Eur J Appl Physiol (2017) 117:1907–1915
DOI 10.1007/s00421-017-3679-9

ORIGINAL ARTICLE

Beta$_2$-adrenergic stimulation increases energy expenditure at rest, but not during submaximal exercise in active overweight men

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Received: 4 March 2017 / Accepted: 5 July 2017 / Published online: 12 July 2017

Abstract
Purpose β_2-Agonists have been proposed as weight-loss treatment, because they elevate energy expenditure. However, it is unknown what effect β_2-agonists have on energy expenditure in overweight individuals. Furthermore, the influence of β_2-agonist R- and S-enantiomer ratio for the increased energy expenditure is insufficiently explored.
Methods Nineteen males were included in the study of which 14 completed. Subjects were 31.6 (±3.5) years [mean (±95% CI)] and had a fat percentage of 22.7 (±2.1)%. On separate days, subjects received either placebo or inhaled racemic (*rac*-) formoterol (2 × 27 µg). After an overnight fast, energy expenditure and substrate oxidation were estimated by indirect calorimetry at rest and during submaximal exercise. Plasma (*R*,*R*)- and (*S*,*S*)-formoterol enantiomer levels were measured by ultra-performance liquid chromatograph–mass spectrometry.
Results At rest, energy expenditure and fat oxidation were 12% ($P \leq 0.001$) and 38% ($P = 0.006$) higher for *rac*-formoterol than placebo. Systemic (*R*,*R*):(*S*,*S*) formoterol ratio was correlated with change in energy expenditure at rest in response to *rac*-formoterol ($r = 0.63$, $P = 0.028$), whereas no association was observed between fat percentage and *rac*-formoterol-induced change in energy expenditure. During exercise, energy expenditure was not different between treatments, although carbohydrate oxidation was 15% higher ($P = 0.021$) for *rac*-formoterol than placebo. *Rac*-formoterol-induced shift in substrate choice from rest to exercise was related to plasma ln-*rac*-formoterol concentrations ($r = 0.75$, $P = 0.005$).
Conclusion Selective β_2-adrenoceptor agonism effectively increases metabolic rate and fat oxidation in overweight individuals. The potential for weight loss induced by β_2-agonists may be greater for *R*-enantiopure formulations.

Keywords Sympathomimetics · Substrate choice · Adrenergic · Energy consumption · Respiratory exchange ratio

Abbreviations

BW	Body weight
β_2-agonist	Beta$_2$-adrenoceptor agonist
CI	Confidence interval
DXA	Dual-energy X-ray absorptiometry
EE	Energy expenditure
$\dot{V}O_{2max}$	Maximal oxygen uptake
RER	Respiratory exchange ratio
MDL	Method detection limit
RSD	Relative standard deviation
SD	Standardized mean difference
UPLC–MS/MS	Ultra-performance liquid chromatography–mass spectrometer

Introduction

The prevalence of obesity is increasing worldwide accompanied by several diseases such as diabetes and

Communicated by Jean-René Lacour.

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hypertension (Ng et al. 2014). One potential approach to combat obesity is to target β_2-adrenoceptors with selective agonists. While the main clinical application of β_2-adrenoceptor agonists (β_2-agonists) is to relieve the bronchoconstriction associated with respiratory disease, animal studies have provided evidence for β_2-agonists as repartitioning agents, since chronic treatment elicits muscle hypertrophy and reduces fat mass (Emery et al. 1984; Hulot et al. 1996; Ryall et al. 2006). Likewise, Hostrup et al. (2015) demonstrated equivalent action of chronic β_2-adrenergic stimulation in humans observing 1.4 kg of fat loss after 4 weeks of daily treatment with oral terbutaline complemented by an elevation of lean body mass by 1.7 kg. However, while the potential of β_2-adrenergic stimulation in the fight against obesity is palpable, chronic use of β_2-agonists may be associated with adverse effects. Chronic high-dose treatment with older generation β_2-agonists such as terbutaline and clenbuterol demonstrate functionally impairing hypertrophy and collagenous infiltrations in heart muscle of animals due to unwanted activation of β_1-adrenoceptors (Jeppsson et al. 1986; Gregorevic et al. 2005; Lynch and Ryall 2008). Nevertheless, with the introduction of the newer generation β_2-agonists with superior affinity and selectivity for the β_2-adrenoceptor as compared to the common short-acting β_2-agonists, salbutamol and terbutaline (Baker 2010), this adverse effect is less pronounced.

The new generation long acting β_2-agonist formoterol has shown some potential as a body weight-reducing agent. Lee et al. (2013) observed a 13% increase in energy expenditure following oral ingestion of 96 µg formoterol in lean young men. However, it is unknown what effect formoterol elicits in overweight subjects. It has been reported that the increase in plasma glycerol, a marker of adipose tissue lipolysis, following treatment with salbutamol is lower in obese compared to lean subjects (Blaak et al. 2004). Furthermore, Schiffelers et al. (2001) observed a dampened increase in energy expenditure upon non-selective β-adrenergic stimulation with isoprenaline at rest in obese compared to lean subjects. β_2-adrenergic stimulation has been demonstrated to lower the respiratory exchange ratio (RER) at rest in lean subjects, indicating an elevated fat oxidation (Lee et al. 2013). On the other hand, Kalsen et al. (2014) observed a higher RER in lean subjects upon β_2-adrenergic stimulation with terbutaline during exercise at an intensity corresponding to 70% of maximal oxygen consumption ($\dot{V}O_{2max}$). These findings indicate a transition from increased fat oxidation at rest to an increased carbohydrate oxidation during exercise upon β_2-adrenergic stimulation. However, this warrants further investigation.

β_2-agonists are normally administered as (1:1) racemic (*rac*) mixtures of *R*- and *S*-enantiomers with stereoselective behavior (Jacobson et al. 2015, 2016). The *R*-enantiomers of β_2-agonists salbutamol and formoterol exhibit activity, while the *S*-enantiomers generally are considered pharmacologically inert at the β_2-adrenoceptor (Källström et al. 1996; Schmidt et al. 2000). Accordingly, *R*-enantiopure formulations of β_2-agonists may be superior agents for weight loss than racemic. However, data are scant regarding the influence of the systemic *R*:*S* ratios on energy expenditure changes induced by β_2-agonists in humans.

The purpose of the present study was, therefore, to examine the effect of β_2-adrenergic stimulation on energy expenditure and substrate oxidation mediated by the selective agonist *rac*-formoterol in inhaled therapeutic doses at rest and during exercise in active overweight men. A secondary purpose was to investigate the relation between (*R*,*R*):(*S*,*S*)-formoterol ratio and change in energy expenditure induced by *rac*-formoterol.

Materials and methods

Subjects and ethics approval

Nineteen recreationally active overweight [body mass index (BMI) of 25–36 kg × $(m^2)^{-1}$] men (21–43 years of age) volunteered to participate in the study. Subjects were non-smokers, had no chronic disease or known allergy towards medication, and did not use β_2-agonist or other prescription medication. Before inclusion in the study, subjects met for a screening. The screening was conducted in the morning after an overnight fast and consisted of a medical examination with measurements of resting blood pressures and heart rate, electrocardiography, and body composition in a dual-energy X-ray absorptiometry (DXA) scanner. Furthermore, $\dot{V}O_{2max}$ and performance were measured during an incremental exercise test to exhaustion on a bike ergometer. Subjects were informed about risks and discomforts related to the different tests and procedures of the study. Each subject gave written and oral informed consent prior to inclusion in the study. The study was approved by the ethics committee of Copenhagen (H-1-2012-090) and performed in accordance with the Helsinki II declaration.

Of the 19 subjects that were screened, 14 completed the study. Five subjects dropped out of the study either because of discomfort with blood sampling ($n = 2$), personal reasons ($n = 2$), or schedule problems ($n = 1$). Characteristics of the 14 subjects who completed the study are presented in Table 1.

Experimental intervention

The intervention consisted of two experimental trials in randomized order. At each of the trials, subjects met in

Table 1 Subject characteristics

Parameter	Mean (±95% CI)
Age (years)	31.6 (±3.5)
Height (cm)	180.4 (±2.8)
Weight (kg)	92.3 (±5.1)
Body mass index [kg $(m^2)^{-1}$]	28.5 (±1.9)
Body fat percentage (%)	22.7 (±2.1)
$\dot{V}O_{2max}$ (mL kg^{-1} min^{-1})	44.7 (±2.9)

Values are means (±95% CI) (n = 14)

$\dot{V}O_{2max}$, maximal oxygen consumption

the morning at the clinic after an overnight fast. Upon arrival, subjects inhaled either three puffs of 9 µg formoterol (27 µg) (Oxis Turbohaler®, AstraZeneca, London, UK) or placebo (three puffs from identically looking placebo Turbohaler®, AstraZeneca, London, UK) during supervision and a catheter was inserted in the antecubital vein for blood sampling. Subjects then rested on a bed for 45 min. Thirty minutes into the resting period, after which inhaled formoterol had reached its peak systemic concentrations (Derks et al. 1997), indirect calorimetry was measured breath-by-breath with a gas analyzing system (JAEGER MasterScreen CPX; Viasys Healthcare GmbH, Hoechberg, DE) for 15 min for determination of energy expenditure and oxidation of fat and carbohydrates. Furthermore, a blood sample (9 mL) was drawn and resting heart rate and blood pressure were determined (Omron M7, Omron Healthcare Europe, The Netherlands). Hereafter, subjects inhaled another three puffs of 9 µg formoterol or placebo. Thirty min after administration of the second dose, subjects performed steady-state submaximal cycling at an intensity corresponding to 30% of $\dot{V}O_{2max}$ (55 ± 7 W) (mean ± 95% CI) for 8 min and at 50% (105 ± 9 W) of $\dot{V}O_{2max}$ for 4 min. Indirect calorimetry was measured breath-by-breath during the exercise for determination of energy expenditure and oxidation of fat and carbohydrates. At the end of exercise, a blood sample (9 mL) was drawn.

The two experimental trials were separated by at least 3 days to ensure complete wash-out of formoterol (elimination half-life of formoterol ~10 h) (Lecaillon et al. 1999). Throughout the study, subjects were instructed to maintain their regular level of physical activity and to abstain from caffeine, alcohol, and strenuous exercise 48 h prior to each experimental trial.

Stimulation of β_2-adrenoceptors

β_2-Adrenoceptors were stimulated with the highly selective β_2-agonist formoterol, which has a long duration of action (Baker 2010; Löfdahl and Svedmyr 1989). We followed the guidelines of inhalation of formoterol, which advises to administer maximally 27 µg at one time. As such, we administered 2 × 27 µg formoterol by inhalation separated by an hour. Formoterol or placebo was inhaled from identically looking Turbohalers. Drug administration was administered in a double-blinded manner. Inhalation technique was thoroughly practiced at the screening visit and all subjects were familiar with the side effects of formoterol.

Dual-energy X-ray absorptiometry

Subjects' whole-body lean mass and fat percentage were determined by dual-energy X-ray absorptiometry (Lunar DPX-IQ, Version 4.7 E, Lunar Corporation, Madison, WI, USA). Subjects were placed in the scanner in supine position undressed and euhydrated for 20 min before the scan. To reduce variation, two scans at medium speed were performed according to the manufacturer's guidelines using the medium research analysis software. The scanner was calibrated before scan, using daily calibration procedures (Lunar "System Quality Assurance"). All scans were conducted by the same hospital technician.

Maximal oxygen consumption

$\dot{V}O_{2max}$ was measured breath-by-breath with a gas analyzing system during an incremental bike ergometer test to exhaustion starting at 150 W and increasing 30 W every min until pedaling frequency fell below 70 rpm for more than 5 s (Monark 839E, Vansbro, SE). Criteria for achievement of $\dot{V}O_{2max}$ were a respiratory exchange ratio above 1.10 or no further increase in oxygen uptake despite an increased power output. $\dot{V}O_{2max}$ was defined as the highest value averaged in any 30 s period. The gas analyzing system was calibrated with a 3-L syringe and with gasses of known O_2 and CO_2 concentrations.

Indirect calorimetry

Energy expenditure of fat and carbohydrates was quantified using indirect calorimetry as previously described (Lee et al. 2013). $\dot{V}O_2$ and $\dot{V}CO_2$ were measured with a gas analyzing system. Energy expenditure and oxidation of carbohydrates and fat were calculated using the following equations deduced by Ferrannini (1988) as done in Lee et al. (2013): $EE = (3.91 \times \dot{V}O_2 + 1.1 \times \dot{V}CO_2)/1000 - 3.34 \times 0.14 \times BW/1440$, carbohydrate oxidation $= ((4.55 \times \dot{V}CO_2 - 3.21 \times \dot{V}O_2)/1000 - 2.87 \times 0.14 \times BW/1440) \times 1000$, fat oxidation $= ((1.67 \times \dot{V}O_2 - 1.67 \times \dot{V}CO_2)/1000 - 1.92 \times 0.14 \times BW/1440) \times 1000$. where EE is energy expenditure in kcal min^{-1}, carbohydrate oxidation is in mg min^{-1}, fat oxidation is in mg min^{-1}, and BW is body weight in kg.

Analysis of plasma K^+, glucose, and lactate

Venous blood samples were collected in heparinized syringes and analyzed immediately for plasma K^+, glucose, and lactate in a blood gas analyzer ABL 800 Flex (Radiometer, Copenhagen, Denmark). Plasma K^+, glucose, and lactate were determined for 12 subjects as two subjects refused to have a catheter inserted into their antecubital vein on the second study visit.

Analysis of plasma concentrations of formoterol

Enantioselective formoterol analyses were based on our previous work with salbutamol and salmeterol (Jacobson et al. 2014, 2015, 2016) and undertaken using UPLC–MS/MS (ultra-performance liquid chromatography–mass spectrometer; Waters Acquity® H-class UPLC system coupled to a Waters Xevo® triple quadrupole mass spectrometer; Waters Corporation, Milford, MA) with chromatography performed using an Astec® CHIROBIOTIC™ T2 chiral column (Sigma-Aldrich). In brief, calibration samples were prepared by spiking drug-free human plasma with unlabeled racemic formoterol (*rac*-formoterol fumarate dihydrate; Carbosynth, Compton, UK). Internal standard (*rac*-formoterol-D6; Toronto Research Chemicals, Toronto, Canada) equivalent to 5 ng mL^{-1} was added to each calibration and study sample aliquot (400 µL), and then, dilute ammonia solution (150 µL) was added to each and vortex mixed before the addition of 850 µL of HPLC grade ethyl acetate. This was vortex mixed for 1 min, then centrifuged at 15,000*g* for 5 mins. The organic supernatant was then collected, and the ethyl acetate extraction repeated and the two residues combined; solvent was then evaporated under nitrogen at 40 °C and reconstituted using 80 µL of methanol and vortex mixed prior to analysis via UPLC–MS/MS. *Rac*-formoterol levels were calculated by combining levels for both (*R*,*R*)-formoterol and (*S*,*S*)-formoterol enantiomers. The assay met acceptance criteria demonstrating linearity ($r^2 = 0.9998$), precision less than 15% relative standard deviation (%RSD) over the calibration range, and method detection limit of (MDL) of 30 pg mL^{-1} calculated using the *S*/*N* ratio method with MDL defined as *S*/*N* = 10. Because of destruction of one sample, formoterol concentrations were determined for 12 subjects.

Statistics

Statistical analysis was performed in SPSS version 24 (IBM, Armonk, NY, USA). Data were tested for normality using the Shapiro Wilks test and Q–Q plots. Data were normally distributed and are presented as means with the 95% confidence interval (95% CI), apart from plasma concentration of *rac*-formoterol, which was not normally distributed and is presented as median with interquartile range. To estimate differences between treatments, linear mixed modelling was used for each dependent variable with treatment as a fixed factor, and age and weight as confounding time-invariant covariates (White et al. 1994; Cheymol 2000) as well as random intercept for subjects. In case of repeated measures, time was included in the model as a fixed factor with a random slope for subjects. Pearson's product moment analysis was used to estimate correlation patterns. Magnitudes of the main outcome statistics are presented with the standardized mean difference (SD) to represent clinical significance as described by Cohen (1988) and *P* values to represent probability.

Results

Plasma concentrations of rac-formoterol

The median (±interquartile range) plasma concentration of *rac*-formoterol at the end of the last exercise bout during the formoterol-trial was 68 (±62) pg mL^{-1} with inter-individual variation ranging from 38 to 275 pg mL^{-1}.

Systemic effects

Five subjects experienced tachycardia and tremor after inhalation of *rac*-formoterol. Resting heart rate and systolic blood pressure were 10 (±5) bpm and 7 (±2) mmHg higher ($P \leq 0.001$) for formoterol than placebo, respectively, while diastolic blood pressure was 3 (±3) mmHg lower ($P = 0.03$) for *rac*-formoterol than placebo and with no difference in mean arterial pressure between treatments (Table 2). Plasma concentration of K^+ at rest was lower ($P \leq 0.001$) for *rac*-formoterol than placebo, being 3.5 (±0.2) mM for formoterol and 4.0 (±0.1) mM for placebo, whereas plasma glucose and lactate were

Table 2 Heart rate and systolic and diastolic blood pressure

	Formoterol	Placebo
Heart rate (bpm)	69 (±8)**	59 (±4)
Systolic blood pressure (mmHg)	122 (±5)**	116 (±5)
Diastolic blood pressure (mmHg)	66 (±4)*	69 (±4)
Mean arterial pressure (mmHg)	85 (±4)	85 (±4)

* Different from placebo ($P \leq 0.05$)

** Different from placebo ($P \leq 0.001$). Values are means (±95% CI) ($n = 14$)

higher ($P \leq 0.001$) for *rac*-formoterol than placebo, being 5.4 (±0.2) and 5.0 (±0.2) mM, respectively, and 1.4 (±0.3) and 0.8 (±0.1) mM, respectively.

Energy expenditure and oxidation of fat and carbohydrates at rest

At rest, energy expenditure and fat oxidation were 12% (Cohen's SD 1.35, $P < 0.001$) (Fig. 1a) and 38% (Cohen's SD 1.01, $P = 0.006$) (Fig. 1c) higher for *rac*-formoterol than placebo, respectively, whereas no statistical difference was observed between treatments in oxidation of carbohydrates (Cohen's SD 0.47, $P = 0.179$) (Fig. 1d). Resting ventilation was higher ($P = 0.0002$) for formoterol [9.8 (±0.7)] L min^{-1} than placebo [8.5 (±0.6)] L min^{-1}. The systemic (*R*,*R*):(*S*,*S*) formoterol enantiomer ratio was significantly correlated with the relative *rac*-formoterol-induced change in energy expenditure at rest compared to placebo [$r = 0.63$ (±0.33), $P = 0.028$] (Fig. 1b), whereas neither fat percentage ($r = 0.11$, $P > 0.50$) nor $\dot{V}O_{2max}$ ($r = 0.04$, $P > 0.50$) predicted any relevant change.

Energy expenditure and oxidation of fat and carbohydrates during exercise

During exercise, energy expenditure was not different between treatments (Cohen's SD 0.34, $P = 0.215$) (Fig. 2a). However, oxidation of fat tended to be lower for *rac*-formoterol than placebo (−13%) (Cohen's SD 0.40, $P = 0.139$) (Fig. 2b), whereas oxidation of carbohydrates was 15% higher for *rac*-formoterol compared to placebo (Cohen's SD 0.64, $P = 0.021$) (Fig. 2c). Ventilation during exercise was higher ($P < 0.0001$) for formoterol [43.2 (±2.9)] L min^{-1} than placebo [38.6 (±3.0)] L min^{-1}. Increase in RER from rest to exercise was higher for *rac*-formoterol than placebo (treatment by time interaction for RER, $P = 0.006$) (Fig. 2d). *Rac*-formoterol-induced change in RER compared with placebo was significantly related

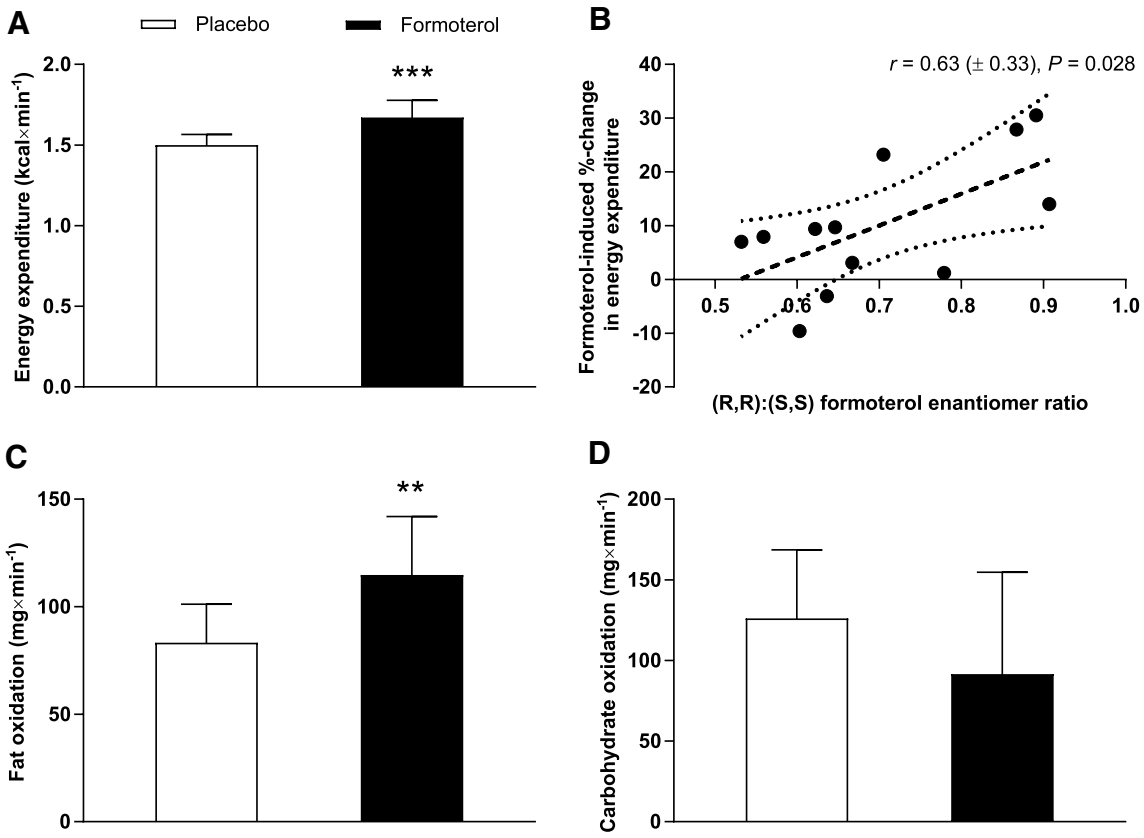

Fig. 1 **a** Mean (±95% CI) ($n = 14$) values of energy expenditure at rest for *rac*-formoterol (*black bars*) and placebo (*white bars*). **b** Bivariate correlation between relative *rac*-formoterol-induced % change in energy expenditure compared with placebo at rest versus the systemic (*R*,*R*):(*S*,*S*) formoterol enantiomer ratio. Individual values ($n = 12$). *Dotted grey lines* show the 95% CI of the *dashed black regression line*. **c**, **d** Mean (±95% CI) ($n = 14$) values of fat and carbohydrate oxidation at rest for formoterol and placebo. **Different from placebo ($P \leq 0.01$). ***Different from placebo ($P \leq 0.001$)

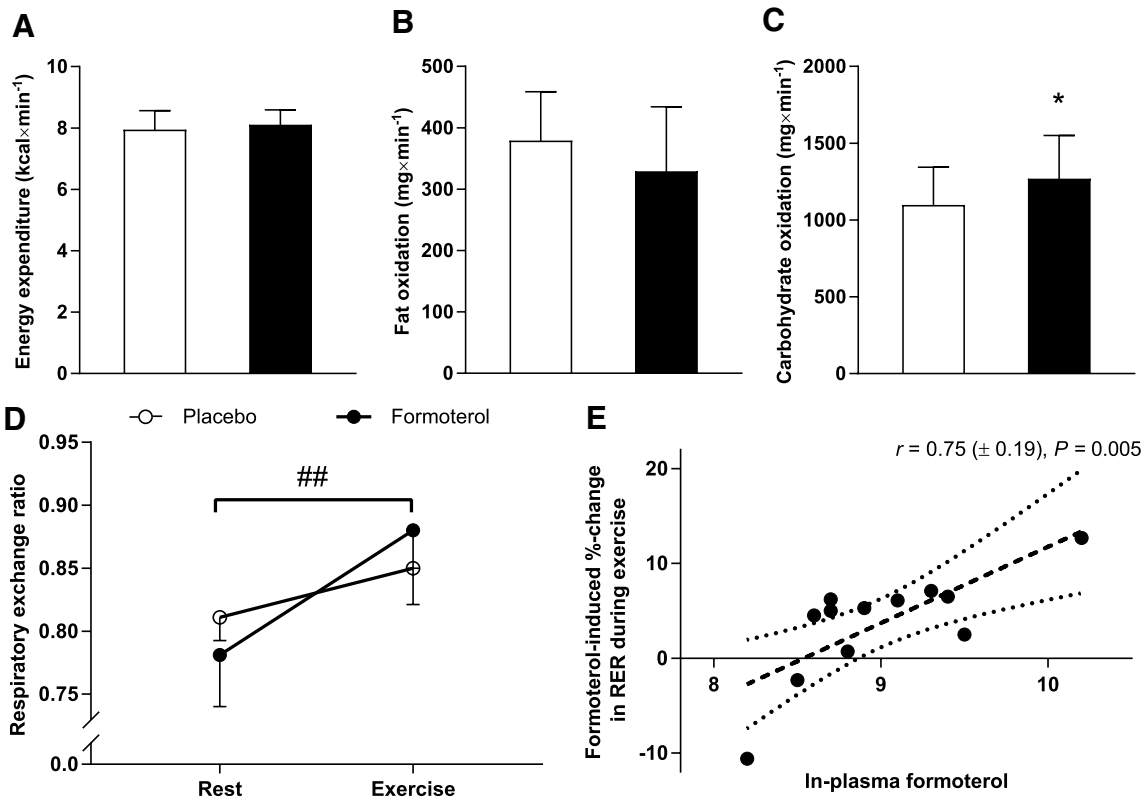

Fig. 2 **a**–**c** Mean (±95% CI) (n = 14) values of energy expenditure (**a**) and oxidation of fat (**b**) and carbohydrates (**c**) during exercise for *rac*-formoterol (*black bars*) and placebo (*white bars*). **d** Mean (±95% CI) (n = 14) respiratory exchange ratio (RER) for *rac*-formoterol and placebo at rest and during exercise. **e** Bivariate correlation between relative *rac*-formoterol-induced % change in RER versus the natural log (ln) plasma concentration of *rac*-formoterol during exercise. Individual values (n = 12). *Dotted grey lines* indicate the 95% CI of the *dashed black regression line*. *Different from placebo ($P \leq 0.05$). #Significant ($P \leq 0.01$) interaction (treatment by time)

to plasma ln-*rac*-formoterol during exercise [r = 0.75 (±0.19), P = 0.005] (Fig. 2e).

Discussion

The novel findings of the present study were that β_2-adrenergic stimulation mediated by the selective agonist *rac*-formoterol in inhaled therapeutic doses increased resting energy expenditure in active overweight men. During exercise, on the other hand, *rac*-formoterol did not increase energy expenditure or oxidation of fat, but increased oxidation of carbohydrates. Furthermore, the present study indicates that neither fat percentage nor $\dot{V}O_{2max}$ is predictive of the *rac*-formoterol-induced increase in energy expenditure at rest, but is rather related to the systemic (*R*,*R*):(*S*,*S*) formoterol enantiomer ratio.

The application of selective β_2-adrenoceptor agonists as weight-loss treatment has been suggested by several authors (Lund and Gillum 2016; Astrup 1995), since chronic treatment with β_2-adrenoceptor agonists effectively increases thermogenesis and reduces fat mass in a variety of mammalian species, including humans (Hostrup et al. 2015; Lynch and Ryall 2008; Lee et al. 2013). However, the doses shown to reduce fat mass in previous studies were supratherapeutic and may, therefore, given side effects associated with long-term high-dose treatment (Burniston et al. 2006), not be feasible in a clinical setting. In the present study, we observed that β_2-adrenergic stimulation mediated by low-dose and therapeutic inhalation of *rac*-formoterol (27 µg) elevated resting energy expenditure by 12% active overweight men, which is on par with that observed by Lee et al. (2013) after oral administration of *rac*-formoterol in markedly higher doses (160 µg) in healthy lean subjects. The similarity in response between the markedly lower doses of *rac*-formoterol administered in the present study as compared to Lee et al. (2013) is most likely related to the higher systemic bioavailability of the inhaled route of administration compared to the oral route. The systemic bioavailability ratio between inhaled and oral administration of β_2-agonists has been shown to be approximately 4:1 (Elers et al. 2012; Dyreborg et al. 2016). Although we observed no significant correlation between the ln-plasma

concentration of *rac*-formoterol and energy expenditure ($r = 0.39$, $P = 0.21$, data not shown), we found that the (*R*,*R*):(*S*,*S*) formoterol enantiomer ratio could predict 40% of the response (Fig. 1b). This finding is consistent with the observation that the activity of *rac*-formoterol resides with the (*R*,*R*)-enantiomer, which has been shown to be 1000 times more potent than the (*S*,*S*)-enantiomer (Källström et al. 1996; Schmidt et al. 2000). While it has been speculated that the β_2-adrenergic-induced increase in energy expenditure is lowered in obese individuals (Lee et al. 2013), we observed no association between *rac*-formoterol-induced change in energy expenditure at rest and subjects' percent body fat or fitness level ($\dot{V}O_{2max}$). However, the current study is limited by subjects' fat percentage being located within a narrow interval in the lower end of the overweight spectrum. The association in mention might have emerged with a greater number of subjects with a more diverse fat percentage distribution, but more studies are needed to elucidate that.

The mechanisms underlying the β_2-adrenergic-induced increase in energy expenditure at rest are possibly multifactorial. β_2-adrenoceptors are highly abundant in skeletal muscle and β_2-adrenergic stimulation augments activity of muscle Na^+/K^+ ATPase and Ca^{2+} ATPase (Clausen and Flatman 1980; Hostrup et al. 2014), thereby increasing ATP consumption. In support of the former, we observed that plasma K^+ was lowered by formoterol at rest. β_2-adrenergic stimulation has also been shown to increase leakage of Ca^{2+} from the sarcoplasmic reticulum (Suko et al. 1993), causing involuntary activation of the myosin ATPase. In addition, β_2-adrenergic stimulation induces chronotropy and inotropy of the heart, thus increasing energy consumption of cardiomyocytes. However, although we observed that *rac*-formoterol increased heart rate by 10 beats $\times$ min^{-1}, which is consistent with several reports (Lee et al. 2013; Gross et al. 2008; Whale et al. 2008), such increase would only increase myocardial oxygen consumption by approximately 2.5 $mLO_2 \times min^{-1}$ (Simonsen and Kjekshus 1978), why the cardiac contribution to the formoterol-induced increase in energy expenditure at rest possibly only constitutes a neglectable fraction. Finally, β_2-adrenergic stimulation may activate brown adipose tissue and induce thermogenesis (Cao et al. 2001). As such, Ernande et al. (2016) demonstrated an increased activation of brown adipose tissue upon β_2-adrenergic stimulation in mice, which was mediated through β_2-adrenergically induced increases in perfusion rather than direct activation of brown adipose tissue β_2-adrenoceptors. However, in contrast, Vosselman et al. (2012) found no effect of unselective beta-adrenergic stimulation with isoprenaline on brown adipose tissue activation measured through flourodeoxyglucose uptake, leaving the effect of beta$_2$-adrenergic stimulation on brown adipose tissue unresolved.

Unlike the increased energy expenditure observed at rest upon β_2-adrenergic stimulation, we observed no differences between *rac*-formoterol and placebo during exercise. This might be attributable to exercise-induced release of adrenaline and noradrenaline (Kalsen et al. 2016), thus acting as competitors for formoterol on the β_2-adrenoceptors. In addition, some of the abovementioned β_2-adrenergically activated processes, such as heart muscle and skeletal muscle activity, are also increased with exercise, thus reducing the difference in energy expenditure between placebo and β_2-adrenergic stimulation as the intensity of the exercise increases.

The *rac*-formoterol-induced increase in resting energy expenditure observed in the present study was paralleled by a concurrent increase in oxidation of fat by 38%. The reason for this has still not been determined with certainty, but presented in the following are two possible mechanisms that could contribute to this increase. First, β_2-adrenergic stimulation has been shown to mobilize free fatty acids from white adipose tissue (Hoeks et al. 2003) and activate hormone-sensitive lipase in skeletal muscle (Jocken et al. 2008). The Randle cycle suggests that glucose oxidation is suppressed with increasing levels of plasma free fatty acids via inhibition of pyruvate dehydrogenase and phosphofructokinase activity (Randle et al. 1963; Randle 1964, 1995; Hue et al. 1988). Unfortunately, we did not measure free fatty acids in plasma, which is a limitation of the study. Second, brown adipose tissue is mainly fueled by fat oxidation (Haman et al. 2002; Bakker et al. 2014), why a potential *rac*-formoterol-induced activation of brown adipose tissue thermogenesis also may contribute to the lowered RER observed at rest upon β_2-adrenergic stimulation in the present study. However, more studies are warranted on this matter before anything can be concluded about the role of brown fat in β_2-adrenergically induced increase in energy expenditure.

Notably, we observed that *rac*-formoterol increased fat oxidation at rest, while carbohydrate oxidation was elevated during exercise compared to placebo. The observation that the relative *rac*-formoterol-induced change in RER during exercise was related to the systemic concentrations of *rac*-formoterol may imply that the β_2-adrenergic mediated response in substrate oxidation is dose-dependent. However, it cannot be excluded that the *rac*-formoterol-induced transition towards a higher carbohydrate oxidation during exercise could be attributed to exercise-induced changes in the pharmacokinetics of formoterol. Lee et al. (2013) observed no increase in carbohydrate oxidation at progressively greater dosages of *rac*-formoterol, why it seems unlikely that the higher carbohydrate oxidation observed during exercise with *rac*-formoterol was caused by an increased amount of inhaled formoterol or an exercise-induced change in the pharmacokinetics of

formoterol. It has been demonstrated that β_2-adrenoceptors play a role in regulation of glycogenolysis during exercise via cAMP/PKA-mediated phosphorylation of glycogenolytic and glycolytic enzymes. For instance, stimulation of β_2-adrenoceptors with selective agonist terbutaline was shown to increase rate of glycogenolysis during exercise and carbohydrate oxidation (Kalsen et al. 2014; Hostrup et al. 2014), which was accompanied by an increased RER the first 20 min of the exercise bout (Kalsen et al. 2014). In addition, adrenergic stimulation with adrenaline has been shown to increase the activity of glycogen phosphorylase in rodents (Jensen and Dahl 1995), which provides a possible explanation behind the increased glucose oxidation during exercise upon β_2-adrenergic stimulation observed in the current study.

Conclusions

In conclusion, β_2-adrenergic stimulation with the selective agonist *rac*-formoterol in therapeutic inhaled doses increases resting energy expenditure and fat oxidation in active overweight individuals irrespective of fat percentage and fitness level. The formoterol-induced increase in energy expenditure was partially related to the systemic (*R*,*R*):(*S*,*S*) enantiomer formoterol ratio, which implies that the potential of β_2-agonists, such as formoterol, as weight-loss agents may be greater for *R*-enantiopure formulations, especially in patients with lower *R*,*R*:*S*,*S* ratios. As such, there would be a strong rationale to provide a chiral switch enantiopure formulations rather than racemic mixtures in future intervention trials. Notably, when subjects performed exercise at submaximal intensity, the *rac*-formoterol-induced increase in energy expenditure was blunted and *rac*-formoterol increased fat oxidation at rest, while carbohydrate oxidation was elevated during exercise.

Acknowledgements The authors would like to thank Jens Lund for many fruitful scientific discussions during the writing process and Dr. David Nichols (Central Science Laboratory, University of Tasmania) for conducting the UPLC–MS/MS instrumental analyses.

Author contribution statement JO collected, analyzed, and interpreted the data, and drafted the manuscript. GJ developed and supervised the enantioselective UPLC–MS/MS analytical component of the study, including assay performance, analysis and reporting of results, as well as contributing to the final manuscript. CN undertook the UPLC–MS/MS sample preparation and method UPLC–MS/MS optimization, and reviewed the manuscript. VB was the responsible medical doctor of the study and performed medical examination of the subjects, and contributed to interpretation and review of the manuscript. AK, MK, and SJ conducted the human experiments and contributed to interpretation of the data and in drafting of the manuscript. JB and MH designed the study and contributed to data collection, analysis, interpretation, and in drafting of the manuscript. All authors approved the final version of the manuscript.

Compliance with ethical standards

Funding The study was supported by the Danish Ministry of Culture. The funder had no role in the design of the study, the collection, analysis, and interpretation of the data or the writing of the manuscript.

Conflict of interest The authors declare that they have no conflicts of interest with the contents of this article.

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