Association between cholesterol synthesis/absorption markers and effects of cholesterol lowering by atorvastatin among patients with high risk of coronary heart disease

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Abstract No indices are currently available to facilitate clinicians to identify patients who need either statin monotherapy or statin–ezetimibe combined treatment. We aimed to investigate whether cholesterol synthesis and absorption markers can predict the cholesterol-lowering response to statin. Total 306 statin-naïve patients with high risk of coronary heart disease (CHD) were treated with atorvastatin 20 mg/day for 1 month. Cholesterol synthesis and absorption markers and LDL cholesterol (LDL-C) levels were measured before and after treatment. Atorvastatin decreased LDL-C by 36.8% (range: decrease of 74.5% to increase of 31.9%). Baseline cholesterol synthesis marker lathosterol and cholesterol absorption marker campesterol codetermined the effect of atorvastatin treatment. The effect of cholesterol lowering by atorvastatin was significantly associated with baseline lathosterol levels but modified bidirectionally by baseline campesterol levels. In patients with the highest baseline campesterol levels, atorvastatin treatment decreased cholesterol absorption by 46.1%, which enhanced the effect of LDL-C lowering. Atorvastatin treatment increased cholesterol absorption by 52.3% in those with the lowest baseline campesterol levels, which attenuated the effect of LDL-C reduction. Especially those with the highest lathosterol but the lowest campesterol levels at baseline had significantly less LDL-C reduction than those with the same baseline lathosterol levels but the highest campesterol levels (27.3% versus 42.4%, P = 0.002). These results suggest that combined patterns of cholesterol synthesis/absorption markers, rather than each single marker, are potential predictors of the LDL-C-lowering effects of atorvastatin in high-risk CHD patients.—Qi, Y., J. Liu, C. Ma, W. Wang, X. Liu, M. Wang, Q. Lv, J. Sun, J. Liu, Y. Li, and D. Zhao. Association between cholesterol synthesis/absorption markers and effects of cholesterol lowering by atorvastatin among patients with high risk of coronary heart disease. J. Lipid Res. 2013. 54: 3189–3197.

Supplementary key words cholesterol synthesis marker • cholesterol absorption marker • low density lipoprotein cholesterol

Low density lipoprotein cholesterol (LDL-C) lowering by a statin is widely accepted as the first-line treatment in the primary and secondary prevention of coronary heart disease (CHD) (1–3). However, many patients may not achieve a satisfactory target level of serum LDL-C under statin monotherapy to minimize the risk of CHD events (4–6). Although serum cholesterol levels come from both synthesis and absorption, statins mainly reduce the cholesterol synthesis (7–11). Therefore, the combination therapy of a statin with an inhibitor of cholesterol absorption from dietary and biliary sources, such as ezetimibe, has been recommended (7, 12). Currently, use of either statin monotherapy or a statin–ezetimibe combination is indiscriminate (13), because no indices have so far been identified that can discriminate between the individual contributions of cholesterol synthesis and cholesterol absorption to cholesterol levels. Growing evidence suggests that circulating lathosterol and desmosterol levels can serve as cholesterol synthesis markers, and circulating campesterol and sitosterol levels as markers of fractional cholesterol absorption (9–11, 14–22). These markers are possible predictors for the effect of LDL-C lowering by a

Abbreviations: ANCOVA, one-way analysis of covariance; BMI, body mass index; BP, blood pressure; CHD, coronary heart disease; FPG, fasting plasma glucose; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; TC, total cholesterol; TG, triglyceride.

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statin or by combined treatment of a statin with ezetimibe. Some studies have reported that LDL-C lowering by statins did associate with baseline levels of high or low cholesterol synthesis and absorption (14–17). Patients with low levels of endogenous cholesterol synthesis exhibited a poor response to statin treatment. On the other hand, LDL-C reduction through inhibiting cholesterol synthesis by statin may also be associated with an increase in cholesterol absorption (18–21). However, conclusive evidence regarding the association of cholesterol synthesis and absorption markers with cholesterol lowering by statins so far is lacking. There is also a paucity of published data on the interactive influences of baseline cholesterol synthesis and absorption markers on their own changes after statin treatment and the effect of cholesterol lowering by statin.

Therefore, this study aimed to examine whether the response to LDL-C lowering by statins among patients with high-risk of CHD is predetermined, at least in part, by baseline patterns of cholesterol synthesis and cholesterol absorption. We also aimed to determine how the potential reciprocal interplay between cholesterol synthesis and cholesterol absorption affects each other after statin treatment, as well as the lowering effect of LDL-C by statin. Addressing these issues will promote rational drug use and guide future clinical treatment.

METHODS

Study patients and design

Study patients were consecutively recruited in outpatient clinics of two hospitals from Shanxi and Henan provinces between October 2008 and June 2009. Patients of age 30–70 years were included in the study if they met the criteria for lipid-lowering drug therapy according to the National Cholesterol Education Program Adult Treatment Panel III report (NCEP ATP III) (1) and if they had no previous treatment with statins or other related lipid-lowering agents. The inclusion criterion was either i) LDL-C levels ≥ 2.6 mmol/l with a history of CHD and diabetes mellitus; or ii) LDL-C levels ≥ 4.1 mmol/l with two or more risk factors, including current smoking, hypertension, low HDL cholesterol (HDL-C) levels, a family history of premature CHD, and age. CHD was defined as a history of myocardial infarction, angina, coronary artery procedures (angioplasty or bypass surgery), or evidence of clinically significant myocardial ischemia. Diabetes mellitus was defined by fasting plasma glucose (FPG) levels ≥ 7.0 mmol/l or by currently being on glucose-lowering medical treatment. Current smoking was defined as smoking one or more cigarettes per day for more than 3 months. Hypertension was defined as systolic blood pressure (BP) ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg and/or current antihypertensive treatment. Low HDL-C levels were defined as HDL-C levels < 1.04 mmol/l. A family history of premature CHD was defined as CHD in males with a first-degree relative < 55 years of age and CHD in females with a first-degree relative < 65 years of age. Age in years was dichotomized by the cutoff of 45 in men and 55 in women.

Patients were excluded if they had i) acute ST-elevation myocardial infarction within the last three months, serious heart failure, autoimmune disease, severe arrhythmia, a malignant tumor, or a history of hypothyroidism or nephritic syndrome; ii) serious trauma or major surgery within the last three months; iii) impaired hepatic/renal function (defined as alanine aminotransferase levels 1.5 times higher than the upper limit of normal levels and creatinine levels higher than 177 μmol/l); iv) treatment by drugs that interact with statins; or v) not complying with the study medication.

Atorvastatin was administered to qualified patients at a dose of 20 mg per day for one month. Patients were called once per week to inquire about dosage and timing of atorvastatin intake, and it was ascertained whether they experienced symptoms related to an adverse drug reaction. All study patients were recruited from Chinese rural areas, and they had no previous therapies by statin or other related lipid-lowering agents. Additionally, high statin dosages may not be a viable option because increasing doses may increase the risk of adverse drug reactions and reduce medication adherence. The initial dose of atorvastatin of 20 mg per day was chosen because this dosage is generally adopted in China (23). All patients were advised to comply with normal diets and routine lifestyles. They were educated regarding the reasons for taking lipid-lowering treatment and the importance of maintaining treatment as a means to improve drug adherence. Accordingly, a total of 363 patients were recruited at baseline to receive atorvastatin therapy for one month. After excluding patients who took atorvastatin irregularly (n = 30) and were lost (n = 15) to follow-up, the remaining 318 patients completed the reexaminations and had normal hepatic function after four weeks. Of these, 306 patients with complete information at baseline and at one month were analyzed in this study. This study was approved by the Clinical Research Ethics Committee of Beijing An Zhen Hospital, and written informed consent was obtained from each patient.

Data collection

A standard questionnaire was used at baseline to collect information regarding demographic characteristics, smoking status, family history of premature CHD, and medical history of diabetes mellitus and hypertension. During a physical examination, anthropometric indices (body weight, height, waist circumference, and hip circumference) and BP were measured. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. BP was measured in the right arm at a sitting position with a regular mercury sphygmomanometer after resting for at least 5 min. The mean of three consecutive BP readings was used.

Venous blood samples were collected after an overnight fast at baseline and follow-up for the laboratory measurements, including serum levels of lipids, FPG, and cholesterol synthesis and absorption markers. Total cholesterol (TC), triglyceride (TG), and FPG levels were determined by enzymatic methods (Human Diagnostics, Wiesbaden, Germany), and LDL-C and HDL-C levels were tested by homogeneous methods (Sekisui Medical Co., Japan). Cholesterol synthesis and absorption markers were quantitatively determined from fasting serum by gas chromatography. For preprocessing, the following protocol was used. Serial dilutions of squalene, cholesterol, desmosterol, lathosterol, campesterol, and sitosterol (Sigma, St. Louis, MO) were prepared in differently labeled solutions, and then were sealed in numbered ampoules and stored at −20°C. As the internal standard, 5α-cholestanol (0.01301 g; Sigma) was diluted to 25 μl using hexane (chromatographically pure; Merck, Darmstadt, Germany). As for serum sample preparation, 100 μl serum samples, 100 μl internal standard, 1,000 μl absolute ethanol (chromatographically pure), and 960 μl 8.90 mol/l potassium hydroxide (analytically pure) were successively added to a 2 ml glass vial. After vortexing for 15 s, saponified for 1 h at 67°C, and then extracted by adding 1,000 μl water (Millipore, Billerica, MA) and 2,000 μl hexane; this extracting procedure was conducted twice. The nonsaponifiable serum materials in the
upper layer were transferred into new clean tubes, washed by water to neutralize any acid therein, and then dried with nitrogen. Next, 200 μl silylating agents (hexamethyldisiloxane/trimethylchlorosilane/pyridine, 3:1:9, v/v; Supelco, Bellefonte, PA) were added to these nonsaponifiable serum materials and coinubated at 67°C for 1 h. After removing the residual silylating agents by drying under a steady stream of nitrogen, the resulting substances were solubilized in 300 μl of hexane, which was used for gas chromatography analysis. Gas chromatography was performed at an HP-5 quartz capillary column (5% phenyl-methyl silicone; Agilent Technologies, Santa Clara, CA) (30 m × 0.32 mm × 0.25 μm) under the following conditions: temperature at the starting point, 150°C; retention, 3 min; temperature-programmed rate, 30°C/min to 250°C and 5°C/min to 280°C; retention, 30 min; temperature of the flame ionization detector, 290°C; sample injection temperature, 290°C; sample introduction pressure, 15 psi; and splitless mode sampling, 1 μl. High-purity nitrogen (99.999%) was adopted as the carrier gas. An Agilent 7890 gas chromatograph was used for image acquisition. All measurements were conducted by the same technician with the same instrument. Cholesterol synthesis markers (desmosterol and lathosterol) and cholesterol absorption markers (campesterol and sitosterol) were measured by the single internal standard curve method. Each run quantified desmosterol, lathosterol, campesterol, and sitosterol levels in an increasing order of retention. Cholesterol synthesis markers and absorption markers were expressed as absolute values. Intra-assay coefficients of variation for cholesterol synthesis and cholesterol absorption markers were from 0.98% to 2.71%, and interassay coefficients of variation for cholesterol synthesis and absorption markers were from 1.26% to 5.83%.

**Sample size estimation**

Based on previous reports (9) that the range of the correlation coefficients between baseline synthesis and absorption markers and cholesterol levels is 0.17 to 0.42, the estimated sample size will be 293 if the correlation coefficient is 0.17, and 45 if the coefficient is 0.42, assuming α (probability of type I error) equals 0.05 and delta (admissible error) equals 0.10. The actual sample size of 306 in this study is within these limits and lends confidence to our findings.

**Statistical analysis**

Continuous variables are expressed as the mean (SD) in case of normal distribution, and are otherwise expressed as medians (interquartile ranges). Differences between two groups were determined by two-sided t-test or by the Mann-Whitney test. ANOVA was used to identify differences across three or more groups. Categorical variables are expressed as numbers (percentages) and were compared by chi-square test.

Baseline cholesterol synthesis and absorption markers were treated as continuous variables (per SD increase) in quantitative analysis and categorical variables (quartiles and combined patterns) in qualitative analysis, to investigate the association of baseline cholesterol synthesis and absorption markers with LDL-C baseline levels and its changes in LDL-C levels.

In quantitative analyses, partial Pearson correlations were determined for baseline cholesterol synthesis and absorption markers with LDL-C baseline levels and its changes after statin treatment (percentage reduction) after adjusting for age and sex. The association of baseline cholesterol synthesis and absorption markers with LDL-C baseline levels and its changes were analyzed in a multiple linear regression model, with LDL-C baseline levels and its changes as the dependent variable and with age, sex, BMI, and baseline cholesterol synthesis and absorption markers as independent variables. Because only the cholesterol synthesis marker lathosterol and absorption marker campesterol were significantly associated with LDL-C baseline levels and its changes (see supplementary Tables I and II), the cholesterol synthesis marker desmosterol and absorption marker sitosterol were not used in further analyses.

In qualitative analyses, baseline lathosterol and campesterol levels in mg/dl were categorized into quartiles, with 0.41, 0.64, and 1.05 as cutoffs for lathosterol and 0.19, 0.29, and 0.46 for campesterol. This rendered a total of 16 (4 × 4) combined patterns of baseline cholesterol synthesis and absorption. Across these combined patterns, one-way ANCOVA with Bonferroni correction was adopted to compare the distributions of baseline LDL-C levels and its changes after one month of atorvastatin treatment after adjusting for age, sex, and BMI. In addition, a percentage reduction in LDL-C levels greater than 30% was regarded as the target of statin treatment. To adjust the influence of the regression to the mean (24, 25), ANCOVA was adopted to adjust baseline level of lathosterol and campesterol.

Given the potential impact of the status of diabetes mellitus (26), comparisons of baseline levels and changes in cholesterol synthesis and absorption were performed between patients with and without diabetes mellitus. Further, the association of combined patterns of baseline cholesterol synthesis and absorption with changes in LDL-C levels was analyzed after adjusting for age, sex, BMI, and the status of diabetes mellitus. Additionally, the association of combined patterns with changes in LDL-C levels was reanalyzed in subgroups with or without diabetes mellitus.

P < 0.05 was considered statistically significant, except for Bonferroni correction. Data were managed and analyzed using SPSS software for Windows (version 13.0; SPSS Inc., Chicago, IL). Sample size estimation was calculated by PASS software for windows (version 08.0.3, Hintze, J. PASS 2008; NCSS, LLC, Kaysville, UT).

**RESULTS**

A total of 306 patients with a mean age of 55.9 ± 8.9 years were enrolled, and 149 women (48.7%) were included. The baseline characteristics of the study patients are summarized in Table 1. The mean baseline LDL-C levels were 3.72 ± 0.81 mmol/l.

**Effects of LDL-C lowering by atorvastatin**

Atorvastatin treatment resulted in a mean reduction in LDL-C levels of 36.8% compared with baseline, and it ranged from −74.5% to +31.9%, corresponding to absolute changes from −4.74 mmol/l to +1.45 mmol/l (Fig. 1). The reduction in LDL-C levels was less than 30.0% in 35.3% of the patients, and less than 40.0% in 52.6% of the patients.

**Association of baseline cholesterol synthesis/absorption markers with baseline LDL-C levels**

For the qualitative analyses, baseline mean LDL-C levels significantly increased with quartile levels of the baseline cholesterol synthesis marker lathosterol and cholesterol absorption marker campesterol after adjusting for age, sex, and BMI by ANCOVA (Table 2). Patients in the highest quartile of baseline lathosterol levels had the highest baseline LDL-C levels, which were 15.6% higher than those in the lowest quartile (P < 0.001). In quantitative
analyses, baseline LDL-C levels were positively and significantly correlated with baseline levels of lathosterol and campesterol, with partial correlation coefficients of 0.162 ($P = 0.005$) and 0.178 ($P = 0.002$), respectively (supplementary Table I). This association was also identified in multiple linear regression analyses, with partial correlation coefficients of 0.162 ($P = 0.005$) and 0.178 ($P = 0.002$), respectively (supplementary Table I). This association was also identified in multiple linear regression analyses, with partial correlation coefficients of 0.162 ($P = 0.005$) and 0.178 ($P = 0.002$), respectively (supplementary Table I).

When the combined patterns of lathosterol and campesterol were examined, patients with higher levels of lathosterol and campesterol had higher baseline LDL-C levels. Patients in the group with the highest synthesis of these markers, indicated by lathosterol and campesterol levels, were signifi cantly associated with the effect of LDL-C reduction by atorvastatin. This association was preserved even after adjusting for baseline LDL-C. The results showed that the association between baseline cholesterol synthesis and absorption codetermined baseline LDL-C levels.

### Association of baseline cholesterol synthesis/absorption markers with LDL-C reduction by atorvastatin

Baseline levels of cholesterol synthesis and absorption markers, indicated by lathosterol and campesterol levels, were significantly associated with the effect of LDL-C reduction by atorvastatin treatment. This association was preserved even after adjusting for baseline LDL-C. The results showed that the association between baseline cholesterol synthesis and the effect of LDL-C lowering by statin treatment was modified by baseline cholesterol absorption (Fig. 3). Among patients with high baseline campesterol levels (the two upper quartile groups), the effect of atorvastatin on LDL-C levels was mainly determined by baseline levels of the cholesterol synthesis marker lathosterol, with a 26.3% reduction in LDL-C levels in patients with the same baseline lathosterol levels but with the highest campesterol levels ($P = 0.002$). When a 30% reduction in LDL-C levels was regarded as a target of atorvastatin treatment, patients with the highest synthesis but the lowest absorption of cholesterol at baseline, or patients with the lowest synthesis but the highest absorption of cholesterol at baseline were less likely to reach that target (Table 3).

We performed a subgroup analysis among 14 patients who had no reduction or even slightly elevated LDL-C levels after one month of atorvastatin treatment. The distribution of these 14 patients among 16 combined patterns of baseline synthesis and absorption of cholesterol showed that 50.0% of them were in the group with the lowest cholesterol synthesis at baseline, and 14.3% of them were in the groups with highest synthesis/lowest absorption of cholesterol.

Additionally, there were no significant differences in baseline levels and changes in lathosterol and campesterol between patients with and those without diabetes (supplementary Table III). Further, the association of baseline cholesterol synthesis/absorption markers with LDL-C reduction by atorvastatin was still significant after adjusting for age, sex, BMI, and diabetes mellitus. Additionally, this

### Table 1. Baseline characteristics among 306 patients

| Characteristic | Total (n = 306) | Men (n = 157) | Women (n = 149) | $P^{*}$ |
|---------------|----------------|---------------|-----------------|--------|
| Age, year, mean (SD) | 55.9 ± 8.9 | 53.4 ± 9.3 | 58.5 ± 7.8 | <0.001 |
| BMI, kg/m², mean (SD) | 25.6 ± 3.0 | 25.7 ± 3.0 | 25.4 ± 2.9 | 0.457 |
| Family history of premature CHD, n (%) | 40 (13.1) | 26 (16.6) | 14 (9.4) | 0.063 |
| Current smoking, n (%) | 66 (21.6) | 60 (38.2) | 6 (4.0) | <0.001 |
| Hypertension, n (%) | 179 (58.5) | 92 (58.6) | 87 (58.4) | 0.970 |
| Diabetes, n (%) | 189 (61.8) | 91 (58.0) | 98 (65.8) | 0.160 |
| CHD, n (%) | 60 (19.6) | 34 (21.7) | 26 (17.4) | 0.354 |
| FPG, mmol/l, mean (SD) | 7.5 ± 4.4 | 7.9 ± 5.4 | 7.2 ± 5.0 | 0.191 |
| TG, mmol/l, mean (SD) | 6.43 ± 2.23 | 6.49 ± 2.76 | 6.36 ± 1.49 | 0.612 |
| LDL-C, mmol/l, mean (SD) | 3.72 ± 0.81 | 3.73 ± 0.85 | 3.71 ± 0.76 | 0.815 |
| HDL-C, mmol/l, mean (SD) | 1.16 ± 0.42 | 1.05 ± 0.38 | 1.28 ± 0.44 | <0.001 |
| TG, mmol/l, median (IQR) | 1.84 (1.25, 2.70) | 1.77 (1.24, 2.57) | 1.88 (1.29, 2.81) | 0.493 |
| Lathosterol, mg/dl, mean (SD) | 0.55 ± 0.13 | 0.60 ± 0.13 | 0.49 ± 0.14 | 0.072 |
| Campesterol, mg/dl, mean (SD) | 0.28 ± 0.11 | 0.29 ± 0.12 | 0.27 ± 0.11 | 0.333 |

Data are expressed as number (percentage) for categorical variables, as mean (standard deviation) for continuous variables in case of normal distributions, and as medians (IQR ranges) otherwise. IQR, interquartile range.

* $P$ values were calculated between men and women.
association was reanalyzed in subgroups with or without diabetes, and the findings were similar in nondiabetic patients. However, in diabetic patients, the trend of LDL-C-lowering effects across different patterns of cholesterol synthesis and absorption was consistent with that of the overall population, but significance was not reached after Bonferroni correction.

Changes in cholesterol synthesis/absorption markers from baseline levels after atorvastatin treatment

The impact of baseline cholesterol synthesis and absorption markers on their own changes after atorvastatin treatment was further examined by quantifying lathosterol and campesterol levels after one month of treatment. Changes in cholesterol synthesis and absorption markers clearly reflected the inhibitory effect of atorvastatin on cholesterol synthesis, as well as the feedback effect of cholesterol absorption. The effect of inhibiting cholesterol synthesis by treatment with atorvastatin was highly associated with baseline levels of the cholesterol synthesis marker lathosterol. Patients in the highest quartile of baseline lathosterol levels had a 5.6 times higher lathosterol reduction than patients in the lowest quartile of baseline lathosterol (Table 2). No significant differences were found in the percentage of lathosterol reduction by atorvastatin across baseline campesterol levels (Fig. 4A).

Changes in campesterol levels after atorvastatin treatment were bidirectional (Table 2). They were significantly increased at lower baseline campesterol levels but were decreased at higher baseline levels. In the group with the

![Figure 2](image)

**Fig. 2.** Baseline levels of LDL-C by baseline cholesterol synthesis and absorption patterns. Q, quartile. Data are expressed as means for continuous variables. Data were compared by ANCOVA with Bonferroni correction, after adjusting for age, sex, and BMI. **P < 0.003 (0.05/15). P-values were calculated between subgroups with the highest lathosterol and campesterol patterns and other subgroups. Subgroups with the highest lathosterol and campesterol patterns were defined as patients with the highest quartiles of lathosterol and campesterol.
DISCUSSION

The present study found significantly varied responses of LDL-C lowering to one month of atorvastatin treatment in 306 statin-naive patients with high CHD risk. The varied responses of LDL-C lowering were significantly associated with the combined patterns of the cholesterol synthesis marker lathosterol and the cholesterol absorption marker campesterol at baseline. The study results showed that the inhibiting effects of atorvastatin on cholesterol synthesis and related LDL-C lowering were significantly associated with baseline levels of the cholesterol synthesis marker lathosterol. However, the LDL-C-lowering effect of atorvastatin was modified bidirectionally by the baseline cholesterol absorption marker campesterol. Cholesterol absorption decreased among patients with higher baseline cholesterol absorption after statin treatment, which enhanced the effect of LDL-C lowering. Cholesterol absorption significantly increased among patients with lower baseline cholesterol absorption after statin treatment, which greatly attenuated the effect of LDL-C reduction. In brief, our findings suggest that the joint effects of cholesterol synthesis and absorption status at baseline and after statin treatment codetermined the effect of LDL-C lowering by a complicated reciprocal interplay between cholesterol

lowest campesterol levels, patients had a mean increase of 52.3% in campesterol levels (ranging from 42.7% to 74.0%), regardless of baseline lathosterol levels (Fig. 4B), which reduces the effect of LDL-C lowering by atorvastatin. Patients with the highest baseline campesterol levels had a mean decrease of 46.1% in campesterol levels (ranging from 25.5% to 65.8%), which enhances the effect of LDL-C lowering by atorvastatin. Decreased campesterol levels after statin treatment in the high baseline campesterol patients were positively associated with the baseline status of lathosterol. Patients with the highest lathosterol levels had a 65.8% decrease in campesterol levels, which was 1.6 times higher than those with the lowest lathosterol levels. These results indicated that the effect of atorvastatin on lowering LDL-C levels was a combined effect of cholesterol synthesis and absorption status.

In addition, we compared changes in campesterol levels between the quartiles of baseline campesterol after adjusting for baseline campesterol to account for the effect of regression to the mean. We found there was still a significant difference in changes in campesterol between the quartiles of baseline campesterol after adjusting for baseline levels ($P < 0.001$). The difference in changes in lathosterol levels between the quartiles of baseline levels was also significant after adjusting for baseline lathosterol levels ($P = 0.004$).

| TABLE 3. Proportion of patients with a reduction in LDL-C levels less than 30% across baseline cholesterol synthesis/absorption patterns |
|---------------------------------------------------------------|
| **Distribution (%, n)**          | **Baseline Campesterol Quartiles** |
| **Lowest** | **Median** | **Higher** | **Highest** |
| Baseline Lathosterol Quartiles | 37.5 (6/16) | 24.0 (6/25) | 38.9 (7/18) | 55.6 (10/18) |
| Median          | 40.0 (8/20) | 44.4 (12/27) | 50.0 (7/14) | 37.5 (6/16) |
| Higher          | 42.9 (9/21) | 45.5 (5/11) | 21.4 (6/28) | 25.0 (4/16) |
| Highest         | 57.9 (11/19) | 28.6 (4/14) | 31.3 (5/16) | 14.8 (4/27) |

Highest synthesis was defined as the highest quartile of lathosterol, higher synthesis as third quartile, median synthesis as second quartile, and lowest synthesis as the lowest quartile. Highest absorption was defined as the highest quartile of campesterol, higher absorption as third quartile, median absorption as second quartile, and lowest absorption as the lowest quartile.
Several studies reported that baseline cholesterol synthesis and absorption markers had no predictive value for LDL-C response to statins after adjusting for baseline LDL-C level by regression analysis (18–20). One fact that coexisted in all of these previous studies was that both cholesterol synthesis and absorption markers were treated as independent continuous variables. However, when the change in the cholesterol absorption marker after statin treatment is bidirectional, it masks the real relationship between baseline cholesterol synthesis/absorption and LDL-C response to statin. An alternative explanation may be that it is inappropriate to treat baseline LDL-C as a confounding variable when its levels depend directly on the status of cholesterol synthesis/absorption. Moreover, most previous studies adopted the ratio of cholesterol absorption marker to TC as an indicator of the changes in cholesterol absorption after statin treatment (17–20), leading us to speculate that this ratio might not be a proper indicator for cholesterol absorption changes given the relative changes between numerator and denominator after statin treatment.

To find the reason why the cholesterol absorption marker campesterol was reduced after statin treatment among patients with high baseline campesterol level, especially in the setting of high baseline level of cholesterol synthesis marker lathosterol, needs further study. Although Miettinen et al. (27) reported that statin monotherapy leads to a compensatory increase in cholesterol absorption, studies by van Himbergen et al. (18) and us showed that there was a wide individual change in campesterol levels after statin treatment, that the changes were bidirectional, and that baseline levels of campesterol were the strongest predictor of changes in campesterol, suggesting that cholesterol absorption is changed in totally opposite ways after atorvastatin treatment. However, we cannot exclude the possibility that when baseline cholesterol absorption is high enough, the feedback effect of cholesterol absorption will be limited, as LDL-C is reduced by inhibiting the cholesterol synthesis (8, 9). Another possibility is that there are independent regulatory mechanisms.
for cholesterol synthesis and absorption in spite of the balanced reciprocal effect between each other. Despite all of this, it is so far difficult to speculate on the underlying molecular mechanism causing these responses, and further studies are warranted.

Our findings suggest that classifying individuals on the basis of cholesterol synthesis marker and absorption markers can potentially explain the large variation in individual responses to LDL-C-lowering therapy. Patients with high levels of cholesterol synthesis and absorption displayed the most effective LDL-C lowering by statin monotherapy, whereas patients with a low level of cholesterol synthesis and a high level of cholesterol absorption, or with a high level of cholesterol synthesis and a low cholesterol absorption responded poorly to statin monotherapy. These findings indicate the need for combination therapy of statin with cholesterol absorption inhibitors in certain individuals.

Despite the clear strengths of the present study, including the recruitment of subjects free of any previous lipid-lowering treatment, and the assessment of joint patterns of cholesterol synthesis and absorption, our study has several limitations. First, data on normal food intake before enrollment were unavailable, and some studies have even shown that the relationship between dietary intake and blood sterol concentrations is not strong (28, 29). In addition, the typical average dietary cholesterol intake was 150 mg/day in rural Chinese populations (30), which was lower than the target of therapeutic lifestyle changes according to NCEP ATPIII (less than 200 mg/day). Second, there may be other explanations for the individual variation of the LDL-C response to atorvastatin treatment, including altered expression of the cytochrome P450 3A4 and 2C9 pathways and variation in LDL receptor degradation by proprotein convertase subtilisin-like kexin type 9 (PCSK9) (31–35). However, these factors are likely to be responsible only for a small fraction of the observed variation (36, 37). Third, despite plasma plant sterols being currently used as markers of cholesterol absorption, Jakulj and colleagues contrastingly stated that these surrogate markers were poor to represent cholesterol absorption according to the gold-standard stable isotope method (38). Because of the substantial interindividual variation in human intestinal cholesterol absorption, analysis of cholesterol absorption using the stable isotope procedure at a single time point may not reflect 24-h absorption (39). Fourth, given that statin treatment may cause a reduction in biliary cholesterol secretion (40–42), plasma plant sterols may not be appropriate for estimating the total amount of cholesterol absorption. However, our study used campesterol levels as a surrogate to reflect the capacity of cholesterol absorption, and it clearly indicated that changes in campesterol were associated with the effect of LDL-C lowering, even though the role of biliary cholesterol secretion is yet to be defined, which was consistent with a recent study by van Himbergen et al. (18). Our findings require validation in larger well-designed studies using the isotope-labeling method. Fifth, the changes in campesterol might have been due to regression to the mean. Multiple measurements can be used to reduce variability at the study design stage (24), but we did not perform multiple measurements. Therefore, when taking into account the influence of this factor in data analysis, we compared the changes in campesterol levels between the quartiles of baseline campesterol after adjusting for baseline campesterol levels. We found there was still a significant difference in the adjusted changes in campesterol between the quartiles of baseline campesterol, suggesting that this chance occurrence is unlikely to have affected our results. Sixth, the sample sizes in subgroup analyses of this study may not have been large enough to draw firm conclusions. This highlights the need for extensive replication and clinical validation of our results in larger studies. Finally, the fact that our study patients were of Chinese descent may limit the generalization of our findings, indicating the need for further confirmation in other ethnic populations.

Taken together, our results suggest that cholesterol synthesis and absorption markers and their joint patterns might be useful predictors of the cholesterol-lowering response to statins, and thereby, they imply that the combined patterns of cholesterol synthesis and absorption may be a tool that can be exploited in clinical practice to tailor divergent responses to statin treatment, thus providing a personalized medicine approach to select the optimal cholesterol-lowering agents for individual patients. Considering that all study patients were at high risk for CHD, our results might provide a novel insight into the statin administration in the primary and secondary prevention of CHD.

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