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Formoterol, a Highly β_2-Selective Agonist, Induces Gender-Dimorphic Whole Body Leucine Metabolism in Humans☆

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ARTICLE INFO

Article history:
Received 14 September 2014
Accepted 19 December 2014

Keywords:
Beta-agonist
Formoterol
Skeletal muscle
Leucine turnover
Sarcopenia

ABSTRACT

Objective. Formoterol is a β_2-selective agonist that enhances protein anabolism in rodents. Whether formoterol imparts anabolic benefits in humans is unknown. The objective of the study was to investigate the effects of formoterol on whole body protein rates of turnover, oxidative loss and synthesis.

Design. Open label intervention study.

Patients. Fifteen healthy adults (8 men).

Measurements. Volunteers were treated with oral formoterol 160 μg/day for one week. Changes in leucine turnover (LRa; index of protein breakdown), oxidation (Lox; irreversible protein loss) and incorporation into protein (LIP; index of protein synthesis) were assessed using the whole body 1-[^{13}C]leucine turnover technique before/after treatment.

Results. LRa, Lox and LIP correlated significantly with lean body mass (LBM). LRa, adjusted for LBM was significantly higher ($P < 0.05$, 160 ± 6 vs 109 ± 3 μmol/min) in men but not fractional Lox and LIP (expressed as a proportion of LRa). Formoterol reduced LRa (−9 ± 4%) in men but stimulated LRa (9 ± 3%) in women. Formoterol significantly reduced ($P < 0.05$) fractional Lox, an effect greater in women (−4 ± 1 vs −1 ± 1 %). It stimulated fractional LIP in women (Δ4 ± 1%, $P < 0.05$) but not in men (Δ1 ± 1%). Formoterol induced an absolute anabolic effect that was greater in women (30 vs 8%). Heart rate, systolic and diastolic blood pressures were unaffected.

Conclusion. In a therapeutic dose, formoterol stimulates protein anabolism in humans. It induced gender-dimorphic effects on protein turnover and on the partitioning of amino acids from oxidative loss toward protein synthesis, effects that are greater in women than in men. Formoterol holds promise as a treatment for sarcopenia.

Abbreviations: β-ARs, β-adrenoceptors; BP, blood pressure; EE, energy expenditure; HR, heart rate; LBM, lean body mass; LIP, leucine incorporation into protein; Lox, leucine oxidation; LRa, leucine rate of appearance; KIC, ketoisocaproic acid.

☆ None of the authors have any conflicts of interests.

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http://dx.doi.org/10.1016/j.metabol.2014.12.005
0026-0495/

1. Introduction

Skeletal muscle mass is a major determinant of health and physical function. A sedentary life style leads to muscle loss, resulting in reduced physical fitness and increasing cardiovascular morbidity and mortality [1,2]. Functional impairment associated with muscle loss is particularly pronounced in women [3], possibly due to lower peak muscle mass than in men. Current therapeutic options for treatment of sarcopenia are limited.

The sympathetic nervous system is a major regulator of muscle mass mediated by β-adrenoceptors (ARs). β-AR subtype distribution varies according to organs. β_1-ARs are chiefly located in the heart and white adipose tissue while β_2-ARs are predominantly found in airways and skeletal muscle [4]. In animals, β_2-selective agonist induces skeletal muscle hypertrophy through cyclic AMP and AKT mediated-pathways [4,5]. These observations suggest that β_2-AR mediated activation of muscle anabolic pathways may prevent or reverse sarcopenia [6]. However, β_2-AR therapeutic activation has been hindered by the limited specificity of traditional β_2-agonists, which cross-stimulate cardiac β_1-ARs, resulting in tachycardia.

Formoterol is a new generation, potent and highly β_2-selective adrenergic agonist, approved for the treatment of asthma and chronic obstructive airway disease. It is 15 times more potent than salbutamol in inhaled forms, and up to 50 times more potent when administered orally. It is also up to 100 times more β_2-selective than traditional β_2-agonists [7]. We have recently reported that formoterol in therapeutic dosage (160 μg/day) augmented resting energy expenditure (EE) and fat utilization in healthy men without inducing tachycardia [8]. Whether it stimulates protein anabolism in humans has not been investigated.

In this study, we have investigated the effects of a therapeutic dose of formoterol on whole body protein metabolism in healthy men and women.

2. Subjects and Methods

Fifteen adults in good health with no chronic medical conditions aged 29 ± 1 years (F = 7, BMI: 23 ± 1 kg/m^2) participated in an open-label intervention study. They were recruited through local advertisement. Subjects attended two visits one week apart, and were evaluated before and after 1 week of oral formoterol treatment at 160 μg daily, a dose based on a dose-finding study showing efficacy in elevating resting EE without increasing heart rate [8]. Formoterol (Atock®) was sourced from Astellas Pharma Inc., Japan. The Human Research Ethics Committee, St. Vincent's Hospital, approved the studies, and all subjects provided written informed consent (Australian New Zealand Clinical Trials Registry: ACTRN12610000161022).

2.1. Assessment of Leucine Turnover

The effect of formoterol on protein metabolism was investigated using the whole body leucine turnover study [9]. The technique is based on the principle that skeletal muscle, the largest component of protein mass in the body, exists in a dynamic state of continuous synthesis and degradation [10]. Leucine derived from protein breakdown is either oxidized and irreversibly lost or reincorporated into protein. As leucine is an essential amino acid, tracking the fate of leucine in the fasting state reflects the dynamic process of whole body protein turnover, protein oxidation and protein synthesis [9].

Participants attended the Garvan Institute of Medical Research at 8 am after an overnight fast. The final dose of formoterol was administered at 7 am. Body weight and height were measured on the same electronic scale. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters. Body composition was measured by multi-frequency bioimpedance analysis (InBody 320 Body Composition S7 Analyzer; Biospace, Sweden). Blood pressure (BP) and heart rate were measured in the supine position after 20 minutes of resting (Omron HEM-780 blood pressure monitor). Two intravenous cannulae were inserted into each arm for isotope infusion and blood sampling.

Whole body protein turnover was assessed using a primed constant infusion of 1-[^{13}C]leucine, as previously described [11]. Participants rested for 20 minutes before two baseline blood and breath samples were collected (−10 and 0 minutes). A 0.1 mg/kg priming dose of $NaH^{13}CO_3$ was administered, immediately followed by a 3-h primed constant infusion of 1-[^{13}C]leucine (prime, 0.5 mg/kg; infusion, 0.5 mg/kg · h). $NaH^{13}CO_3$ and 1-[^{13}C]-leucine boluses and infusions were prepared under sterile conditions by a certified compound chemist. The amount of $NaH^{13}CO_3$ and 1-[^{13}C]-leucine infused was 30 mg and 150–220 mg, dependent on the weight of the participant, respectively. The calculated amount was dissolved in 60 ml normal saline and further filtered through a 0.2 μm filter (Medipark) into a 50 ml syringe immediately prior to injection on the study day. Blood and breath samples were collected at 140, 160 and 180 minutes, when steady state was previously confirmed to be achieved during the third hour of infusion. Breath samples were collected by blowing through a straw into an Exetainer screw-capped glass vial (Labco Ltd, High Wycombe, UK). Blood samples were placed on ice, plasma separated and stored at −80 °C until analysis.

CO_2 production rates were measured by a ventilated hood system (Parvomedic metabolic monitor, Parvo Medics, UT) during two 20-minute periods and averaged. Resting EE and substrate utilization were calculated, as previously described [12], using the equations of Ferrannini. The mean day-to-day intrasubject CV for resting EE in our hands is approximately 4%.

2.2. Calculation of Whole Body Protein Turnover

Whole body protein turnover was calculated using the reciprocal pool method [13], which is based on the principle of steady-state kinetics, whereby the rate of appearance of a substrate equals its rate of disposal. As leucine is either oxidized or reincorporated into protein, it allows the calculation of rates of leucine appearance (LRa) (an index of protein turnover), leucine oxidation (Lox) (an index of oxidative loss of protein), and leucine incorporation into protein (LIP) (an index of protein synthesis). As leucine is transaminated to ketoisocaproic acid (KIC) intracellularly, which equilibrates