

ORIGINAL ARTICLE

Beta₂-agonist increases skeletal muscle interleukin 6 production and release in response to resistance exercise in men

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Objective: Several tissues produce and release interleukin-6 (IL-6) in response to beta₂-adrenergic stimulation with selective agonists (beta₂-agonists). Moreover, exercise stimulates muscle IL-6 production, but whether beta₂-agonists regulate skeletal muscle production and release of IL-6 in humans in association with exercise remains to be clarified. Thus, we investigated leg IL-6 release in response to beta₂-agonist salbutamol in lean young men at rest and in recovery from resistance exercise.

Design: The study employed a randomized controlled crossover design, where 12 men ingested either salbutamol (16 mg) or placebo for 4 days, followed by the last dose (24 mg) administered 1½ h before exercise. Arterial and femoral venous plasma IL-6 as well as femoral artery blood flow was measured before and ½–5 h in recovery from *quadriceps* muscle resistance exercise. Furthermore, *vastus lateralis* muscle biopsies were collected ½ and 5 h after exercise for determination of mRNA levels of IL-6 and Tumor Necrosis Factor (TNF)-α.

Results: Average leg IL-6 release was 1.7-fold higher ($p = 0.01$) for salbutamol than placebo, being 138 ± 76 and 79 ± 66 pg min⁻¹ (mean ± SD) for salbutamol and placebo, respectively, but IL-6 release was not significantly different between treatments within specific sampling points at rest and after exercise. Muscle IL-6 mRNA was 1.5- and 1.7-fold higher ($p = 0.001$) for salbutamol than placebo ½ and 5 h after exercise, respectively, whereas no significant treatment differences were observed for TNF-α mRNA.

Conclusions: Beta₂-adrenergic stimulation with high doses of the selective beta₂-agonist salbutamol, preceded by 4 consecutive daily doses, induces transcription of IL-6 in skeletal muscle in response to resistance exercise, and increases muscle IL-6 release in lean individuals.

KEYWORDS

beta₂, beta₂-adrenoceptor, beta-adrenoceptor, cytokine, SABA

1 | INTRODUCTION

A considerable body of evidence highlights the β_2 -adrenoceptor as a promising target in muscle wasting disorders, type 2 diabetes, and obesity.^{1–3} This is substantiated by studies showing that selective β_2 -adrenergic receptor agonists (β_2 -agonists) induce muscle hypertrophy^{4–6} and increase energy expenditure,^{2,7} fat oxidation,^{2,7} protein turnover,^{8,9} and glucose disposal in humans.¹⁰ Moreover, studies indicate that β_2 -agonists regulate the production of cytokines, including interleukin-6 (IL-6),¹¹ which possesses pro-inflammatory and anti-inflammatory actions, as well as being involved in cell growth and metabolism.^{12–15} In vitro studies have demonstrated that β_2 -agonists induce transcription of IL-6 in a variety of cell lines.^{11,16,17} In accordance, administration of non-selective beta-agonist isoproterenol has been shown to increase systemic levels of IL-6 in fasting men and women.¹⁷ In addition, clinical trials indicate that IL-6 release from adipose tissue contributes substantially to the beta-agonist-induced rise in systemic IL-6 levels in resting obese individuals,¹⁷ likely explaining the higher beta-adrenergic IL-6 response observed in obese than in lean individuals.¹⁸ Nevertheless, the observation that selective beta-agonist administration also raises systemic IL-6 levels in very lean individuals^{18,19} suggests that other tissues are involved.

Skeletal muscle is the largest tissue in lean individuals²⁰ and has a high abundance of beta-adrenergic receptors, of which the β_2 -subtype accounts for approximately 80%–90%.^{21,22} Although unexplored in human skeletal muscle, β_2 -adrenergic stimulation induces IL-6 mRNA in skeletal muscle of mice and in myoblasts,²³ indicating that skeletal muscle may contribute to the β_2 -agonist-induced elevations in systemic IL-6 levels observed in humans. Nevertheless, to the best of our knowledge, no studies have investigated whether β_2 -agonists regulate skeletal muscle production and release of IL-6 in humans.

While significant advances have been made in understanding the regulation of IL-6, less is known about the inflammatory cytokine tumor necrosis factor (TNF)- α . It has been suggested that the IL-6 produced in skeletal muscle during exercise inhibits expression of TNF- α .²⁴ In accordance, the overexpression of IL-6 markedly reduces serum TNF- α concentrations in transgenic mice with viral myocarditis,²⁵ and IL-6 and TNF- α gene expression is differentially regulated in response to elevated intracellular calcium levels, with increasing and decreasing mRNA of IL-6 and TNF- α , respectively, in human skeletal muscle cell cultures.²⁶ Similarly, infusion of recombinant IL-6 during exercise ablates endotoxin-induced increases in plasma TNF- α concentrations in humans.²⁷ Moreover,

while knee-extensor exercise stimulates the expression and release of IL-6 from the contracting muscles, no such changes are evident for TNF- α ,²⁴ further lending support to the hypothesis that IL-6 and TNF- α are differentially regulated. Studies in cell lines have suggested that the β_2 -adrenergic receptor inhibits transcription of TNF- α ,^{11,28–33} underlying the relevance of assessing whether β_2 -agonists regulate TNF- α expression in human skeletal muscle.

While skeletal muscle contributes to a negligible amount of the IL-6 found in the circulation at rest, contracting skeletal muscles produce and release larger amounts of IL-6 during exercise in an intensity- and duration-dependent manner, contributing to the systemic increase in IL-6.³⁴ Moreover, part of the exercise-induced increase in systemic IL-6 is related to beta-adrenergic signaling,³⁴ making it relevant to investigate a potential interaction between exercise and β_2 -agonist on skeletal muscle IL-6 release. In addition, because β_2 -agonists have been shown to augment adaptations in muscle mass and strength associated with resistance training,^{4,6,27} it is particularly interesting to assess whether β_2 -agonist affects muscle IL-6 in response to resistance exercise.

In the present study, we investigated the effect of β_2 -agonist on muscle IL-6 release in lean young men at rest and after resistance exercise. Furthermore, we examined the effect of β_2 -agonist on muscle IL-6 and TNF- α mRNA after resistance exercise. We hypothesized that β_2 -agonist would induce a higher net leg IL-6 release than placebo.

2 | MATERIALS AND METHODS

2.1 | Study design

The study employed a randomized double-blinded placebo-controlled crossover design and is part of a larger project investigating β_2 -adrenergic effects on skeletal muscle hypertrophy and metabolism induced by selective β_2 -agonist. A part pertaining to β_2 -adrenergic-induced changes in muscle protein turnover rates,³⁵ glucose metabolism,³⁶ and hypertrophy³⁷ have been described elsewhere. The outcome measures for the present part are arterial and venous IL-6 concentrations, a-v IL-6 difference, IL-6 release, and mRNA levels of IL-6 and TNF- α .

The study was approved by the Committee on Health Research Ethics of the Capital Region of Denmark (H-1-2012-119) and performed in accordance with the standards set by the Declaration of Helsinki. The study was registered in ClinicalTrials.gov (NCT02551276).

2.2 | Subjects and eligibility criteria

Lean young men were recruited for the study in the Capital Region of Denmark via advertising and social networks. Eligibility criteria were 18–40 years of age, an active lifestyle (>3 h of weekly physical activity), non-smoker, no known chronic disease, and no signs of allergy toward the study drug or other prescription medication. Subjects were informed about the potential risks and discomforts related to the study, and each subject gave written and oral informed consent prior to inclusion. Twelve subjects completed the study. Subjects were 23 ± 4 years (mean \pm SD), 181 ± 6 cm height, and had a lean body mass of 61 ± 5 kg and body fat percentage of $12 \pm 5\%$. A CONSORT flow-diagram is shown in Figure 1.

2.3 | Experimental protocol

The experimental protocol has been described in detail previously⁹ and was undertaken in the Department of Nutrition, Exercise, and Sports, University of Copenhagen. In short, subjects ingested either beta₂-agonist (16 mg salbutamol) or placebo tablets daily for 4 days, and met for two experimental trials in the morning after an overnight fast and ingested a final dose of tablets (24 mg salbutamol or placebo) in conjunction with a standardized meal (energy: 369 kcal; protein: 12 g; carbohydrate: 67 g; fat: 3 g). After 90 min of rest, subjects performed two sets of 10 repetition knee-extensor exercise at an intensity corresponding to 50% of 3 repetition maximum (RM), followed by eight sets of 12 repetitions of knee-extensor exercise at an intensity corresponding to 12 RM (75 ± 7 kg) with 2 min of recovery between each set. Intensity and recovery time were duplicated for each subject during the two trials. After the exercise, subjects remained inactive in a bed for 5 h. Biopsies were sampled from the *vastus lateralis* muscle $\frac{1}{2}$ and 5 h after resistance exercise using a 4-mm Bergström biopsy needle (Stille) with suction.³⁸ These time points were chosen based on the previous

observations of IL-6 mRNA being increased immediately after knee-extensor exercise and increasing progressively up to 3 h in recovery.²⁴ Brachial arterial and femoral venous blood samples were drawn in EDTA tubes (9 ml) prior to exercise as well as $\frac{1}{2}$ –5 h following exercise and blood flow was measured at the same time points by ultrasound Doppler. Subjects were told to refrain from caffeine, nicotine, and alcohol 24 h before each trial, as well as from strenuous exercise 48 h before.

2.4 | Study drugs

Beta₂-adrenoceptors were stimulated with the highly selective beta₂-agonist salbutamol (Ventolin®, 4 mg tablets; GlaxoSmithKline), which has a duration of action of 6–8 h³⁹ and a plasma elimination half-life of 3–4 h. Salbutamol concentrations peak systemically 1½–3 h after oral administration.⁴⁰ Identical looking lactose monohydrate tablets were used for placebo treatment. Drugs were delivered by the Regional Pharmacy of Copenhagen and were administered randomly in a double-blinded manner. A block-randomization sequence was generated in SPSS version 21 (IBM). Each trial was preceded by a 4-day lead-in period with oral salbutamol (16 mg daily) or placebo treatment. The two trials were separated by 3–6 weeks to minimize potential confounding carry-over effects of salbutamol.⁴¹ Eight of the 12 subjects experienced common side effects of salbutamol during the first 2 days of treatment, including tremors ($n = 7$) and palpitations ($n = 6$).⁸

2.5 | Plasma concentration of IL-6

The plasma concentration of IL-6 was determined in arterial and femoral venous plasma ($n = 10$ subjects, plasma yield from 2 subjects was inadequate to allow measurements) in triplicates (mean CV: 5%), using Human IL-6 quantikine ELISA kit (R&D systems, Biotechne, #HS600C) according to the manufacturer's protocol.

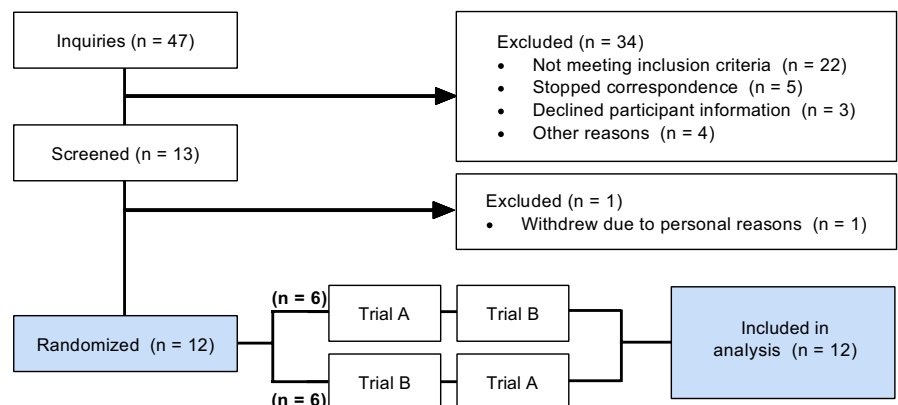


FIGURE 1 CONSORT flow diagram

2.6 | RNA isolation, reverse transcription, and real-time PCR

The method for RNA isolation, reverse transcription, and real-time PCR has been described previously.^{42,43} Total RNA was isolated from ≈ 20 mg wet wt muscle tissue using a modified guanidinium thiocyanate–phenol–chloroform extraction method from Chomczynski and Sacchi (1987) as described by Pilegaard et al.⁴³ except for the use of a TissueLyser (TissueLyser II; Qiagen) for homogenization.^{43,44} Superscript II RNase H- and Oligo dT (Invitrogen) were used to reverse transcribe mRNA to cDNA. Quantification of cDNA as a measure of mRNA content of a given gene was performed by real-time PCR using an ABI 7900 sequence-detection system (Applied Biosystems). Self-designed probes (Table 1) and 5'-6-carboxyfluorescein (FAM)/3'-6-carboxy-N,N,N',N'-tetramethylrhodamine (TAMRA) labeled TaqMan probes were designed from human-specific databases from ensemble (www.ensembl.org/homo_sapiens/info/index) using Primer Express 3.0 software (Applied Biosystems) and were obtained from TAG Copenhagen.

Real-time PCR was performed in triplicates in a total reaction volume of 10 μ l using Universal Mastermix with UNG (Applied Biosystems). Mean CV of triplicates was 18 and 13% for IL-6 and TNF- α , respectively. The obtained cycle threshold values reflecting the initial content of the specific transcript in the samples were converted to a relative amount by using standard curves constructed from serial dilution of a pooled sample made from all samples. Target mRNA content was normalized to single-stranded (ss) DNA content in each sample determined by using OliGreen reagent (Molecular Probes) as previously described.⁴⁵

2.7 | Blood flow measurement

Femoral arterial blood flow was measured with ultrasound Doppler (Vivid E9, GE Healthcare) equipped with a linear probe operating at an imaging frequency of 4.0–8.0 MHz and Doppler frequency of 4.3 MHz. Blood flow was measured in the common femoral artery between the inguinal ligament and the bifurcation into the superficial and profundus branches to avoid sampling sites with turbulent flow. Recordings were obtained at the lowest possible insonation angle, which was always below 60°. Sample volume was

as high as possible within the confines of the vessel wall and always centered in the middle of the artery. Blood flow measurements were calculated from Doppler traces with a low-velocity filter rejecting velocities below 1.8 m s⁻¹, and averaged over 8 heart cycles at the time of blood sampling. Arterial diameter was assessed in triplicates during systole at rest based on longitudinal two-dimensional images.

2.8 | Calculations

Net leg IL-6 release was calculated as femoral venous [IL-6] minus arterial (systemic) [IL-6], multiplied by the femoral plasma flow (Fick's principle), because the amount of IL-6 bound to blood cells can be neglected.³⁹ The plasma flow was calculated as blood flow \times (1 – hematocrit).

2.9 | Statistics

Statistical analyses were performed in SPSS version 25 (IBM). Sample size was calculated for a larger part of the study which has been published elsewhere,⁸ and was estimated based on the expected change in fractional protein synthesis rate after beta₂-agonist treatment reported in previous studies.^{46,47} Thus, no a-priori power calculation was performed for the outcome measures in the present study, which were explorative in nature. Data were normally distributed based on Q-Q plots and the Shapiro–Wilk's test. To estimate differences between treatments, two-tailed linear mixed modeling was used with treatment as a fixed effect and a random effect for subjects. In addition, age and lean body mass were included in the model as time-invariant covariates because they may confound the effect of beta₂-agonist.^{48,49} In case of repeated measures, sampling time was included in the model as a fixed effect for a full factorial design. In case of significant main effects for repeated measures, post hoc testing was conducted and *p*-values were adjusted with the Benjamini–Hochberg procedure. Data are presented as mean \pm SD and effect size as mean changes with 95% confidence interval (CI) with exact *p*-values (unless lower than 0.001) to represent probability for fixed effect parameter estimates. *p*-Values ≤ 0.05 were considered significant.

TABLE 1 Primers used for real-time PCR

Target gene	Sense primer	Antisense primer	TaqMan probe
IL-6	5'TCTCAGCCCTGAGAAAGGAGACA 3'	5'CATCTTTGGAAGGTTTCAGGTTGT 3'	5'ACATGTGTGAAAGCAGCAAAGAGGCACTG 3'
TNF- α	5'TCTGGCCCAGGCAGTCAGAT 3'	5'AGCTGCCCTCAGCTTGA 3'	5'CAAGCCTGTAGCCCATGTTGTAGCAAACC 3'

FIGURE 2 Arterial (A) and femoral venous (B) plasma interleukin 6 (IL-6) at rest as well as ½ and 5 h in recovery from quadriceps resistance exercise in lean young men. Individual values for salbutamol and placebo are presented ($n = 10$)

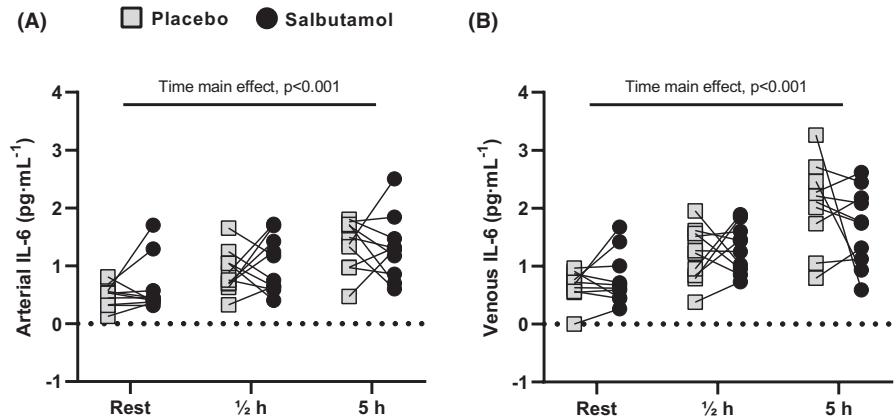
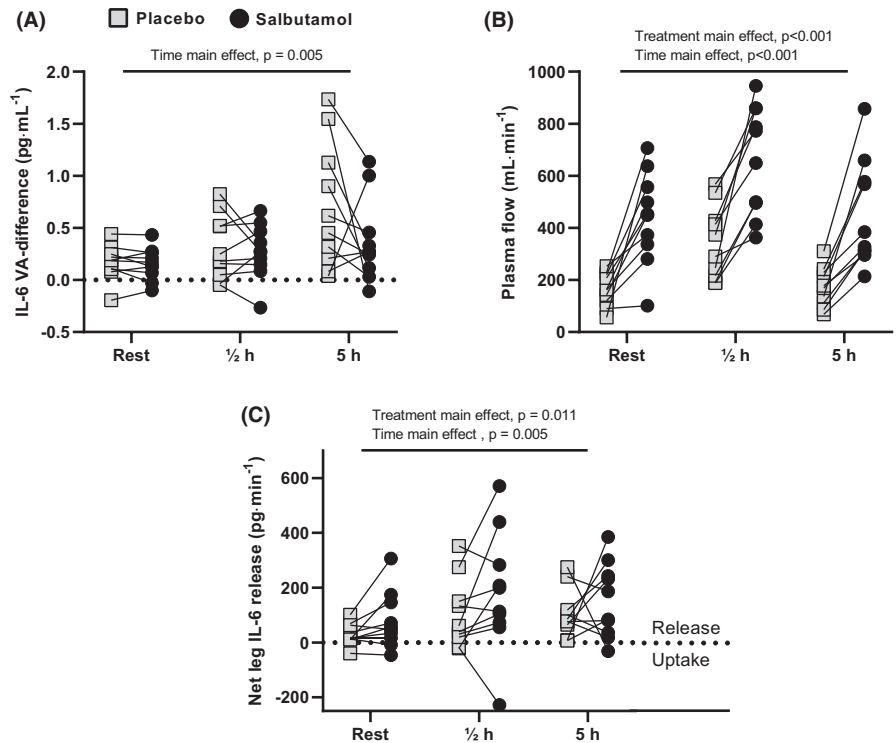


FIGURE 3 Femoral venous-to-arterial interleukin 6 (IL-6) concentration difference (A), femoral artery plasma flow (B), and net leg release of IL-6 (C) at rest as well as ½ and 5 h in recovery from quadriceps resistance exercise in lean young men. Individual values for salbutamol and placebo are presented ($n = 10$)



3 | RESULTS

3.1 | Arterial and femoral venous plasma concentrations of IL-6

No treatment difference or treatment by time interaction was observed for arterial plasma IL-6 concentrations or femoral venous plasma IL-6 concentrations (Figure 2A). For arterial plasma IL-6 concentrations, there was a main effect of time ($p < 0.001$), where concentrations increased by 0.40 pg ml^{-1} (95% CI: 0.28 to 0.53; $p < 0.001$) from rest to ½ h in recovery from exercise, and further by 0.37 pg ml^{-1} (95% CI: 0.17 to 0.57; $p = 0.002$) from ½ h to 5 h in recovery from exercise for both treatments. Similarly, a main effect of time was evident for femoral venous plasma IL-6 concentrations

($p < 0.001$) which increased from rest to ½ h in recovery from exercise by 0.53 pg ml^{-1} (95% CI: 0.38 to 0.72; $p < 0.001$), and further by 0.61 pg ml^{-1} (95% CI: 0.39 to 0.83; $p < 0.001$) from ½ h to 5 h in recovery from exercise for both treatments (Figure 2B).

3.2 | Leg arteriovenous difference and net leg IL-6 release

For leg IL-6 v-a difference, the effect of salbutamol vs. placebo was -0.13 pg ml^{-1} (95% CI, -0.28 to 0.02) with no significant main effect of treatment ($p = 0.089$) and no apparent treatment differences within specific sampling points (Figure 3A). There was a main effect of time ($p = 0.005$) for Leg IL-6 v-a difference, which increased

by 0.13 pg ml^{-1} (95% CI: 0.04 to 0.22; $p = 0.018$) from rest to $\frac{1}{2}$ h in recovery from exercise, and further by 0.24 pg ml^{-1} (95% CI: 0.01 to 0.48; $p = 0.043$) from $\frac{1}{2}$ h to 5 h in recovery from exercise for both treatments. For femoral arterial plasma flow, there was a main effect of treatment ($p < 0.001$), where plasma flow was approximately two-fold higher for salbutamol than placebo at rest and $\frac{1}{2}$ –5 h in recovery from exercise (mean difference: 290 ml min^{-1} , 95% CI, 213 to 368; $p < 0.001$) (Figure 3B). For leg IL-6 release, there was a main effect of treatment ($p = 0.011$) where IL-6 release was 1.7-fold higher for salbutamol than placebo (mean difference 59 pg min^{-1} , 95% CI, 14 to 104; $p = 0.011$) (Figure 3C). There was a main effect of time ($p = 0.005$) for leg IL-6 release, which increased by 87 pg min^{-1} (95% CI: 32 to 142; $p = 0.007$) from rest to $\frac{1}{2}$ h in recovery from exercise for both treatments, but not further from $\frac{1}{2}$ h to 5 h in recovery from exercise ($p = 0.647$). No treatment by time interactions was observed in leg IL-6 v-a difference and release.

3.3 | Muscle content of mRNA IL-6 and TNF- α

For muscle mRNA content of IL-6, there was a main effect of treatment ($p = 0.001$), with the effect of salbutamol vs. placebo being 0.10 IL-6 mRNA/ssDNA (95% CI: 0.05 to 0.16; $p = 0.001$) (Figure 4). IL-6 mRNA content was 1.5-fold higher at $\frac{1}{2}$ h ($p = 0.036$) and 1.7-fold higher at 5 h ($p = 0.027$) in recovery from exercise compared with placebo. There was also a main effect of time ($p < 0.001$), where IL-6 mRNA levels declined by 0.10 IL-6 mRNA/ssDNA (95% CI: -0.15 to -0.01 ; $p < 0.001$) from $\frac{1}{2}$ h to 5 h in recovery from exercise for both treatments. For muscle TNF- α mRNA content, there was no treatment main effect

($p = 0.107$) but an overall main effect of time ($p < 0.001$) where levels declined by 0.23 TNF- α mRNA/ssDNA (95% CI: -0.33 to -0.13 ; $p < 0.001$) from $\frac{1}{2}$ h to 5 h in recovery from exercise for both treatments.

4 | DISCUSSION

The most important findings of the present study were that the selective beta₂-agonist salbutamol induced an elevated leg IL-6 release at rest and in recovery from resistance exercise, as well as increased skeletal muscle mRNA levels of IL-6 in recovery from resistance exercise in lean young men.

A strength of the present study was the sampling of arterial and femoral venous blood in combination with measurements of femoral artery blood flow, allowing estimation of in vivo leg IL-6 release to the systemic circulation at rest and in recovery from exercise. The observation that selective beta₂-agonist induced greater leg IL-6 release and IL-6 mRNA levels extends previous findings^{18,19} by demonstrating that skeletal muscle contributes to IL-6 production and release in response to beta₂-adrenergic stimulation in lean individuals. Other human studies have highlighted adipose tissue as the main site of the beta-adrenergic-induced rise in systemic IL-6 levels, thus explaining the greater rise in IL-6 levels observed in obese than lean individuals upon beta₂-agonist treatment.¹⁸ Although we cannot preclude a contribution of leg adipose tissue for the beta₂-agonist-induced increase in leg IL-6 release, this seems unlikely given the low fat percentage of the subjects (12% body fat) in the present study. Furthermore, the leg IL-6 release values observed were associated with large inter-subject variability with one subject displaying a large

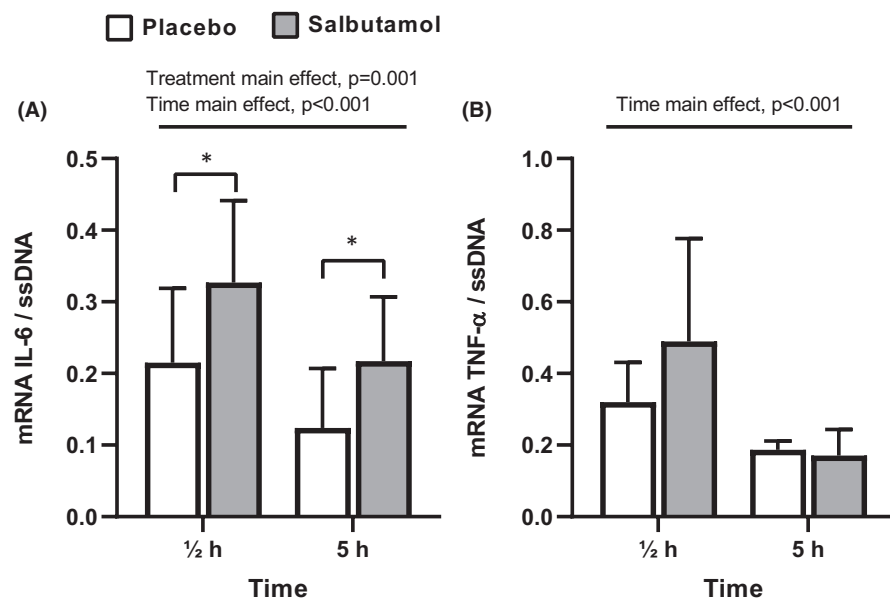


FIGURE 4 Muscle interleukin-6 (IL-6) (A) and tumor necrosis factor (TNF- α) (B) mRNA content for salbutamol and placebo $\frac{1}{2}$ and 5 h in recovery from resistance exercise in lean young men ($n = 12$). *Different from placebo ($p < 0.05$). Data are mean (bars) and SD (error bars)

salbutamol-induced decline in leg IL-6 release in recovery from exercise.

The observation of no effect of salbutamol on arterial levels of IL-6 compared with placebo was unexpected, as studies have demonstrated higher levels of IL-6 in arterial-ized venous blood after treatment with non-selective beta-agonist isoproterenol in fasting lean individuals at rest¹⁸ and in mixed venous blood of elite athletes in recovery from exercise after combined treatment with several selective beta₂-agonists.¹⁹ Differences in beta₂-adrenoceptor pharmacodynamics of the agonist used⁵⁰ and dosing regimen applied might explain the discrepancies. However, the latter is unlikely, as the dose of salbutamol administered in the present study was considerably higher than the inhaled doses used for treatment purposes ($\approx 1\text{--}400\ \mu\text{g}$ for salbutamol) and has been shown to elicit a pronounced physiological response.⁸ Nonetheless, the observation that salbutamol did not increase systemic IL-6 levels in the present study, regardless of leg IL-6 release being larger for salbutamol than placebo, suggests a higher IL-6 uptake and/or elimination via degradation in other tissues or renal filtration with salbutamol treatment.⁵¹ However, the fact that we employed resistance exercise may also explain why we did not observe a rise in systemic IL-6 that has been observed in protocols of longer durations. For example, Steensberg et al.²⁴ observed that systemic IL-6 increased in a duration-dependent manner in a protocol utilizing 180 min of knee-extensor exercise, being markedly longer than the protocol employed in the present study. Therefore, the salbutamol-induced increase in leg IL-6 release in the present study may have been too small to change the systemic concentration or the present study may have been underpowered to detect changes of such small magnitude.

The quadriceps resistance exercise undertaken induced a 2- to 3-fold increase in net leg IL-6 release and an approximately 2-fold increase in arterial plasma IL-6 levels $\frac{1}{2}$ –5 h in recovery from exercise compared with rest regardless of concomitant treatment with salbutamol. Thus, we did not observe any indication that the effect of selective beta₂-agonist on leg IL-6 release was confounded by the resistance exercise undertaken. While IL-6 release from exercising muscles following resistance exercise, to the best of our knowledge, has not been determined previously, the observed increase in arterial plasma IL-6 levels to around $1.0\text{--}1.5\ \text{pg ml}^{-1}$ is lower than those observed following whole-body resistance exercise ($\approx 3\text{--}7\ \text{pg ml}^{-1}$).^{52,53} This is likely explained by between-study differences in exercise intensity and duration, as well as the number of muscle groups exercised (e.g., only the quadriceps muscles in the present study).³⁴ The exercise-induced rise in systemic IL-6 levels evoked by resistance exercise is lower than that observed after prolonged cycling exercise,³⁴ where systemic levels of IL-6 can increase 10-fold. This

is consistent with the lower net leg IL-6 release observed in recovery from the quadriceps resistance exercise in the present study than the values observed in recovery from cycling exercise in previous studies.⁵⁴

The observation that the greater leg IL-6 release with salbutamol treatment was associated with higher muscle IL-6 mRNA levels in recovery from resistance exercise extends findings in rodents and cell lines. In mice, non-selective beta-adrenergic stimulation with infusion of epinephrine increased muscle IL-6 mRNA levels 40-fold.²³ But in contrast to studies in cell lines showing that beta₂-agonists potently downregulate TNF- α expression in response to lipopolysaccharide stimulation in human monocytes^{11,33} and other cell types,^{30–32} we observed no apparent effect of salbutamol in muscle TNF- α mRNA content $\frac{1}{2}$ –5 h in recovery from exercise, which would indicate that the beta₂-agonist-induced increase in muscle IL-6 does not inhibit TNF- α transcription.

A limitation of the present study was that the sample size may have been insufficient to detect differences in systemic concentrations of IL-6 and leg IL-6 release at individual time points. Nevertheless, we were able to demonstrate an overall effect of salbutamol treatment on IL-6 release, which was paralleled by an increase in IL-6 mRNA, suggesting that beta₂-agonist treatment increases muscle IL-6 production in human skeletal muscle. As we did not include female participants, we can not conclude if this effect of beta₂-agonists on muscle IL-6 production is comparable between sexes, although some studies do report sex-independent responses to beta₂-agonist treatment.^{19,55}

5 | CONCLUSION

The present findings were that selective beta₂-agonist salbutamol at oral doses increased leg IL-6 release at rest and in the recovery period after quadriceps resistance exercise in lean young men, but with no apparent increase in systemic IL-6 levels. Furthermore, salbutamol increased IL-6 mRNA levels in skeletal muscle after resistance exercise. Collectively, these findings indicate that beta₂-adrenergic receptors contribute to IL-6 production in human skeletal muscle and that resistance exercise does not confound the effect of beta₂-agonist on muscle IL-6 release.

6 | PERSPECTIVES

The observed increased production and release of IL-6 adds to the complex transcriptional and signaling signature in skeletal muscle upon stimulation of the beta₂-adrenergic receptor^{9,50,51} and suggests a possible contribution of IL-6

in the wide range of whole-body metabolic effects observed with prolonged beta₂-agonist exposure. For example, beta₂-agonist exposure stimulates adipose tissue lipolysis and whole body fat oxidation,^{2,56} and prolonged exposure to beta₂-agonists may be a potential target to combat obesity.^{4,57,58} Indeed, IL-6 too stimulates adipose tissue lipolysis⁵⁹ and infusion of a IL-6 receptor antibody during a weight loss intervention prevents loss of visceral fat mass.⁶⁰ Hence, IL-6 and beta₂-agonist administration display overlap in their whole-body metabolic functions. In the present study, we did not detect increases in systemic IL-6 concentrations, which would likely be necessary for mediating whole-body effects of IL-6. However, the lack apparent increase in systemic IL-6 concentrations are possibly related to an insufficient sample size. Considering the value of strategies that can mitigate obesity, investigations on the possible contribution of IL-6 in the beta₂-agonist induced changes in body composition during prolonged exposure to beta₂-agonists are warranted. Such studies should be conducted with populations including both males and females and with sample sizes sufficient to detect small changes in IL-6 concentrations.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

MH designed the study, participated in the collection of data as well as in data analysis and interpretation, and in the drafting of the manuscript. SJ, JG, and CM participated in data acquisition as well as in data analysis and interpretation, and in critically revising the manuscript. HP and JB contributed to the conception of the study as well as in data analysis and interpretation, and in critically revising the manuscript. All authors approved the final version of the manuscript.


DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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