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ORIGINAL ARTICLE

Formoterol, a highly β_2-selective agonist, increases energy expenditure and fat utilisation in men

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BACKGROUND: The sympathetic nervous system regulates energy metabolism via β-adrenoreceptors. Therapeutic exploitation of previous β_2-adrenegic agonists for metabolic benefits has been hindered by cross stimulation of cardiac β_1-adrenoceptor, causing tachycardia. Formoterol is a novel highly β_2-selective adrenergic agonist and holds promise as a β_2-agonist that could impart selective beneficial metabolic effects.
OBJECTIVE: To investigate the metabolic effects of formoterol on energy and substrate metabolism.
PARTICIPANTS: Healthy volunteers.
DESIGN: (1) Dose-finding study, step-wise incremental design of weekly administration of 80, 160 and 320 μg daily of formoterol in four subjects and, (2) metabolic study, an open-label metabolic evaluation of 1-week treatment in eight men using a dose determined from (1).
MAIN OUTCOME: Resting energy expenditure (EE), diet-induced thermogenesis (DIT) and fat oxidation (Fox) using indirect calorimetry, heart rate and plasma non-esterified fatty acid (NEFA) levels.
RESULTS: In the dose-finding study, all three doses increased resting EE and Fox with the 320 μg dose significantly increasing heart rate. In the metabolic study, the selected 160 μg daily dose increased resting EE by 13 ± 2% ($P<0.001$) and Fox by 23 ± 4% ($P<0.01$), but not DIT. Plasma NEFA levels rose by 16 ± 2% ($P<0.01$). Heart rate did not change significantly. Out of the eight subjects, six reported tremor and palpitation, two lost appetite and one suffered from insomnia.
CONCLUSIONS: At a dose of 160 μg per day, formoterol increases resting EE and fat utilization without inducing tachycardia. From this first metabolic evaluation in humans, we conclude that formoterol imparts beneficial metabolic changes that may be exploited for therapy of obesity.

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Keywords: beta-agonist; formoterol; energy expenditure; diet-induced thermogenesis; brown adipose tissue

INTRODUCTION

The sympathetic nervous system (SNS) is a major regulator of energy balance and substrate utilization. It stimulates energy expenditure (EE) and regulates fat and protein metabolism. Reduction in SNS activity leads to weight gain in animal models,[1] while ablation of adrenergic receptors (ARs) in mice reduces EE and leads to obesity.[2]

There are three types of β-ARs. β_1-ARs control cardiac while β_2-ARs respiratory function. These properties form the pharmacological basis for the clinical use of β_1- and β_2-agonists as inotropic agents and bronchodilators, respectively. β_3-ARs are found chiefly in brown adipose tissue.[3] The metabolic impact of β_2-agonists have received relatively less attention compared with their effect on respiratory function. β_2-ARs are present in adipose tissue and skeletal muscle, and regulate fat and protein metabolism. In animals, β_2-adrenergic stimulation increases EE and enhances lipolysis.[4,5] In contrast, inhibition of the β-adrenergic system by β-blockers blunts EE, fat utilization and leads to obesity.[6] Given the importance of the SNS in energy balance and metabolism, and the adverse metabolic consequences of β-blockade, harnessing the β-adrenergic system by pharmacological activation may have metabolic benefits.

However, to date, metabolic application has been hindered by limited specificity of β_2-agonists, which harbour significant cross stimulation of cardiac β_1-ARs. Salbutamol, one of the most widely used β_2-agonist for the treatment of airway diseases, lacks potency and selectivity for this purpose. Therapeutic doses do not stimulate resting EE or fat utilization whereas supra-physiological doses invariably cause tachycardia.[7]

Formoterol is a new generation, potent and highly β_2-selective adrenergic agonist, currently approved for the treatment of asthma and chronic obstructive airway disease. Selectivity for β_2- over β_1-ARs is 400:1, compared with only 8:1 in the case for salbutamol.[8] The metabolic effects of formoterol in humans have not been studied.

The aim of the current study was to investigate the effects of formoterol on EE and fat metabolism. We hypothesized that formoterol in therapeutic doses enhances EE and fat utilization without inducing significant adverse cardiovascular effects.

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SUBJECTS AND METHODS

Subjects and study design

This study comprised two parts, a dose-finding study to establish a dose of formoterol required to stimulate energy expenditure and fat oxidation, and a metabolic study investigating the effects of formoterol, using a dose defined in Part 1. Given formoterol has a half-life of $\sim$12 h, we estimated steady state to be reached after 3 days. A study duration of 1 week was chosen for formoterol dosing. Formoterol (Atock) was purchased from Astellas Pharma Inc., Japan. The Research Ethics Committee, St Vincent's Hospital, approved the studies, and all subjects provided written informed consent.

Dose-finding study. This was an open-label step-wise sequential design of weekly administration of 80, 160 and 320 μg daily of formoterol with resting EE, Fox and heart rate measured at baseline and at the end of each dose. Four volunteers (two women) aged 30 ± 4 year (BMI (body mass index): 23 ± 2 kg m^{-2}) were recruited through local advertisement. All were healthy subjects and were not taking any medications, other than the oral contraceptive pill by both female volunteers. Volunteers were studied on four occasions, each separated by 1 week (that is, baseline and following escalating dose of formoterol). During each visit, subjects underwent heart rate, blood pressure, body weight and resting indirect calorimetric measurements, as detailed under Clinical Protocol.

Metabolic study. This was an open-label design quantifying changes in EE, Fox and protein metabolism following 1-week treatment with formoterol at a daily dose pre-determined from study 1. Eight men aged 31 ± 2 year (BMI: 24 ± 1 kg m^{-2}) were recruited through local advertisement. All subjects were in good health with no chronic medical conditions and receiving no regular medications. Volunteers were studied on two occasions, separated by 1 week (that is, baseline and following chosen dose of formoterol). In addition to the procedures conducted in the dose-finding study, volunteers also underwent meal study and blood sampling for biochemical analysis, as detailed under Clinical Protocol.

Clinical protocol

Subjects attended the Clinical Research Facility, Garvan Institute of Medical Research, Sydney, Australia, at 0800 hours after an overnight fast. After changing into a hospital gown, body weight was measured on electronic scale. BMI was calculated as body weight in kilograms divided by the square of height in metres. Blood pressure and heart rate were measured in the supine position after 20 min of recumbence (Omron HEM-780 blood pressure monitor, Omron Health-care, Lake Forest, IL, USA). Mean arterial pressure was calculated as previously described.[9]

Indirect calorimetry. EE and substrate utilization were quantified by indirect calorimetry. This was an open circuit, ventilated hood system (Parvo Medics' TrueOne 2400, ParvoMedics, East Sandy, UT, USA). The monitor was calibrated against standard gases before each study. Subjects rested in a supine position for 30 min before measurement commenced. Gas exchange measurements were recorded continuously for 30 min with the first 5 min discarded in final analysis. Resting EE and rates of substrate oxidation were calculated, as previously described,[10] using the equations of Ferrannini [11]: EE (kcal per day) = $(3.91 \times VO_2/1000 + 1.1 \times VCO_2/1000 - 3.34 \times 0.14 \times \text{body weight}) \times 1440$, G (mg min^{-1}) = $(4.55 \times VCO_2/1000 - 3.21 \times VO_2/1000 - 2.87 \times 0.14 \times \text{body weight}/1440) \times 1000$ and L (mg min^{-1}) = $(1.67 \times VO_2/1000 - 1.67 \times VCO_2/1000 - 1.92 \times 0.14 \times \text{body weight}/1440) \times 1000$, where VO_2 is oxygen consumption in ml min^{-1}, VCO_2 is carbon dioxide production in ml min^{-1}, G is carbohydrate oxidation and L is lipid oxidation. The mean day-to-day intrasubject CV for resting EE at our institute is $\sim$4%.[12] Diet-induced thermogenesis (DIT) was quantified by measuring the increase in EE over the 120 min period after a standardized meal (250 ml Ensure: 14.8% protein, 57% carbohydrate, 28.2% fat) at 0, 60 and 120 min.

Biochemical variables. Plasma glucose was determined immediately by the glucose oxidase method using a YSI glucose analyser (model 2300 STAT PLUS 230V, YSI, Inc., Yellow Springs, OH, USA). Non-esterified fatty acids (NEFAs) were determined spectrophotometrically at 550 nm, by enzymatic colorimetry (Wako, Inc., Osaka, Japan). Inter- and intraassay CVs were less than 10% for these assays in our laboratory.

Participants attended the Clinical Research Facility, Garvan Institute of Medical Research at 0800 hours after an overnight fast. An intravenous cannula was inserted into the arm for blood sampling. Participants rested for 20 min before baseline blood samples were collected. Blood samples were placed on ice, plasma separated and stored at −80 °C until analysis.

Statistical analysis

Statistical analysis was undertaken using the statistical software package SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). Data are expressed as mean ± s.e. of the mean for normally distributed continuous variables. The post-prandial incremental area under the curve was calculated by the trapezoidal method, inclusive of the basal period, by subtracting baseline values extrapolated over 120 min from the total post-prandial area. Comparisons between results in dose-finding study were performed using one-way analysis of variance with Bonferroni's correction. Results in metabolic study were analysed using the paired *t*-test. Data that were not normally distributed were log transformed before statistical analysis to achieve a normal distribution but are presented in the text non-transformed. A P value <0.05 was considered statistically significant.

RESULTS

Dose-finding study

Formoterol at daily doses of 80, 160 and 320 μg increased resting EE by 13 ± 2%, 17 ± 3% and 14 ± 1%, respectively, as shown in Figure 1a. There was a commensurate increase in the rate of Fox by 37 ± 13%, 23 ± 8% and 21 ± 12%, as reflected by the reduction in the respiratory quotient (Figure 1b). The mean heart rate (HR) at baseline was 66 ± 2, and increased slightly in a dose-dependent manner, approaching statistical significance with the highest 320 μg dose ($P = 0.05$) of 80 ± 2 b.p.m. (Figure 1c). No subjects developed tachycardia (>100 b.p.m.). The mean arterial pressure did not change during the course of the study (Figure 1d).

Formoterol 160 μg daily was chosen as the dose for the metabolic study, because it increased resting EE and Fox without inducing significant HR elevation.

Metabolic study

EE and substrate metabolism. A daily 160 μg dose of Formoterol significantly increased ($P<0.01$) resting EE by 13 ± 2% (Figure 2a) and decreased respiratory quotient by 5 ± 1% (Figure 2b). Rate of Fox increased by 23 ± 4%. It increased fasting plasma NEFA concentration by 16 ± 2% but did not significantly change fasting blood glucose concentration (4.5 ± 0.1 vs 4.6 ± 0.1 mmol l^{-1}, $P = 0.4$) or the rate of carbohydrate oxidation (103 ± 12 vs 109 ± 12 mmol l^{-1}, $P = 0.4$). There was a positive correlation between Fox and plasma NEFA both at baseline ($R^2 = 0.81$, $P<0.01$) and after formoterol treatment ($R^2 = 0.78$, $P<0.01$).

This dose of formoterol did not change mean basal HR (67 ± 2 vs 72 ± 3 b.p.m., $P = 0.12$), confirming the observation in Part 1.

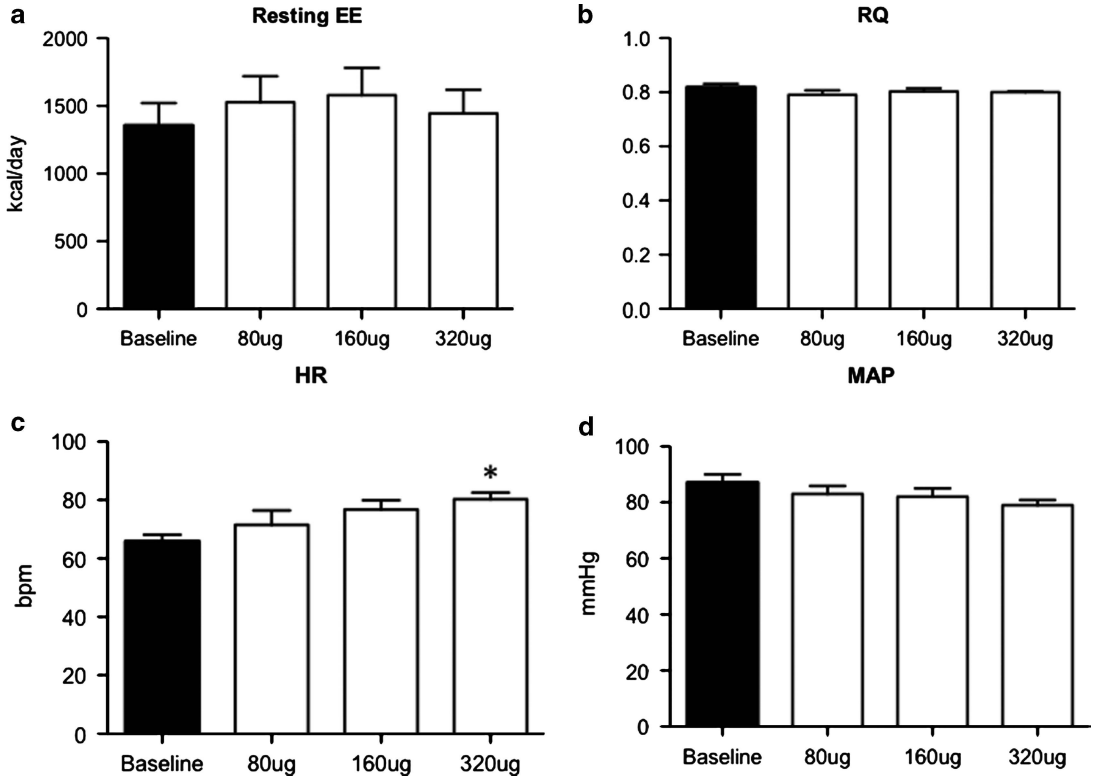

Figure 1. Changes in (**a**) resting EE, (**b**) respiratory quotient (RQ), (**c**) heart rate (HR) and (**d**) mean arterial pressure (MAP) at increasing doses of formoterol in dose-finding study. *$P<0.05$.

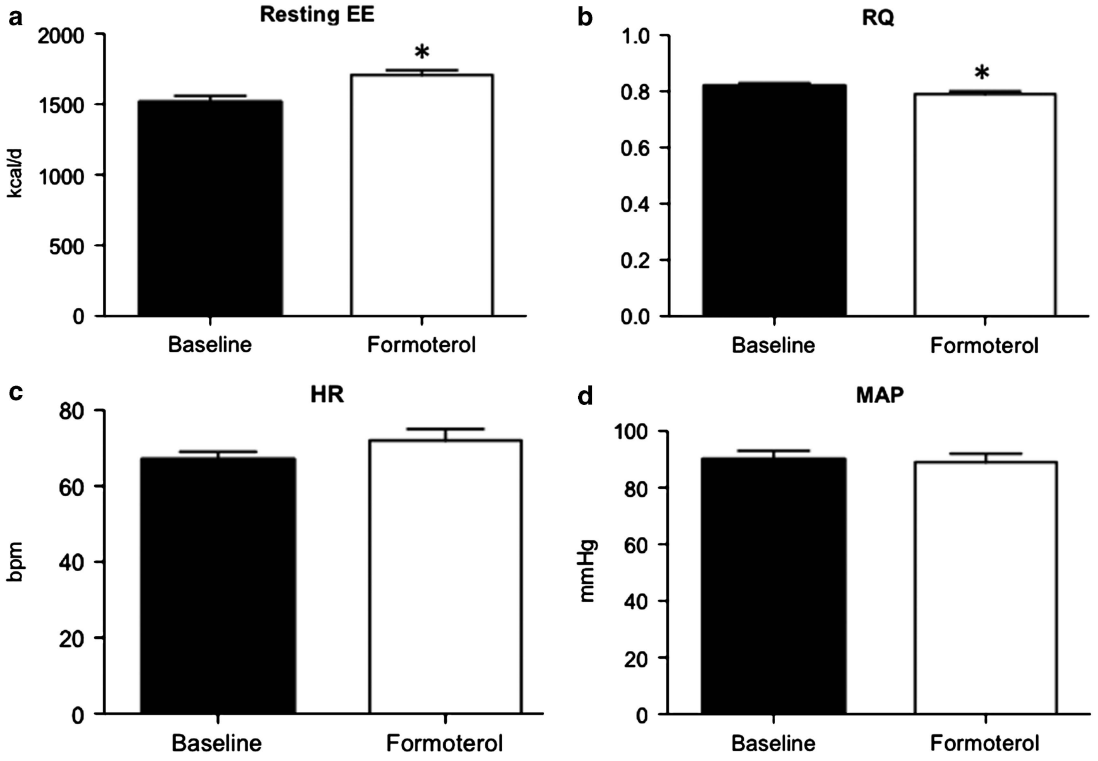

Figure 2. Changes in (**a**) resting EE, (**b**) respiratory quotient (RQ), (**c**) heart rate (HR) and (**d**) mean arterial pressure (MAP) in the fasting state before and after 1 week of formoterol (160 ug per day) treatment. *$P<0.05$.

Diet-induced thermogenesis. Before formoterol treatment, the maximal increase in EE after a standardized meal over the fasting state was $16\pm3\%$. The standardized meal also induced a fall in the rate of Fox of $13\pm8\%$ ($P<0.01$) and an increase in the rate of Cox of $114\pm36\%$ ($P<0.01$).

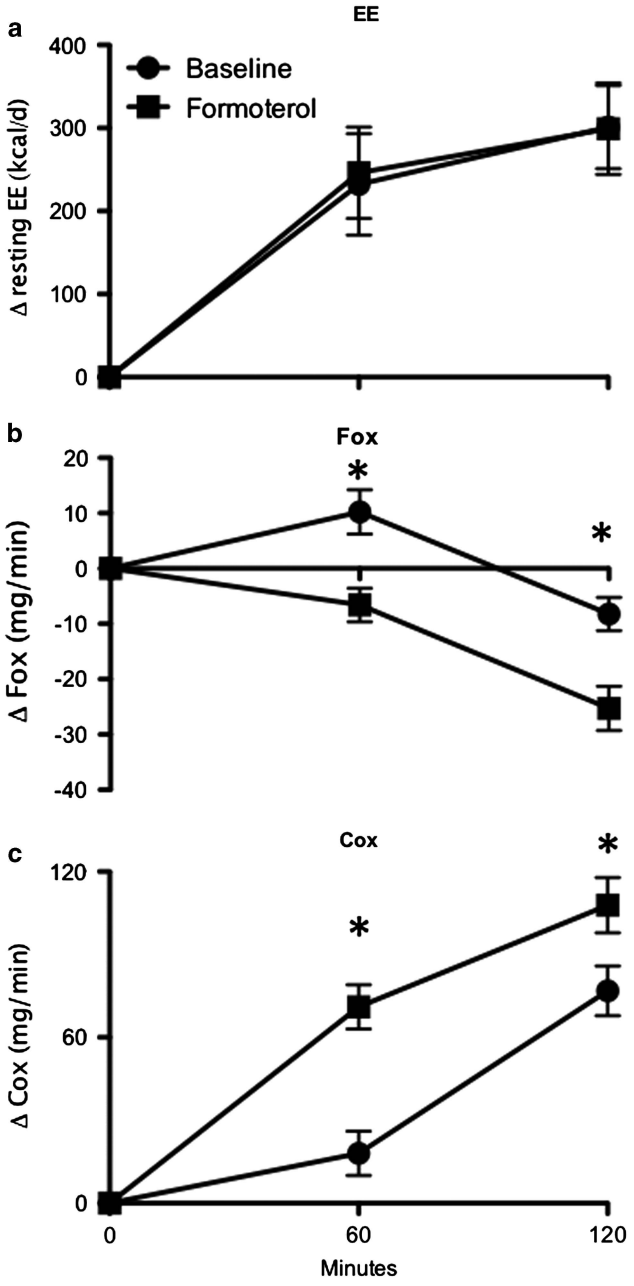

Figure 3. Changes in (**a**) EE, (**b**) Fox, (**c**) carbohydrate oxidation (Cox) following a standardized meal before (circles) and after (squares) 1 week of formoterol (160 ug per day) treatment. *$P<0.05$.

DIT was not significantly increased by formoterol treatment ($14\pm2\%$ vs $16\pm3\%$, $P=0.4$) (Figure 3a). The fall in Fox induced by the standardised meal was greater following formoterol treatment ($16\pm9\%$ vs $38\pm10\%$, $P<0.05$) (Figure 3b). In contrast, the increase in Cox induced by the mixed meal was enhanced by $60\pm17\%$ ($P<0.001$) and $23\pm16\%$ ($P=0.04$), at 60 and 120 min during formoterol treatment (Figure 3c). The mean post-prandial blood glucose level at 120 min was not different before and after formoterol treatment (5.6 ± 0.3 vs 5.9 ± 0.2 mmol l^{-1}, $P=0.2$).

Side effects. Formoterol was well tolerated in both the dose-finding study and the metabolic study. Overall tolerability score was 8 ± 1 (1 to 10 from least to most tolerable). All participants completed the study. In the metabolic study, six subjects reported tremor and palpitation, with severity scores (1 to 10 with increasing severity) of 3 ± 1 and 2 ± 1, respectively. The tremor did not stop volunteers from performing usual daily activities. Palpitations were self-limiting, typically occurring on the first day and not associated with any other symptoms. Two subjects reported loss of appetite and reduced food intake. One subject reported insomnia. Both rated as mild with scores at 1–2/10. No subjects experienced tachycardia (HR > 100 b.p.m.) and the peak heart rate ranged from 61 to 84 b.p.m.

DISCUSSION

This is the first study investigating the metabolic effects of formoterol, at therapeutic dosages of 80, 160 and 320 ug, which are those used in the treatment of asthma and airway conditions. In a dose-finding study, all three doses increased resting EE and Fox without a dose-response relationship. Only the highest dose caused a mild but significant increase in basal heart rate. In the detailed metabolic study, the effects of 160 ug formoterol on fasting resting EE and Fox were replicated. A standardized mixed meal increased EE, Cox and reduced Fox. Although formoterol did not increase DIT, it further increased Cox but suppressed Fox post-prandially. Formoterol did not change fasting or post-prandial glucose status. Formoterol was well tolerated and did not increase basal heart rate significantly.

Despite the potential metabolic benefits of β_2-agonists,[13] systematic metabolic evaluations of commonly prescribed inhaled or oral β_2-agonist have not been undertaken in humans. All previous studies in healthy volunteers investigating the impact of β-agonists on energy and fat metabolism have utilized non-physiological, i.v./s.c. formulations.[7,14–21] Adrenaline, a non-selective β-agonist, increases EE, lipolysis and Fox in humans.[22] But as a non-selective agonist, its cardiovascular effects on heart rate and blood pressure limit its clinical utility. Salbutamol, a widely used β_2-agonist for the treatment of airway diseases, holds potential promise for metabolic exploitation. However, it lacks potency and selectivity for this purpose. Doses required to stimulate resting EE and Fox, $\sim$3–4 times higher than that used for asthma, invariably cause tachycardia.[7]

The main metabolic effects of formoterol were the stimulation of EE and fat utilization. The rise in EE cannot be explained by an increase in myocardial oxygen consumption. The myocardial contribution to EE, estimated by the rate pressure product method,[23] was 2%, 4% and 5% at the three doses, used in the dose-finding study, respectively, as compared with 13–17% with these doses (Figure 1a). The increase in resting EE was accompanied by a stimulation of Fox and not Cox, indicating a selective contribution of increased fat utilization. This was supported by an increase in the appearance of NEFA in plasma after formoterol treatment. Rate of Fox correlated positively with plasma NEFA concentration, suggesting enhanced utilization of fatty acids from augmented lipolysis by formoterol. After a mixed meal, formoterol did not affect DIT but increased the carbohydrate contribution to it, at the expense of further suppression of Fox. Thus, the observation indicates complex effects on resting, post-prandial EE and the underlying components of substrate metabolism, favoring glucose utilization during a meal.

Results arising from the current study may be clinically relevant. With the assumption of steady substrate oxidation rate in the fasting state (8 h per day), an increase in basal Fox rate by 16 mg min^{-1} (Figure 2) could translate into a loss of 2.8 kg of fat, after 1 year of formoterol therapy, assuming no change in energy intake and habitual activity. Weight loss as little as 5% of body weight can improve many obesity-related metabolic conditions. These considerations support the therapeutic potential of formoterol in the management of obesity.

Primary safety concern of formoterol relates to potential cardiac adverse effects. The current study was conducted with careful instructions provided for volunteers to report any cardiac

symptoms during the course of treatment. No subjects developed tachycardia or significant increase in blood pressure. It is possible the lack of significant heart rate increase was a type 2 error. However, the peak heart rate observed at 84 b.p.m. was well-within normality. All subjects rated treatment as well tolerated. In respiratory clinical studies, significant cardiovascular effects have not been reported in patients treated with therapeutic doses of formoterol up to 240 ug daily.[8] The safety of this dosage (160 ug daily, $\sim 2\,\mathrm{ug\,kg^{-1}}$ per day) is further supported by animal studies which reveal no measurable cardiac abnormalities at formoterol doses as high as of $25\,\mu\mathrm{g\,kg^{-1}}$ per day.[24] In addition, β_2-agonism may reduce glucose uptake in muscle, leading to hyperglycaemia.[25] Inhaled formoterol causes transient increase in plasma glucose concentrations in healthy volunteers.[26] Neither fasting nor post-prandial blood glucose levels rose significantly during formoterol treatment in the current study. Future evaluations should consider including measurements of parameters in glucose homoeostasis and biomarkers of insulin sensitivity. Other side effects reported in the current study also include tremor, insomnia and loss of appetite, consistent with the stimulatory effects of β-agonists. Evaluation of food intake, satiety, hunger and physical activity should be undertaken in future studies as sympathetic agonism may impart changes in nutrient sensing, appetite control and activity level. Collectively, the potential metabolic benefits of formoterol have to be balanced against its adverse effects and should be investigated systematically in future studies.

There are several limitations in our study. The sample size is small and limited to only men. It is not blinded and of only a short duration. Receptor desensitisation may occur following chronic sympathetic stimulation.[27] Sustainability of enhanced fat utilization will require confirmation in longer-term studies and in a larger number of subjects. If sustained, evaluation of body composition is required to assess long-term metabolic benefits. The current study does not identify the major tissue sites that contribute to the metabolic changes. Based on animal studies, there is strong evidence that skeletal muscle and adipose tissue are primary targeted organs.[28] It is also possible that formoterol stimulates brown adipose tissue, given the presence of β_2-adrenoceptors in human brown adipose tissue.[3] On the basis of metabolic imaging studies using Positron Emission Tomography, adult humans possess up to 100 g of brown adipose tissue, which can amount to 10% of resting EE,[29] not dissimilar to the observed EE augmentation by formoterol in the current study. In addition, given the abundance of β_2-ARs in skeletal muscle, formoterol may also impart metabolic changes in muscle, augmenting EE and substrate utilization observed on a whole body level. Future studies utilizing tracer techniques could evaluate the impact of formoterol on protein metabolism, and if significant, the therapeutic potential of formoterol extends to the treatment of muscle-wasting disorders and frailty.

In summary, formoterol 160 μg per day increases resting EE and fat utilization in healthy humans. It is well tolerated without inducing tachycardia. From this first proof-of-principle study in humans, we conclude that formoterol stimulates energy metabolism and may induce fat loss. Formoterol may be useful in the management of human obesity.

CONFLICT OF INTEREST

Dr Paul Lee was funded by an Australian National Health Medical Research Council postgraduate scholarship. The other authors declare no conflict of interest.

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