observed by Scheidegger *et al.* (1984) in healthy men who ingested terbutaline tablets at doses of 15 mg × day<sup>-1</sup> for 1–2 weeks. Aside from the more clinically relevant dosing regimen in the current study, we also introduced a long

**Figure 7. Lean mass and insulin-stimulated glucose disposal**

Individual relationship between change in lean mass and change in glucose infusion rate during the last 20 min of a 2-h hyperinsulinaemic-euglycaemic clamp in healthy lean subjects before and after 4 weeks of terbutaline (inhalation, 4 mg × day<sup>-1</sup>; TER, *n* = 13) or placebo (PLA, *n* = 8) treatment. Shaded area represents 95% CI. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



wash-out at the end of the treatment period (48–72 h), in contrast to Scheidegger *et al.*, to assert adaptive effects of prolonged treatment without any confounding acute effects on glucose uptake and metabolism (Onslev *et al.* 2019). While negligible residual levels of terbutaline may still have been present for some subjects in the present study (half-life 4–6 h for inhaled terbutaline (Krogh *et al.* 2017)), such levels are unlikely to have had any meaningful physiological effect considering terbutaline's duration of action of 6 h (Sears & Lötvall, 2005). This is supported by the similar plasma glucose and insulin levels prior to the clamp, along with the observation of no changes in basal metabolic rate (Pre: 1.31 vs. Post: 1.37 kcal  $\times$  min<sup>-1</sup>) and substrate utilization (Jessen *et al.* 2018) from before to after the intervention with terbutaline treatment. In Scheidegger *et al.* (1984), on the other hand, basal metabolic rate was around 8% higher during the hyperinsulaemic-euglycaemic clamp after the treatment period, which possibly contributed to the greater glucose disposal in that study. Notwithstanding, the similar increase in insulin-stimulated whole-body glucose disposal with a period of terbutaline treatment in the two studies suggests that prolonged treatment with beta<sub>2</sub>-agonist enhances the capacity for peripheral glucose disposal during insulin stimulation in humans and highlights a potential therapeutic application of beta<sub>2</sub>-agonists outside of obstructive pulmonary disease treatment.

Consistent with the muscle hypertrophic actions of beta<sub>2</sub>-agonists (Hostrup *et al.* 2020; Hostrup & Onslev, 2021), we observed a 1.1 kg lean mass accretion with terbutaline (Jessen *et al.* 2018) – an effect that mainly relates to an increased rate of myofibrillar protein synthesis (Koopman *et al.* 2010; Lee *et al.* 2015; Hostrup *et al.* 2018b), and, in some instances, also a lowered proteolysis during treatment (Navegantes *et al.* 2000, 2001; Koopman *et al.* 2010). Skeletal muscle is the primary site for insulin-stimulated glucose disposal (DeFronzo *et al.* 1981) and gains in lean mass correlate with increases in insulin sensitivity incurred from a period of resistance training (Miller *et al.* 1984). Therefore, the gain in lean mass observed in the present study may explain part of the increased whole-body insulin-stimulated glucose disposal after a period of terbutaline treatment. In support of this, we observed that change in lean mass correlated with change in glucose infusion rate (Fig. 7A). This coincides with rodent studies showing that increases in insulin sensitivity and glucose tolerance with a period of beta<sub>2</sub>-agonist treatment occur along with an increase in muscle mass (Torgan *et al.* 1993; Jacob *et al.* 1999; Castle *et al.* 2001; Pan *et al.* 2001). While the 1.1 kg increase in lean mass may seem small, it is clinically relevant and equivalent to the around 1.0–1.5 kg gained in lean mass by a period of full-body resistance training of similar

duration also shown to enhance insulin sensitivity in non-type 2 diabetic men (Ismail *et al.* 2019).

Changes in the expression of proteins regulating glucose transport and metabolism in skeletal muscle and adipose tissue may also affect the potential for glucose disposal (Birnbaum, 1989; Charron *et al.* 1989). Indeed, the magnitude of increase in insulin-stimulated glucose disposal exceeds the magnitude of lean mass gain in resistance training studies (Yaspelkis, 2006) and muscle GLUT4 content increases in response to resistance exercise training in parallel with an increase in insulin-stimulated glucose disposal. Likewise, improved insulin sensitivity following aerobic training is associated with qualitative changes in muscle, such as mitochondrial biogenesis. Nevertheless, we observed no apparent effect of terbutaline treatment on muscle content of GLUT4 and hexokinase II, nor in markers of mitochondrial content (i.e. CS and COXIV content). In adipose tissue too, GLUT4 content did not change with terbutaline treatment and none of the changes in protein content contributed to explaining the changes observed in insulin-stimulated glucose disposal. And although we cannot exclude the possibility that the period with terbutaline treatment altered insulin signalling and GLUT4 translocation, recent findings by Meister *et al.* (2022) suggest that beta<sub>2</sub>-agonist treatment (with clenbuterol) augments the capacity of skeletal muscle for glucose uptake via mechanisms unrelated to GLUT4 protein levels and translocation in mice.

A strength of the present study was the strict control of drug intake, which was monitored daily, hence ensuring 100% compliance. It should, however, be noted that the study utilized an unbalanced design with subjects being enrolled in favour of active treatment as we expected a higher drop-out rate due to side effects associated with terbutaline and a larger within-group variability due to individual differences in beta<sub>2</sub>-agonist response. Contrary to our expectations, the attrition rate was similar in the two groups resulting in more subjects in the terbutaline than in the placebo group, and the drop-out of two subjects in the placebo group during the clamp-trials reduced the power for between-group comparisons. Thus, with the effect size and between-group variation observed, at least 10 subjects should have completed the study in the placebo group for a significant between-group interaction.

Taken together, the present study indicates that daily treatment with a selective beta<sub>2</sub>-agonist, at close-to-therapeutic doses, augments insulin-stimulated whole-body glucose disposal, which may in part be attributed to muscle hypertrophy. These observations extend findings in rodents and highlight the therapeutic potential of beta<sub>2</sub>-agonists to enhance the capacity for glucose disposal and whole-body insulin sensitivity in humans.

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## Additional information

### Data availability statement

Data are available by request to the corresponding author.

### Competing interests

The authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

### Author contributions

M.H., J.B., V.B., and J.O. designed the study. S.J., J.O., K.E., T.B.S., and M.H. collected the data. T.B.S. and M.H. performed statistical analyses. All authors contributed to the drafting of the manuscript and approved the final version of the submitted manuscript. All authors are guarantors of this work and had full access to all the data in the study and take responsibility for the manuscript.

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### Keywords

adrenergic, adrenoceptor, clenbuterol, glucose transport, insulin sensitivity, muscle hypertrophy, SABA, salbutamol

### Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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## Translational perspective

The relatively large effect observed on insulin-stimulated whole-body glucose disposal with only a few weeks daily treatment with selective beta<sub>2</sub>-agonist is noteworthy as the subjects included were a homogeneous group of healthy lean young men with a high pre-intervention insulin sensitivity ( $\sim 9.7 \text{ mg} \times \text{kg body weight} \times \text{min}^{-1}$ ) well above insulin-resistant individuals ( $< 4.7 \text{ mg} \times \text{kg body weight} \times \text{min}^{-1}$ ) (Bergman *et al.* 1985; Stern *et al.* 2005). Hence, the effect of beta<sub>2</sub>-agonist may be more pronounced in insulin-resistant subjects – particularly in persons with obesity. Although change in fat mass was not an independent predictor of change in insulin-stimulated glucose disposal in the lean population investigated in this study, it is conceivable that beta<sub>2</sub>-agonist treatment can improve insulin-stimulated glucose uptake in populations with greater potential for reductions in fat mass as beta<sub>2</sub>-adrenergic stimulation induces lipolysis in adipose tissue and accentuates whole-body energy expenditure (Onslev *et al.* 2017; Jessen *et al.* 2020). For the same reason, the beta<sub>2</sub>-adrenergic receptor has been highlighted as a potential target to induce leanness and improve insulin sensitivity (Hostrup & Onslev, 2021). Nevertheless, investigations on the applicability of beta<sub>2</sub>-agonist treatment in such populations are warranted.