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Original Article

The Lipid-lowering Effects of R-bambuterol in Healthy Chinese Volunteers: A Randomized Phase I Clinical Study

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ABSTRACT

Background: Existing treatments are inadequate for patients at high risk of coronary heart disease caused by elevated levels of plasma low-density lipoprotein cholesterol (LDL-C). Bambuterol is a prodrug of β2-agonist commonly used for the treatment of asthma and chronic obstructive pulmonary disease (COPD) with the advantage of once daily dosing and favorable side effect profile. The potential lipid-lowering effects of bambuterol were unclear, possibly due to the racemic bambuterol (rac-bambuterol) that was used in previous studies.

Methods: The lipid-lowering effects of R-bambuterol were examined in a randomized phase I trial in 48 healthy Chinese volunteers aged 18–45 years. Participants were randomly assigned to five groups to receive a single dose (2.5 mg, 5 mg or 10 mg) or multiple doses (5 mg) of oral medications of R-bambuterol, or a single dose of rac-bambuterol (10 mg). Plasma lipid levels were measured at baseline, time to peak concentration (T_{max}) and 24 h after the treatment.

Findings: Administration of a single-dose of R-bambuterol resulted in dose-dependent reductions in the levels of plasma LDL-C, high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA1) at T_{max}. Levels of LDL-C exhibited the most reductions, which were statistically significant in all three single-dose R-bambuterol groups (all P values < 0.05). R-bambuterol was more potent in LDL-C lowering compared to rac-bambuterol at T_{max} ($P = 0.08$). At 24 h after dosing, the significant lipid lowering effects of R-bambuterol sustained for LDL-C ($P = 0.01$), ApoB ($P = 0.001$) and ApoA1 ($P = 0.03$), but not for HDL-C. The ratio of ApoA1/ApoB was marginally increased ($P = 0.06$). In the multiple-dose group, LDL-C levels again were significantly reduced (all P values < 0.05), whereas the ratios of ApoA1/ApoB were marginally increased.

Interpretation: R-bambuterol can lower the plasma levels of LDL-C, and marginally raise the ratio of ApoA1/ApoB (indicator of HDL-C/LDL-C) with both a single dose and multiple doses. R-bambuterol was more potent in LDL-C lowering than rac-bambuterol.

1. Introduction

A high plasma concentration of low-density lipoprotein cholesterol (LDL-C) is one of the major risk factors for coronary heart disease (CHD) (Collaboration CTT, 2010; Reiner et al., 2011). Epidemiological surveys have shown that LDL-C levels were log-linearly correlated with CHD risk in a broad range of cholesterol levels (Grundy et al., 2004). Clinical trials also demonstrated that effective reduction in LDL-C can substantially reduce the risk of CHD (Collaboration CTT, 2010; Anon., 1984; Canner et al., 1986). High potency statins have been used to treat patients with hypercholesterolemia. However, taking statins alone might not always achieve the optimal low level of LDL-C (Kuklina et al., 2009; Karalis et al., 2011). The effect of statins in primary prevention of CHD, i.e. among those with elevated cholesterol levels but without CHD, is also limited (Reiner, 2013). Moreover, 10–20% of the patients taking statins experienced moderate to severe side effects, especially muscle damage (Zhang et al., 2013). It's also known that some patients do not respond to statin treatment. Thus, additional options for the treatment of hypercholesteremia are required.

Bambuterol is a prodrug of the β2-agonist terbutaline. It is commonly prescribed for asthma and chronic obstructive pulmonary disease (COPD) with once daily dosing and documented clinical safety (Olsson and Svensson, 1984; D'Alonzo et al., 1995; Sitar et al., 1993). It has been reported previously that bambuterol increases the level of high-density lipoprotein cholesterol (HDL-C) in patients with hyperlipidemia (FlorÉN et al., 1997) and in patients with type II diabetes mellitus (Bitzén et al., 1993) after six to eight weeks of treatments. However, the cholesterol lowering effect of bambuterol, particularly on the level of LDL-C, was only marginal and inconclusive (FlorÉN et al., 1997; Bitzén et al., 1993). Bambuterol is a chiral drug with R- and S-

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enantiomers (Gazić et al., 2006). The uncertain LDL-C lowering effect of bambuterol in the previous studies might be related to the inefficacy of racemic bambuterol due to the different biological effects of enantiomers (Waldeck, 1993). Animal study clearly suggested a cholesterol lowering effect of R-bambuterol in the treatment of tyloxapol-induced hyperlipidemia in mice (Cheng and Tan, 2009). In addition, the previously inconclusive effects on plasma lipid of bambuterol were shown in long term studies without a demonstration of a dose relationship.

In this study, using single optic pure enantiomers, we examined: (1) dose–response effects of single dose R-bambuterol on plasma lipid levels; (2) the differences of R-bambuterol versus Rac-bambuterol in the effects of the lipid levels; and (3) effects of multiple doses R-bambuterol on the lipid profile in 48 healthy participants of a randomized phase I clinical trial.

2. Methods

2.1. Study Participants

All healthy individuals between the age of 18 and 45 and within the normal range of body mass index (BMI, 19–24) were eligible to participate in the study. Before entering the study, all volunteers were ascertained to be healthy by medical history, physical examination, 12-lead electrocardiography and routine laboratory tests. A total of 48 healthy Chinese volunteers were enrolled. None of the participants used continuous medication such as lipid-lowering drugs or smoked. None of the female participants were taking oral contraceptives or had contraceptive implants. Written consent forms were obtained from all study participants. The study was approved by the ethics committees of 210 Dalian Army Hospital and followed the Good Clinical Practice guidelines and the Declaration of Helsinki.

2.2. Study Design and Procedures

The study was as an open-label, randomized phase I study to assess the pharmacokinetic and safety profiles of R-bambuterol. A sample size of at least 8 healthy volunteers in each group is determined according to the Chinese regulatory requirements, as stipulated by China State Food and Drug Administration (SFDA). All study participants were randomly randomized into five groups using a random number table and assigned to receive single dose or multiple doses of oral medications of R-bambuterol or rac-bambuterol (Key Pharma Ltd., China): (i) single dose of R-bambuterol, 2.5 mg; (ii) single dose of R-bambuterol, 5 mg; (iii) single dose of R-bambuterol, 10 mg; (iv) single dose of rac-bambuterol, 10 mg; (v) and multiple dose of R-bambuterol, 5 mg/day, for 7 days. All medications were identically compressed in tablets.

All study participants stayed in the clinic of the 210 Dalian Army Hospital during the clinical trial. Standard meals were served at designated times during the day. All subjects were fasted overnight for at least 10 h and till 4 h after dosing. Each dose of R- or rac-bambuterol was administrated with 200 mL water. All subjects were asked not to drink more water until 2 h after dosing. For the single dose groups, blood samples were collected at 1 h before dosing and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72 and 96 h after dosing. For the multiple dose group, blood samples were collected at 1 h before dose 4 (day 4) and at 24 h after dose 4 to dose 7 (day 5 to day 8). In addition, blood samples were collected at various time points, i.e. 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24, 48, 72, and 96 h, after dose 7. All laboratory analyses were performed in the South China University of Technology in compliance with Good Laboratory Practice (GLP) guidelines. The trial is registered with China SFDA, number 2010L01319.

2.3. Determination of Drug Concentration

Plasma extraction was performed using a validated method developed in our laboratory. In brief, samples were precipitated by acetonitrile, after which the supernatant was transferred into chromatography vials for UPLC/MS analysis. Albuterol was added into each sample and served as internal standard. R or rac bambuterol was separated on ACQUITY UPLC BEH HILIC column, 2.1 × 100 mm, 1.7 μm, (Waters Inc., MA, US) and analyzed with tandem quadrupole (triple quadrupole) Mass spectrometry Xevo™ TQ-S (Waters Inc.,

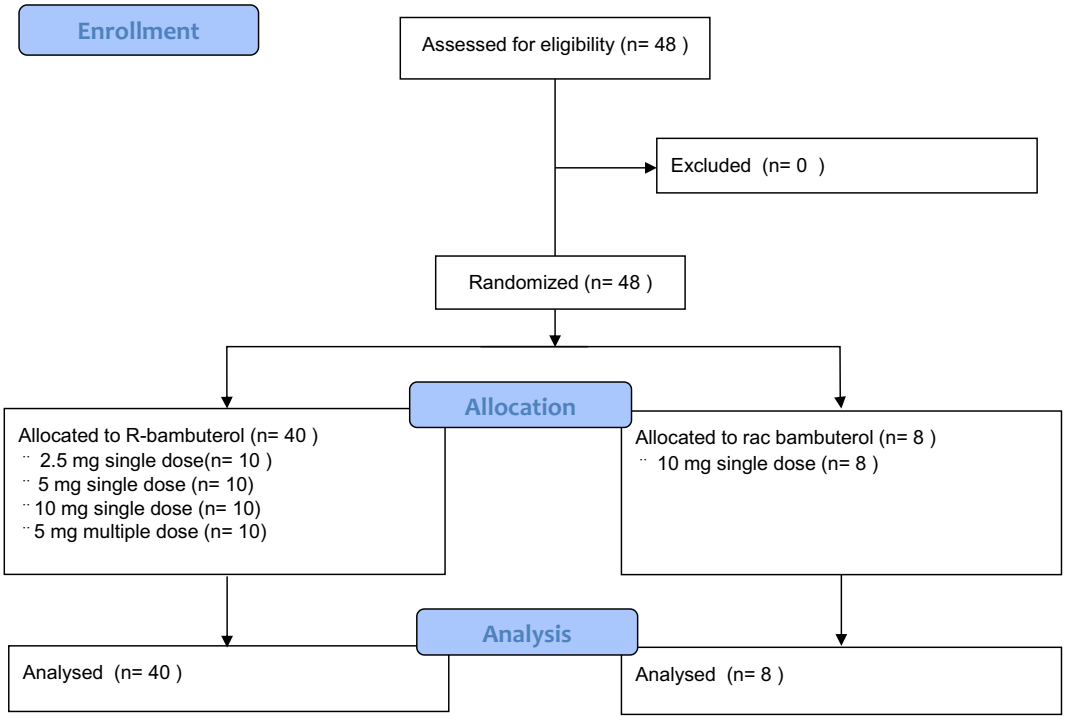

Fig. 1. Study profile.

Table 1
Summary statistics of demographic and baseline characteristics.

	R-bambuterol			Racemic bambuterol
	Single dose			Single dose
	2.5 mg (n = 10)	5.0 mg (n = 10)	10 mg (n = 10)	10 mg (n = 8)
Age (year)	25.1 ± 2.7	22.2 ± 2.5	22.2 ± 3.3	23.1 ± 2.1
Sex				
Male	6	6	6	4
Female	4	4	4	4
BMI (kg/m^2)	21.9 ± 1.6	21.0 ± 1.6	22.0 ± 1.6	21.7 ± 1.9
LDL-C (mmol/L)	2.2 ± 0.8	2.4 ± 0.9	2.3 ± 0.7	2.5 ± 0.8
HDL-C (mmol/L)	1.1 ± 0.2	1.2 ± 0.2	1.1 ± 0.2	1.0 ± 0.2
TC (mmol/L)	3.7 ± 0.8	4.0 ± 0.8	4.1 ± 0.8	3.7 ± 0.8
TG (mmol/L)	1.2 ± 1.0	1.3 ± 0.9	1.8 ± 1.6	1.3 ± 0.9
ApoAI (mmol/L)	1.2 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.1
ApoB (mmol/L)	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.2

* Abbreviations: BMB, bambuterol; BMI indicates body mass index; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein; TC, total cholesterol; TG, triglyceride.
Means ± standard deviation was shown for all continuous variables.

MA, US) by monitoring the response of a specific parent/daughter ion pair (m/z 368.1/294.2).

2.4. Lipid Analysis

LDL-C, total cholesterol (TC), HDL-C and TG were analyzed using enzyme-based colorimetric assay (BioSino Bio-technology and Science Inc., Beijing, China). ApoB and ApoA1 were measured using an immunoturbidimetric assay (BioSino Bio-technology and Science Inc., Beijing, China). All assays were conducted following the manufactures' instruction.

2.5. Statistical Analysis

Percentage change in lipid parameter was calculated for each study participant as the difference between the post-dose value and the baseline value, divided by the baseline value. Lipid levels measured at 1 h before dosing in the single dose groups and at 1 h before dose 3 in the multiple dose groups served as baseline values. Mean percentage changes and standard deviations (SD) were calculated for each treatment group. Differences between treatment groups were assessed by Student's *t*-test. Pearson correlation coefficients were used to assess the correlation between lipid parameters and plasma concentrations of bambuterol. All statistical analyses were conducted using GraphPad Prism version 6 (San Diego, CA). All P values were 2-sided. Values of P less than 0.05 were considered statistically significant.

2.6. Role of the Funding Source

The sponsor supported part of the laboratory work.

3. Results

3.1. Participant Characteristics

Forty-eight eligible healthy subjects were randomly assigned to receive 2.5 mg single-dose R-bambuterol (n = 10), 5 mg single-dose R-bambuterol (n = 10), 10 mg single-dose R-bambuterol (n = 10), 5 mg multiple-dose R-bambuterol (n = 10), or 10 mg single dose rac-bambuterol (n = 8), respectively (Fig. 1). Baseline demographic characteristics and lipid levels for the single-dose groups were listed in Table 1. There were no statistically significant differences in age, BMI and baseline cholesterol levels across different treatment groups. Total cholesterol levels were normal (<5.2 mmol/L) in all except for two subjects whose total cholesterol levels were borderline high (5.2-6.2 mmol/L): one in the 2.5 mg single-dose R-bambuterol group and one in the single-dose rac-bambuterol group. For subjects in the multiple-dose group, blood collection started on the fourth day of treatment and therefore the measurements before dose 4 (day 4) were used as baseline. The levels of LDL-C, HDL-C, TC and TG all appeared to be lower compared to those of the single-dose groups, although these groups were not directly comparable (Suppl. Table 1).

3.2. Drug Responses and Pharmacokinetics of R-bambuterol

During the trial period, no serious adverse events were observed in the participants who received R-bambuterol. No clinically significant dose-dependent changes were found in the tested laboratory indices, i.e. liver function tests, kidney function tests, plasma electrolytes, routine blood tests and urinalysis (data not shown).

The time to peak concentration (T_{max}) and peak plasma concentration (C_{max}) of bambuterol after administration of a single dose of R-bambuterol or rac-bambuterol were shown in Table 2. In the 2.5 mg, 5 mg and 10 mg single-dose R-bambuterol groups, C_{max} increased with the dose (149.2 ng/L, 419.4 ng/L and 1136.0 ng/L, respectively) and the corresponding T_{max} were 1.5, 1.5 and 2 h, respectively. The 10 mg rac-bambuterol group had a more than two fold longer T_{max} and a 47% higher C_{max} of bambuterol than the 10 mg R-bambuterol group (Table 2). At 24 h after dosing, the plasma concentration of R-bambuterol in the 10 mg group dropped to 68.7 ng/L whereas the concentration of its metabolite terbutaline was 1053.0 ng/L.

3.3. Lipid-lowering Effects of Single-dose R-bambuterol at T_{max}

Administration of a single dose of R-bambuterol resulted in dose-dependent reductions in the levels of plasma LDL-C, HDL-C, TC, ApoB, and ApoA1 at T_{max} (Fig. 2). Levels of LDL-C exhibited the most reduction. Percentage changes in LDL-C from the baseline measurements in the 2.5 mg, 5 mg, and 10 mg R-bambuterol groups were -2.9% ($P = 0.05$), -6.7% ($P = 0.05$) and -12.4% ($P = 0.01$), respectively (Fig. 2A). Statistically significant change in HDL-C level at T_{max} was only observed in the 10 mg R-bambuterol group (Fig. 2B, $P = 0.05$). The ratio of ApoA1/ApoB, an indicator of the HDL-C/LDL-C ratio, increased with dose, although the changes from baseline were not statistically significant (Fig. 1G). There is no obvious dose–response relationship for TG

Table 2
Pharmacokinetics of R-bambuterol and rac-bambuterol.

Variables	R-BMB			Rac-BMB	Multiple dose R-BMB (dose 7)
	2.5 mg (n = 10)	5.0 mg (n = 10)	10 mg (n = 10)	10 mg (n = 8)	5 mg (n = 10)
t_{max} (h)	1.5 (0.25–5)	1.5 (0.5–12)	2.0 (0.5–8)	4.5 (0.5–12)	2.0 (0.5–8)
C_{max} (ng/L)	149.2 (110.3–201.8)	419.4 (251.6–699.0)	1136 (660.5–1952)	1668 (740.8–3756)	398.3 (166.2–954.7)
C_{24} (ng/L)	BLQ	32.0 (17.3–39.4)	68.7 (38.2–123.4)	89.8 (42.7–188.8)	62.9 (32.2–122.8)
C_{24} Terbutaline (ng/L)	159.3 (110.3–230.1)	498.9 (362.1–687.3)	1053 (748.6–1480)	702.2 (533.2–924.7)	783.0 (495.6–1237)

T_{max}, time to reach peak concentration, expressed as median (range).
C_{max}, maximum plasma concentration, expressed as the geometric means (95% confidence intervals).

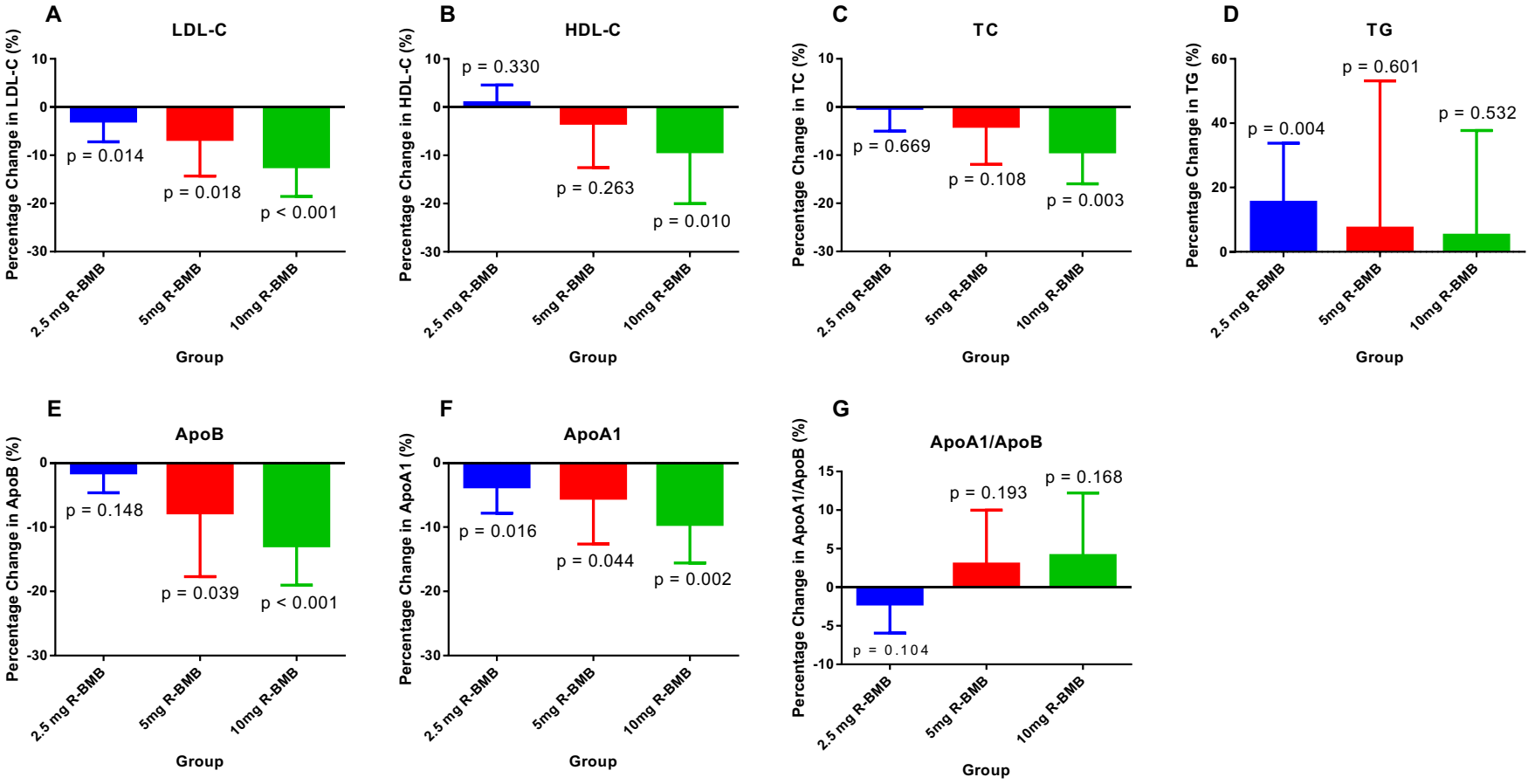

Fig. 2. Lipid lowering effects of a single dose of R-bambuterol at T_{max}. (A) LDL-C; (B) HDL-C; (C) TC; (D) TG; (E) ApoB; (F) ApoA1; (G) ApoA1/ApoB. Each subject received a single dose of R-bambuterol tablets of 2.5 mg, 5 mg or 10 mg orally. Values shown are means of percentage changes. Error bars indicate standard deviation.

(Fig. 2D). Correlations between C_{max} of R-bambuterol and the corresponding lipid levels at T_{max} were examined in all study participants who received a single dose of R-bambuterol (Suppl. Fig. 1). Again, C_{max} of R-bambuterol was significantly correlated with a reduction in LDL-C ($R = -0.39$, $P = 0.03$) and marginally correlated with an increase in the ratio of ApoA1/ApoB ($R = 0.33$, $P = 0.07$).

3.4. Lipid-lowering Effects of R-bambuterol Versus Rac-bambuterol at T_{max}

R-bambuterol appeared to be more potent in lowering plasma lipids than rac-bambuterol, particularly for LDL-C (Fig. 3, $P = 0.08$). At T_{max}, percentage reductions in LDL-C, TC, ApoB and ApoA1 in the 10 mg rac-bambuterol group (6.6%, 5.0%, 7.6% and 5.9%, respectively) were similar to the reductions in the 5 mg R-bambuterol group (6.7%, 4.0%, 7.8% and 5.5%, respectively), but were approximately half of the reductions in the 10 mg R-bambuterol group (12.4%, 9.3%, 12.9% and 9.6%, respectively). Reduction in HDL-C level in the 10 mg rac-bambuterol group, however, was similar to that in the 10 mg R-BMB group at T_{max} (Fig. 3, $P = 0.99$).

3.5. Lipid-lowering Effects of Single-dose R-bambuterol at 24 h

The concentrations of R-bambuterol at 24 h after dosing were less than one tenth of that at T_{max} (Table 2). However, the lipid-lowing effects of R-bambuterol were still observed at 24 h after dosing (Fig. 4). Percentage changes in LDL-C from the baseline measurements in the 2.5 mg, 5 mg, and 10 mg R-bambuterol groups were 1.2%, −1.3% and −8.4%, respectively, with only the 10 mg group showed statistically significant reduction ($P = 0.05$). Levels of ApoA1 and ApoB were also significantly reduced in the 10 mg R-bambuterol group at 24 h after dosing, whereas levels of HDL-C, TC, and TG were not. The ratio of ApoA1/ApoB again showed a marginal increase at 24 h after dosing.

3.6. LDL Particle Size after Single-dose R-bambuterol Treatment

The LDL-C/ApoB ratio, an indicator of compositional changes in LDL particle size, was calculated for all the single-dose R-bambuterol groups (Fig. 5). The mean values of LDL-C/ApoB were not significantly different between different treatment groups before dosing. No significant changes in LDL-C/ApoB ratios were noted at either T_{max} or 24 h after dose administration, which indicated minimal changes in LDL particle sizes in each group.

3.7. Lipid-lowering Effects of Multiple-dose R-bambuterol at 24 h

The lipid-lowering effects of multiple doses of 5 mg R-bambuterol were further studied. In the multiple-dose group, lipid profiles were measured before dose 4 (day 4) and at 24 h after dose 4 to dose 7 (day 5 to day 8). The differences in lipid profile between the measurement before dose 4 (as baseline value) and the measurements at 24 h after dose 4 to dose 7 were calculated and expressed as percentage changes of the baseline value (Fig. 6). No significant reductions in lipid levels were observed at 24 h after dose 4 and dose 5 compared to the lipid levels before dose 4. At 24 h after doses 6 and 7, significant reductions in levels of LDL-C, TC and ApoB were observed (all P values < 0.05). The levels of HDL-C did not significantly decrease at 24 h after doses 6 and 7, which led to marginal increases in ratios of ApoA1/ApoB (Fig. 6).

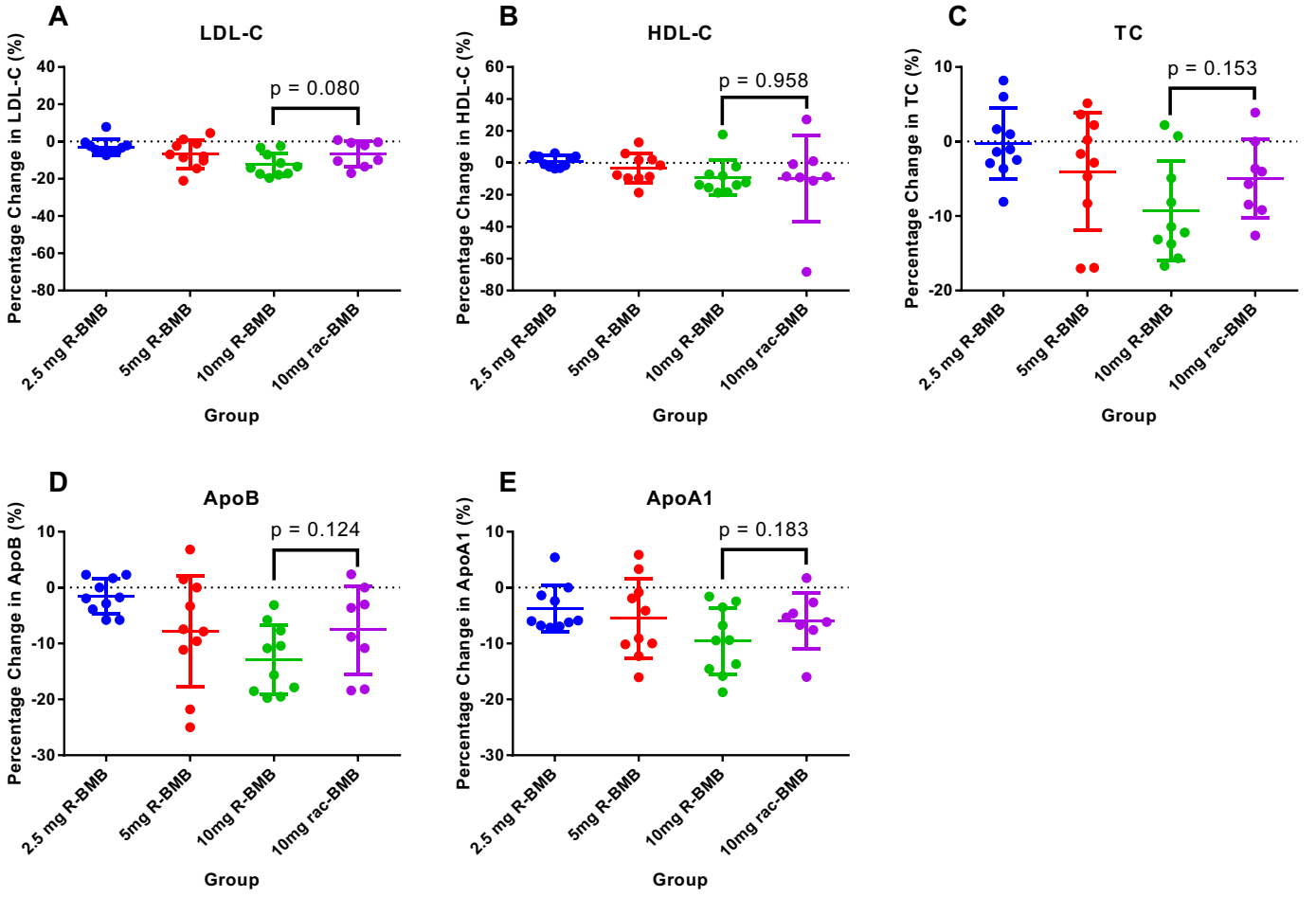

Fig. 3. Different lipid-lowering effects between R-BMB and rac-BMB. (A) LDL-C; (B) HDL-C; (C) TC; (D) ApoB; (E) ApoA1. Each subject received a single dose of R-bambuterol tablets of 2.5 mg, 5 mg or 10 mg, or a single dose of rac-bambuterol. Values shown are individual results with means. Error bars indicate standard deviation.

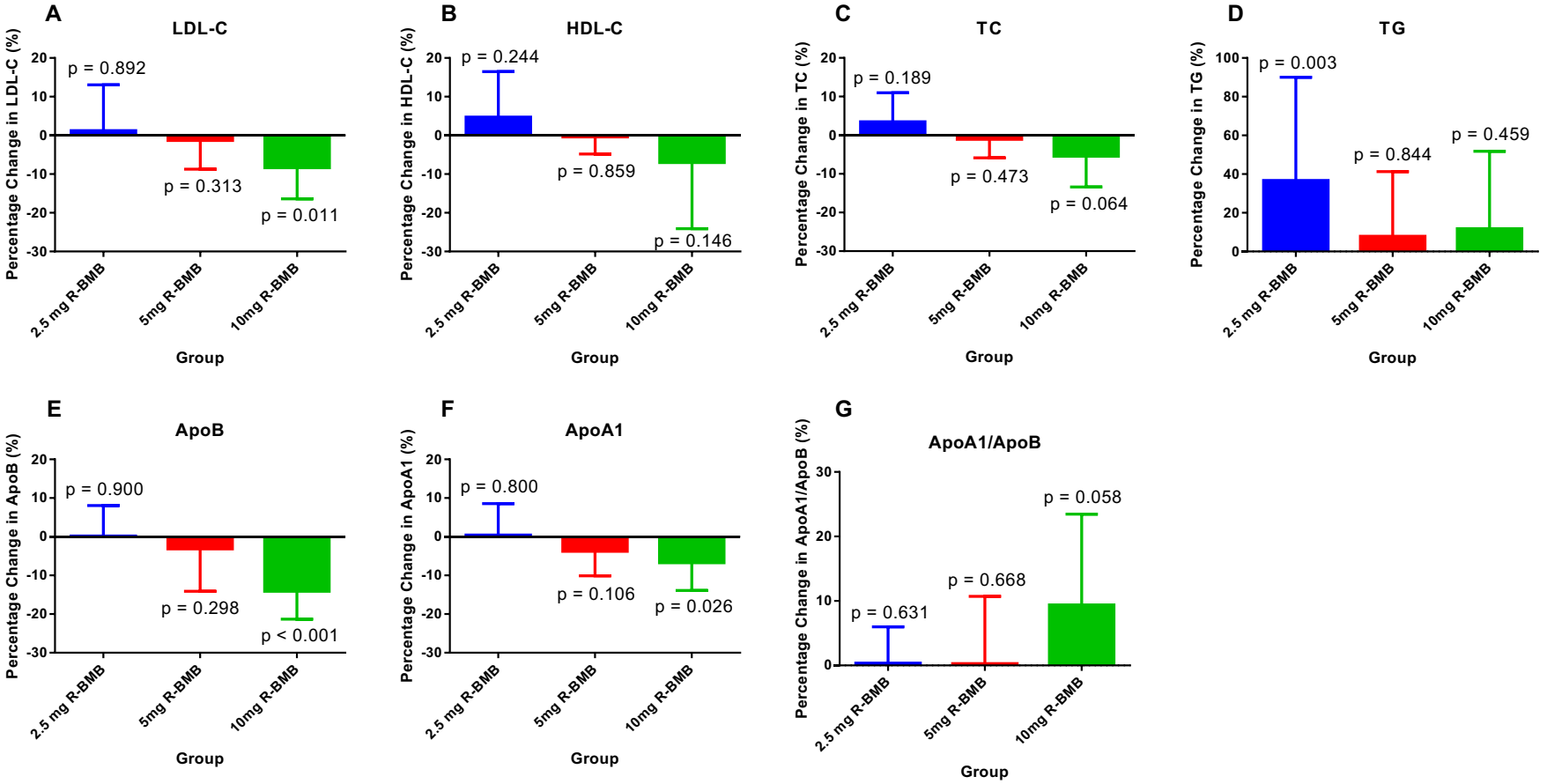

Fig. 4. Lipid-lowering effects of a single dose of R-bambuterol at 24 h after dosing. (A) LDL-C; (B) HDL-C; (C) TC; (D) TG; (E) ApoB; (F) ApoA1; (G) ApoA1/ApoB. Each subject received a single dose of R-bambuterol tablets of 2.5 mg, 5 mg or 10 mg orally. Values shown are means of percentage changes. Error bars indicate standard deviation.

4. Discussion

High plasma level of LDL-C is a major risk factor for coronary heart disease. Conventional cholesterol-lowering treatments such as statin are only modestly effective. Bambuterol is a type of β2-agonist commonly used for the treatment of asthma and COPD with the advantage of once daily dosing and favorable side effect profile (Olsson and Svensson, 1984; D'Alonzo et al., 1995; Sitar et al., 1993). In this open-label, randomized phase I clinical trial, we showed that R-bambuterol significantly lower the plasma levels of LDL-C, and marginally raise the ratio of ApoA1/ApoB, an indicator of HDL-C/LDL-C, in less than 2 h after administration and in a dose-dependent manner. We also showed that R-bambuterol is more potent in cholesterol lowering than rac-bambuterol. The lipid-lowing effects of R-bambuterol sustained after 24 h of treatment and after multiple doses. R-bambuterol therefore is an attractive alternative or complement for other treatments to decrease plasma LDL-C levels, and would be especially beneficial for those patients who also suffering from COPD.

The lipid-lowering effect of R-bambuterol was dose-dependent (Fig. 2). A single dose of R-BMB significantly lowered the levels of LDL-C and marginally raised the ratio of ApoA1/ApoB at T_{max} (less than 2 h), therefore achieved a favorable lipid lowering effect in a short term. Correlation between C_{max} of R-bambuterol and the corresponding lipid levels at T_{max} further supported the lipid-lowering effect of R-bambuterol (Suppl. Fig. 1).

The lipid-lowering effect of R-bambuterol was also observed at 24 h after dosing, which appeared to be weaker. At 24 h, the concentration of plasma R-bambuterol was less than one tenth of that at T_{max} (Table 2). It indicated that multiple mechanisms underlie the lipid-lowering effects of R-bambuterol, namely the initial effects and the lagging effects. The initial effects were possibly related to the inhibition of butyrylcholinesterase (Gazić et al., 2006). Butyrylcholinesterase activity has been associated with cardiovascular risk factors, including the level of LDL-C (Stojanov et al., 2011). The lagging effects were unlikely to be related to terbutaline since the concentration of terbutaline at 24 h after treatment (Table 2) was much lower compared to the previous study (Hooper et al., 1981). In the multiple-dose 5 mg R-bambuterol group, the reduction in LDL-C and the increase in ApoA1/ApoB at 24 h after dose 6 and dose 7 were comparable to those in the single-dose group. These results suggested potential long-term cholesterol-lowering effects of R-bambuterol.

The lipid-lowering effects of R-bambuterol and rac-bambuterol were compared. At T_{max}, percentage changes in LDL-C, TC, ApoB and ApoA1 in the 10 mg rac-bambuterol group were similar to those in the 5 mg R-bambuterol group, but only approximately half of those in the 10 mg R-bambuterol group (Fig. 3), suggesting that R-bambuterol was more potent. R-bambuterol made up half of rac-bambuterol. It is possible that R-enantiomer of bambuterol is the eutomer which accounted for most of the observed cholesterol-lowering effects of rac-bambuterol. Similar findings in previous study in guinea pigs showed that the protective effect of R-bambuterol from histamine induced bronchoconstriction is stronger than that of rac-bambuterol (Cheng and Tan, 2009).

Previously, adverse effects, i.e. fatigue, nausea, palpitations, headache, dizziness and tremor have been reported for rac-bambuterol (Holstein-Rathlou et al., 1986). It has been suggested R-bambuterol has less cardiac inotropic and chronotropic effects than rac-BMB in animals (Cheng and Tan, 2009). In this study, R-bambuterol administered either as single (10 mg) or as multiple doses (5 mg/daily) was well tolerated since the lower dose used compared to previous studies (10 mg/daily versus 20 mg daily). Several studies suggested associations between the regular use of β2-agonist and non-fatal ischemic heart disease (Martin et al., 1998) or asthma death (Cazzola and Matera, 2007). Based on our findings, these adverse effects may be reduced by using R-bambuterol enantiomer which requires only one half of the dose of rac-bambuterol while limiting the possible non-target effects of S-bambuterol.

FlorÉN et al. (1997) reported that rac-bambuterol treatment significantly increases the level of HDL-C, but not significantly change the level of LDL-C. The effect was different from our findings, possibly due to the different experimental settings and the rac-bambuterol administrated. In the previous study, the treatment period was 6–8 weeks, whereas in this study the dosing period of R-bambuterol was only one to seven days. Also, the previous study was conducted among patients with hyperlipidemia, who were also at older age (mean age of 59.3 years). More importantly, R-bambuterol and S-bambuterol might have different or even contradicting biological effects, which have been observed for other racemic drugs (Caner et al., 2004). Nevertheless, the approximately 15% LDL-C lowering and the marginal ApoA1/ApoB increasing effects of R-bambuterol within a short-term treatment observed in this study is noteworthy.

Limitations of the study include the small study population, the lack of control group, and the short term of treatment. More studies with longer periods of treatment and among larger populations are required to define the more accurate effects of R-bambuterol on lipid levels in healthy population. In addition, a phase II trial with high cholesterol patients will be needed for better understanding of the lipid lowering effects by R-bambuterol.

The lipid-lowering effect of R-bambuterol was likely different from that of fibrates and niacin, which also effectively reduced plasma TG levels (Bruckert et al., 2011; Fabbrini et al., 2010). A higher LDL-C/ApoB ratio indicates an increase in the LDL particle size. Unlike statins and fibrates (Watts et al., 2006; Ballantyne et al., 2008), the administration of R-bambuterol did not significantly alter the LDL-C/ApoB ratio (Fig. 4). In addition, oral administration of R-bambuterol in this study did not significantly alter the plasma concentration of glucose or potassium (data not shown), which were found in previous clinic studies when orally administering Rac-bambuterol and other racemic β2-agonists such as albuterol.

In conclusion, this study showed that R-bambuterol can lower the plasma levels of LDL-C, and raise the ratio of ApoA1/ApoB (indicator of HDL-C/LDL-C) when administered in either a single dose or multiple doses. R-bambuterol was more potent in lipid-lowering than rac-bambuterol. Therefore, R-bambuterol might offer an alternative treatment for patients with high LDL-cholesterol level, especially for whom also suffering from COPD.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ebiom.2015.02.006.

Author Contributions

All authors interpreted the data and collaborated in the preparation of the report. WT had primary responsibility for the decision to

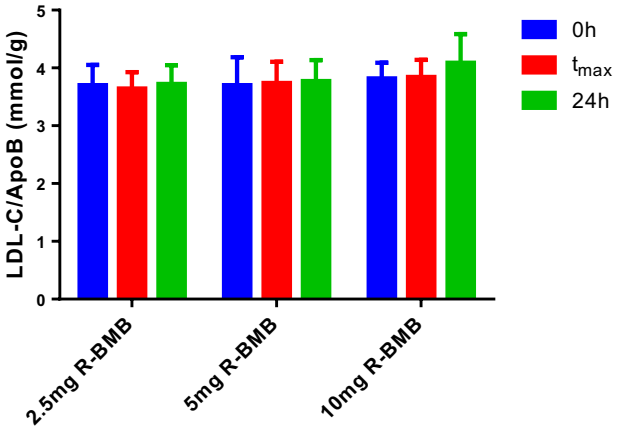

Fig. 5. LDL-C/ApoB ratios in single-dose R-bambuterol groups. LDL-C/ApoB ratios were calculated for time points at 0 h, t_{max} and 24 h. Values shown are means of percentage changes. Error bars indicate standard deviation.

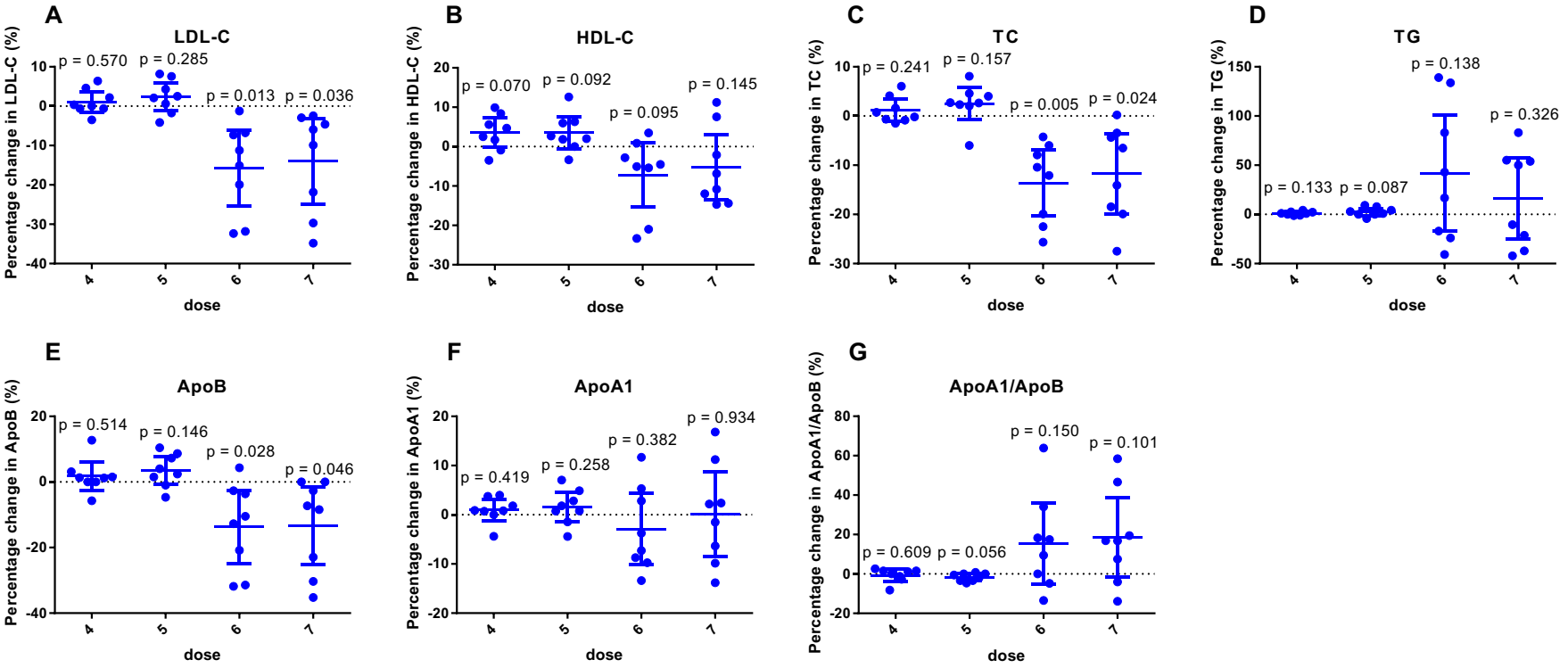

Fig. 6. Cholesterol lowering effects of multiple doses of 5 mg R-BMB. (A) LDL-C; (B) HDL-C; (C) TC; (D) TG; (E) ApoB; (F) ApoA1; (G) ApoA1/ApoB. Each subject received a multiple 5 mg dose of R-bambuterol orally. Percentage changes in lipids were calculated as the percentage change within each group using values at 1 h before dose 3 as baseline and 24 h after each dose. Values shown are individual results with means. Error bars indicate standard deviation.

submit for publication, and all authors vouch for the completeness and accuracy of the data and analyses. TZ and CZ were responsible for the pharmacokinetic data; and WT, TZ, QC, and SB were responsible for the design and execution of the phase 1 trial. TZ, QC, and SB were responsible for the collection and collation of the clinical data, YY, HX, LZ, and JZ were responsible of laboratory analysis, YY and LQ were responsible for the statistical analysis, and WT, YY, and LQ were responsible for literature search and writing.

Disclosures

All authors declare that they have no conflicts of interest.

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