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# Gas Chromatography and Flame-Ionization Detection of Non-Cholesterol Sterols as Indicators of Cholesterol Absorption and Synthesis in 158 Chinese Individuals with Normolipidemia, Hyperlipidemia, and Familial Hypercholesterolemia

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**Conflict of interest:** None declared

**Background:** There are limited studies on the effects of cholesterol homeostasis in populations at high risk for cardiovascular disease. We aimed to use gas chromatography and flame-ionization detection (GC-FID) of non-cholesterol sterols as indicators of cholesterol absorption and synthesis. Sterol indicators of cholesterol absorption included campesterol, stigmasterol, and sitosterol. Sterol indicators of cholesterol synthesis included squalene, 7-lathosterol, and desmosterol.

**Material/Methods:** A total of 158 participants were enrolled in 3 groups: healthy control (n=64), hyperlipidemia (n=69), and familial hypercholesterolemia (FH, n=25). Age, sex, blood pressure, blood glucose, and lipoprotein were collected, and cholesterol absorption and synthesis markers were determined by GC-FID.

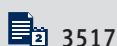
**Results:** All 6 cholesterol concentration indicators, except squalene, were significantly different among the 3 groups (all  $P < 0.05$ ); whereas in the ratio to cholesterol (%), sterols/cholesterol, only desmosterol and lathosterol were significantly different ( $P < 0.05$ ). Multifactorial regression analysis showed that triglycerides, total cholesterol, and desmosterol were independent risk factors affecting the development of hyperlipidemia ( $P < 0.05$ ). The efficacy of the ROC curve for the diagnosis of dyslipidemia was also higher for all 3 indices (Model 1, AUC=0.960). Model 1 was superior to Model 2 for the 6 indicators of cholesterol. For the FH and dyslipidemia groups, the 6-indicator model (Model 3) was shown to have a good diagnostic value (AUC=1.000).

**Conclusions:** The 6 sterol indicators of cholesterol absorption and synthesis had a dynamic course in all study participants. Desmosterol was an indicator of dyslipidemia. The combined use of the 6 sterol indicators differentiated between healthy individuals and patients with dyslipidemia and FH.

**Keywords:** Cholesterol • Metabolism • Absorption • Synthesis

**Abbreviations:** TC – total cholesterol; TG – triglycerides; HDL – high-density lipoprotein; LDL-C – low-density lipoprotein cholesterol; FPGc – fasting plasma glucose; FH – familial hypercholesterolemia

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/934471>



## Background

Dyslipidemia is an important risk factor for atherosclerotic cardiovascular disease; however, the rate of lipid reduction in dyslipidemia is low. Cholesterol in the body comes from absorption and synthesis, and detecting the characteristics of absorption and synthesis of cholesterol is important for understanding individual differences in cholesterol metabolism and guiding clinical drug use [1]. Various markers of cholesterol uptake and synthesis maintain a steady-state of its metabolism through negative regulatory mechanisms that provide a better picture of cholesterol metabolic function than do conventional lipids. Studies have shown inconsistent lipoprotein abnormalities across different patterns of cholesterol homeostasis. The 24-dehydrocholesterol/sitosterol ratio can be used to assess patient dyslipidemia and to guide treatment after disease progression [2]. A study showed that abnormal concentrations of low synthetic and high absorption markers of cholesterol in the blood reflect an impaired homeostatic environment, which is an important independent prognostic factor for the prevalence of cardiovascular disease in the population. Therefore, it is crucial to capture the pattern of cholesterol homeostasis in different populations of patients with cardiovascular disease [3]. The Scandinavian Simvastatin Survival Study [4] showed that a reduction in major coronary events in one-third of patients come from the cholestanol/cholesterol ratio and that cholesterol synthesis markers are decreased and cannot be explained from baseline lipids. The gas chromatography and flame-ionization detection (GC-FID) method has good accuracy and precision in the determination of squalene and 5 non-cholesterol sterols, with good stability at 12 h [5]. Therefore, this study aimed to use GC-FID of non-cholesterol sterols as indicators of cholesterol absorption and synthesis in 158 Chinese individuals with normolipidemia, hyperlipidemia, and familial hypercholesterolemia. Sterol indicators of cholesterol absorption included campesterol, stigmasterol, and sitosterol. Sterol indicators of cholesterol synthesis included squalene, 7-lathosterol, and desmosterol.

## Material and Methods

Written informed consent was obtained from each patient and healthy participant. All methods were carried out following relevant guidelines and regulations, and all experimental protocols were approved by the Ethics Committee of Anzhen Hospital.

### Study Groups

Sixty-nine patients with hyperlipidemia, including 30 men and 39 women, were selected from the outpatient department of Beijing Anzhen Hospital, affiliate of Capital Medical University. The average patient age was  $50.3 \pm 5.9$  years. Dyslipidemia was

defined as total cholesterol  $>5.72$  mmol/L and/or triglyceride  $>1.7$  mmol/L [6]. The control group consisted of 64 healthy participants, including 16 men and 48 women with an average age of  $49.58 \pm 7.18$  years. Patients with liver, kidney, endocrine, and metabolic diseases, which affect blood lipid metabolism, were excluded. Healthy participants had normal blood lipid levels and were qualified by physical examination. Other diseases were excluded on physical examination, and the liver and kidney functions were determined to be normal. A total of 25 patients (13 males and 12 females, ranging in age from 6 to 53 years) with a clinically confirmed familial hypercholesterolemia (FH) were enrolled in the Atherosclerosis Outpatient Clinic. The diagnostic criteria for familial hypercholesterolemia included (1) blood cholesterol concentration  $>7.3$  mmol/L in adults and  $>6.57$  mmol/L in children; (2) the occurrence of yellow tendon tumors in the patient or in first-degree relatives; (3) hypercholesterolemia in first-degree relatives; and (4) hypercholesterolemia detected in childhood in the patient's family members. The diagnosis of FH was made by the presence of inclusion criterion 1 and any of criteria 2 and 4. Blood pressure was measured with a tabletop sphygmomanometer, and body mass index (BMI) was calculated as weight/height ( $\text{kg}/\text{m}^2$ ) [7].

### Biochemical Index Determination

After centrifugation at 4000 r/min for 10 min, the upper plasma was collected and stored at  $-80^\circ\text{C}$ . Total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein cholesterol (LDL-C), and fasting plasma glucose (FPGc) were routinely detected after fasting. Each of the above factors was tested on a fully automated biochemical instrument, and the Friedewald formula was used to calculate LDL-C [8].

### Determination of Cholesterol Absorption and Synthetic Markers

The GC-FID method, which we had established earlier, was used to detect cholesterol synthesis markers (squalene, lathosterol, and desmosterol) and absorption markers (campesterol, stigmasterol, and sitosterol) [5]. For eliminating the effects of continuous cholesterol change, the absorption rate was represented by plant sterols/cholesterol and cholesterol synthesis rate by precursor concentration of cholesterol/cholesterol (unit:  $\mu\text{mol}/\text{mmol},\%$ ). The procedure was as follows: 1 mL of serum was kept frozen at  $-80^\circ\text{C}$  for the determination of cholesterol absorption synthetic markers. The serum underwent saponification by alkaline alcohol solution, extraction by hexane, and silylation before treatment on the machine; the data were analyzed by GC-FID. The conditions for detection included a HP-5 quartz capillary column ( $30 \text{ m} \times 0.32 \text{ mm} \times 0.25 \mu\text{m}$ ); an initial column temperature of  $150^\circ\text{C}$  was held for 3 min, a programmed ramp-up rate of  $30^\circ\text{C}/\text{min}$  to  $250^\circ\text{C}$ , then to

280°C at 5°C/min, was held for 30 min; the hydrogen FID temperature was 290°C; and the inlet temperature was 290°C. The inlet pressure was 15 psi, the non-split mode was used, and the injection volume was 1 µL (increasing). In this study, the relationship between cholesterol absorption and synthesis was investigated in terms of the ratio of campesterol/cholesterol, ratio of sitosterol/cholesterol, and relationship between 7-lathosterol/cholesterol.

## Reagents

Assay kits for total cholesterol, triglycerides, high-density lipoprotein, blood glucose, and alanine aminotransferase (ALT) were obtained from the Zhongsheng Co. Cholesterol, 5 $\alpha$ -cholestane, desmosterol, lathosterol, squalene, campesterol, stigmasterol, and sitosterol were obtained from Sigma-Aldrich. KOH (analytical pure), anhydrous ethanol (chromatographic pure), and n-hexane (chromatographic pure) were obtained from Beijing Chemical Reagent Company. The HMDS: TMCS: pyridine ratio was 3: 1: 9 and was obtained from Supelco Co. The experimental water used was ultrapure water obtained from Millipore.

## Equipment

The following equipment and devices were used: a CRONY 630 semi-automatic biochemical analyzer (CRONY Instruments, Italy); high-speed centrifuge (Shanghai Medical Analysis Instrument Factory); gas chromatograph 7890A (Agilent); electronic balance CP225D (Sartorius, accurate to 0.00001 g); nitrogen blower N-EavPTM111 (Organomation Associates, Inc); centrifuge TDL-60C (Shanghai Anting Scientific Instrument Factory); Hh-4 digital display constant temperature water bath (Jiangsu Jintan Ronghua Instrument Manufacturing Co); LTD. KQ3200 ultrasonic cleaner (Kunshan Ultrasonic Instrument Co); LTD.DHC-90a electric thermostatic drying oven (Shanghai Jinghong Experimental Equipment Co); and LTD.GHK-500 type hydrogen and air generator (Beijing Oriental Jinghuayuan Technology Co, Ltd).

## Statistical Methods

The mean±standard deviation was used to express continuous data that conformed to a normal distribution. For data with a non-normal distribution, we used the median (25%, 75%) to express the data. For the comparison of 3 and more sets of continuous data, the chi-squared test was used; for the comparison of 2 sets of continuous data, the *t* test or non-parity test was used. For count data, the chi-squared test was used. Risk factors for the development of hyperlipidemia were tested using multifactorial regression analysis. The diagnostic value of cholesterol absorption and synthesis markers was also calculated using receiver operating characteristic (ROC) curves. *P*<0.05 was considered statistically significant.

## Results

### Comparison of Basic Group Data

A total of 158 participants were included in this study. There were 64 healthy participants in the control group, with 16 (25.0%) men and 48 (75.0%) in women; 69 patients in the simple dyslipidemia group, with 30 (43.5%) men and 39 (56.5%) women; and 25 patients in the familial hypercholesterolemia group, with 13 (52.0%) males and 12 (48.0%) females. The age distribution of healthy individuals and patients with simple dyslipidemia was 51.0 (45.0, 55.0) years and 52.0±5.8 years, respectively, with no significant difference between the 2 groups (*z*=-0.043, *P*=0.966). The age data of patients with familial hypercholesterolemia were incomplete, and only the approximate range of 6 to 53 years was known. The BMI values of healthy individuals and patients with simple dyslipidemia were 24.33 (21.66, 27.69), and 26.01±3.58, respectively, with no significant difference between the 2 groups (*P*=0.066). Among the lipid-related parameters, HDL-C, LDL-C, triglycerides, and total cholesterol were significantly different overall between the 3 groups (all *P*<0.05). The 2-way comparison showed that there was a statistically significant difference between the 2 groups (all *P*<0.05), except for the HDL-C comparison between the control group and the 2 patient groups (*P*=0.570). Detailed results are shown in **Table 1**.

### Concentration and Ratio of Cholesterol Synthesis and Absorption Markers to Cholesterol in the 3 Groups

Subsequently, we compared and analyzed the concentrations and ratios of cholesterol synthesis and absorption markers to cholesterol in healthy participants and patients with dyslipidemia and FH. The results showed that in the concentration distribution of 5 cholesterol markers (except for squalene [mg/dL]), the comparisons among the 3 groups showed overall statistically significant differences (all *P*<0.05). Moreover, the sum of the 6 indicators was also statistically different among the 3 groups (*P*<0.001). Among sterols/cholesterol (%), only desmosterol (%) and lathosterol (%) were significantly different among the 3 groups (*P*<0.001). Detailed results are shown in **Table 2**.

### Correlation Analysis of Cholesterol Synthesis and Absorption Markers

We then performed a correlation analysis of the ratios of cholesterol absorption and synthesis indicators to cholesterol concentrations in the 3 groups. The results showed that in the healthy control group, the indicators with negative correlations included desmosterol vs squalene (*r*=-0.264, *P*=0.035), lathosterol vs campesterol (*r*=-0.400, *P*=0.001), and lathosterol vs sitosterol (*r*=-0.255, *P*=0.042). Positive correlations were found for campesterol vs sitosterol (*r*=0.715, *P*<0.001) and

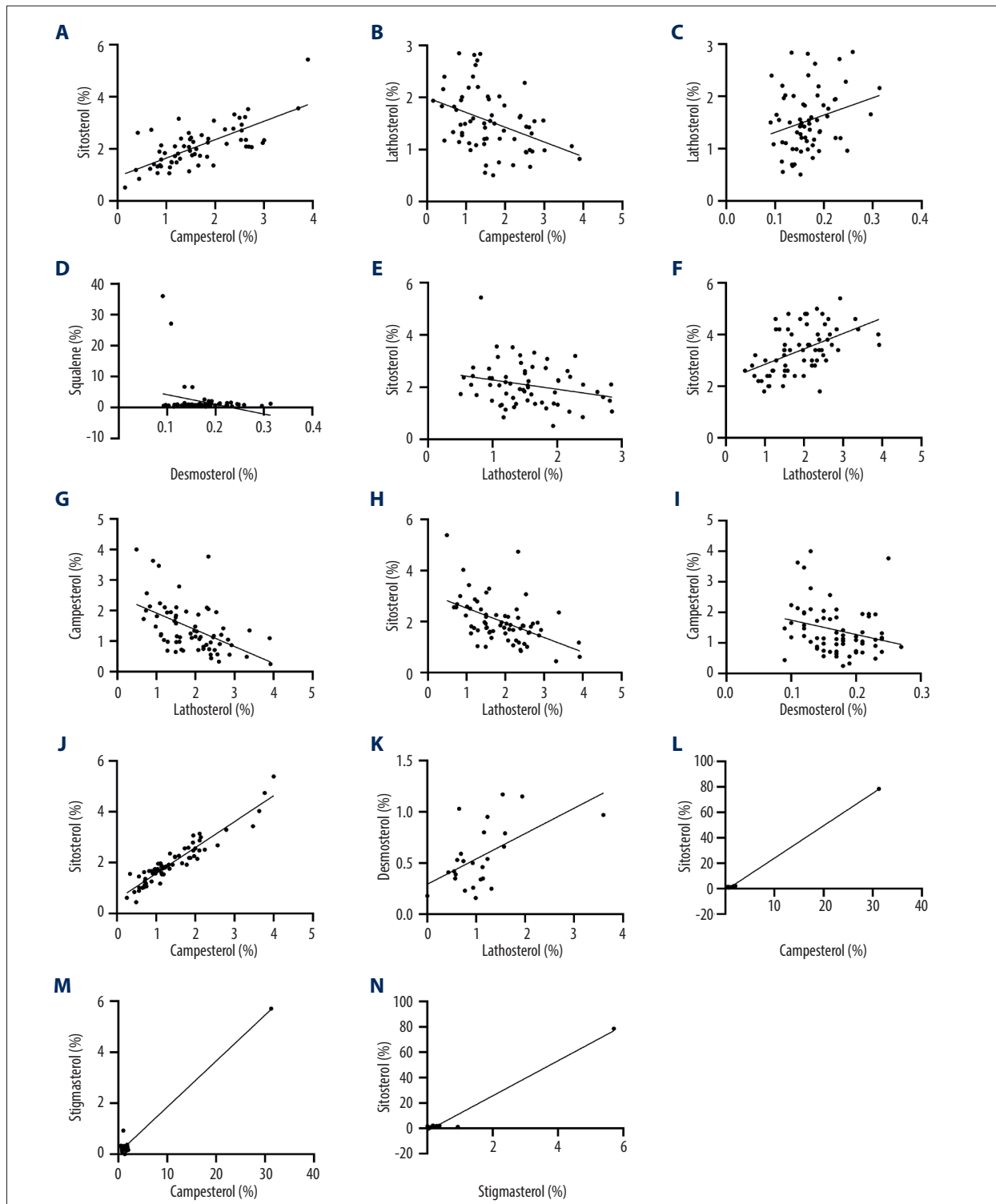
**Table 1.** Levels of high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, total cholesterol, and fasting plasma glucose and blood pressure in healthy group and dyslipidemia and familial hypercholesterolemia groups.

Item	Healthy group (n=64)	Dyslipidemia group (n=69)	FH group (n=25)	F/z	P
Age	51.0 (45.0, 55.0)	52.0±5.8	6-53	-0.043	0.966
Sex (male/female)	16/48	30/39	13/12	7.573	0.023
BMI	24.33 (21.66, 27.69)	26.01±3.58	–	-1.84	0.066
HDL-C (mmol/L)	1.42 (1.30, 1.65)	1.31 (1.13, 1.60)	62.37±32.65	241.69	<0.001
LDL-C (mmol/L)	3.03±0.53	3.38 (3.10, 4.09)	553.70±167.18	756.54	<0.001
TG (mmol/L)	1.20 (0.80-1.40)	2.10 (1.60, 3.50)	108.41±116.50	683.37	<0.001
TC (mmol/L)	4.75±0.65	5.35 (4.89, 6.15)	650.86±179.02	908.11	<0.001
FPG (mmol/L)	5.00 (4.76, 5.36)	5.40 (4.93, 6.00)	–	-3.487	<0.001
SBP (mm Hg)	120.0 (110.0, 120.0)	120.0 (110.0, 130.0)	–	-1.766	0.077
DBP (mm Hg)	80.0 (70.0, 80.0)	80.0 (70.0, 90.0)	–	-1.258	0.208

HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; TG – triglycerides; TC – total cholesterol; FPG – fasting plasma glucose; SBP – systolic blood pressure; DBP – diastolic blood pressure; FH – familial hypercholesterolemia.

**Table 2.** Concentration and ratio of cholesterol synthesis and absorption markers to cholesterol in healthy group and dyslipidemia and familial hypercholesterolemia groups.

Sterols	Concentration (mg/dL)				
	Healthy group (n=64)	Dyslipidemia group (n=69)	FH group (n=25)	F	P
Squalene	0.121 (0.099, 0.152)	0.160 (0.113, 0.254)	0.207 (0.119, 0.583)	0.075	0.928
Desmosterol	0.024±0.007	0.029±0.008	0.322±0.150	260.252	<0.001
Lathosterol	0.207 (0.159, 0.256)	0.322±0.126	0.653±0.416	46.503	<0.001
Campesterol	0.212 (0.156, 0.317)	0.210 (0.140, 0.345)	0.647 (0.382, 1.142)	31.747	<0.001
Stigmasterol	0.049 (0.045, 0.055)	0.051±0.011	0.153 (0.102, 0.230)	27.224	<0.001
Sitosterol	0.305 (0.220, 0.389)	0.340 (0.239, 0.496)	0.802 (0.553, 1.379)	15.16	<0.001
Total	0.995 (0.814, 1.171)	1.212 (0.974, 1.482)	2.826 (2.130, 5.187)	28.439	<0.001
Sterols	Sterols/cholesterol (%)				
	Healthy group (n=64)	Dyslipidemia group (n=69)	FH group (n=25)	F	P
Squalene	0.080 (0.066, 0.111)	0.089 (0.059, 0.153)	0.043±0.017	1.177	0.311
Desmosterol	0.016 (0.014, 0.019)	0.017±0.004	0.050 (0.035, 0.080)	100.475	<0.001
Lathosterol	0.154±0.034	0.191±0.075	0.099 (0.063, 0.127)	14.625	<0.001
Campesterol	0.148 (0.099, 0.240)	0.118 (0.087, 0.193)	0.105 (0.077, 0.163)	1.202	0.303
Stigmasterol	0.032 (0.028, 0.039)	0.028±0.007	0.022 (0.017, 0.032)	1.267	0.285
Sitosterol	0.208 (0.148, 0.251)	0.184 (0.155, 0.245)	0.129 (0.094, 0.174)	1.56	0.213
Total	0.672 (0.549, 0.800)	0.664 (0.575, 0.787)	0.458 (0.382, 0.565)	0.222	0.802



**Figure 1. Correlation of cholesterol absorption factors and synthesis factors in the 3 groups. (A-E)** Health group; **(F-J)** hyperlipidemia group; **(K-N)** familial hypercholesterolemia group. **(A)** campesterol (%) vs sitosterol (%); **(B)** campesterol (%) vs lathosterol (%); **(C)** desmosterol (%) vs Lathosterol (%); **(D)** desmosterol (%) vs squalene (%); **(E)** lathosterol (%) vs sitosterol (%). **(F)** lathosterol (%) vs desmosterol (%); **(G)** lathosterol (%) vs campesterol (%); **(H)** lathosterol (%) vs sitosterol (%); **(I)** desmosterol (%) vs campesterol (%); **(J)** campesterol (%) vs sitosterol (%); **(K)** lathosterol (%) vs sitosterol (%); **(L)** campesterol (%) vs sitosterol (%); **(M)** campesterol (%) vs stigmasterol (%); **(N)** stigmasterol (%) vs sitosterol (%).

**Table 3.** Results of multifactorial regression analysis.

Item	B	SE	Wald	Sig.	Exp (B)	95% CI EXP (B)	
						LL	UL
Sex	0.509	1.021	0.248	0.618	1.663	0.225	12.312
Age (year)	0.025	0.065	0.142	0.706	1.025	0.902	1.165
BMI (kg/m <sup>2</sup> )	0.005	0.109	0.002	0.965	1.005	0.812	1.244
TG (mmol/L)	5.919	1.415	17.497	<0.001	372.170	23.238	5960.466
TC (mmol/L)	4.384	1.419	9.552	0.002	80.197	4.973	1293.413
HDL-C	-0.758	1.109	0.467	0.494	0.468	0.053	4.119
LDL-C	-0.697	1.318	0.280	0.597	0.498	0.038	6.593
FPG (mmol/L)	0.910	0.540	2.842	0.092	2.485	0.862	7.164
SBP (mmHg)	-0.028	0.060	0.227	0.634	0.972	0.864	1.093
DBP (mmHg)	-0.007	0.081	0.007	0.933	0.993	0.847	1.164
Squalene (%)	-0.081	0.084	0.935	0.334	0.922	0.782	1.087
Desmosterol (%)	-35.604	13.950	6.514	0.011	<0.001	<0.001	<0.001
Lathosterol (%)	0.794	0.781	1.034	0.309	2.212	0.479	10.211
Campesterol (%)	-0.457	0.883	0.268	0.605	0.633	0.112	3.572
Stigmasterol (%)	-0.413	1.689	0.060	0.807	0.662	0.024	18.136
Sitosterol (%)	0.460	0.989	0.216	0.642	1.584	0.228	11.006
Constant	-26.228	8.761	8.963	0.003	<0.001		

BMI – body mass index; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; TG – triglycerides; TC – total cholesterol; FPG – fasting plasma glucose; SBP – systolic blood pressure; DBP – diastolic blood pressure; FH – familial hypercholesterolemia; SE – standard error.

desmosterol vs lathosterol ( $r=0.262$ ,  $P=0.037$ ) in the control and dyslipidemia groups (Figure 1A-1E).

In the dyslipidemia group, positive correlations included lathosterol vs desmosterol ( $r=0.520$ ,  $P<0.001$ ) and campesterol vs sitosterol ( $r=0.943$ ,  $P<0.001$ ). Negative correlations were observed for desmosterol vs campesterol ( $r=-0.261$ ,  $P=0.031$ ), lathosterol vs campesterol ( $r=-0.511$ ,  $P<0.001$ ), and lathosterol vs sitosterol ( $r=0.495$ ,  $P<0.001$ ) (Figure 1F-1J).

In the FH group, the indicators with significantly different correlations were all positive. Among the indicators with correlations were desmosterol vs lathosterol ( $r=0.555$ ,  $P=0.004$ ), campesterol vs stigmasterol ( $r=0.986$ ,  $P<0.001$ ), campesterol vs sitosterol ( $r=0.998$ ,  $P<0.001$ ), and stigmasterol vs sitosterol ( $r=0.989$ ,  $P<0.001$ ) (Figure 1K-1N).

### The Results of Multifactorial Regression Analysis

We performed a multifactorial regression analysis on the control group and dyslipidemia group. The results showed that

triglycerides (mmol/L;  $P<0.001$ ), total cholesterol (mmol/L;  $P=0.002$ ), and desmosterol (%;  $P=0.011$ ) were independent risk factors for dyslipidemia in the patients. Details are shown in Table 3.

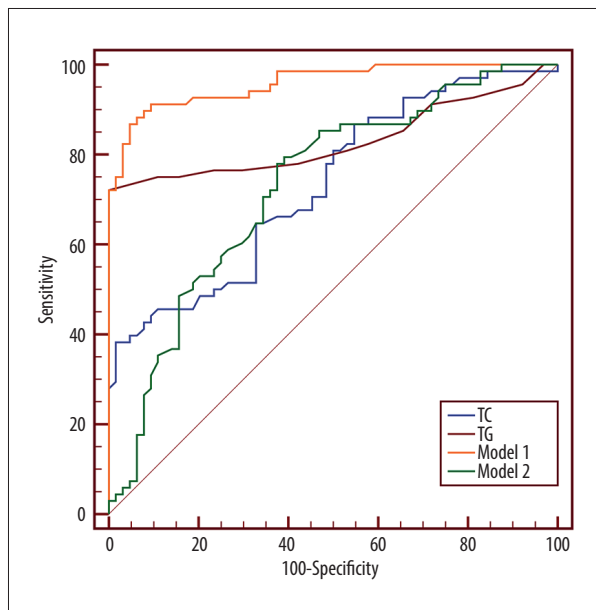
### ROC Curve Results for Healthy Control and Dyslipidemia Groups

We used ROC curves to investigate the diagnostic value of with triglycerides (mmol/L), total cholesterol (mmol/L), and desmosterol (%) in the dyslipidemia group. We also used logistic regression analysis to construct a joint diagnostic model, Model 1, for the 3 groups; it was also compared with Model 2, which was constructed by combining 6 catabolic and synthetic markers of cholesterol with the ratio of cholesterol (%). In this study, triglycerides (mmol/L) had the best diagnostic value (AUC=0.833, sensitivity=72.46%, specificity=100%) when a single factor diagnosis was performed. However, all 3 indicators were not as good as the diagnostic value of Model 1 (AUC=0.960, sensitivity=86.76%, specificity=95.31%), and there were statistically significant differences between Model

**Table 4.** Results of receiver operating characteristic curve for healthy group (n=64) and dyslipidemia group (n=69).

Item	AUC	SE	95% CI	z	P	Youden Index	Associated criterion	Sensitivity (%)	Specificity (%)
TC (mmol/L)	0.730	0.043	0.646-0.803	5.356	<0.001	0.361	>5.73	37.68	98.44
TG (mmol/L)	0.833	0.038	0.759-0.892	8.675	<0.001	0.725	>1.7	72.46	100.00
Desmosterol (%)	0.524	0.051	0.435-0.611	0.470	0.638	0.106	>0.19	30.88	79.69
Model 1	0.960	0.015	0.910-0.986	30.362	<0.001	0.821	>0.61	86.76	95.31
Model 2	0.728	0.044	0.644-0.802	5.138	<0.001	0.404	>-0.17	77.94	62.50

Model 1: TC+TG+desmosterol; Model 2: squalene+desmosterol+lathosterol+campesterol+stigmasterol+sitosterol. AUC – area under the ROC curve; SE – standard error; TC – total cholesterol; TG – triglycerides.



**Figure 2.** Receiver operating characteristic curve comparing the dyslipidemia group and healthy group. Model 1: total cholesterol + triglycerides + desmosterol (%); Model 2: squalene (%) + desmosterol (%) + lathosterol (%) + campesterol (%) + stigmasterol (%) + sitosterol (%).

1 and the other 3 groups. A model consisting of 6 synthetic and catabolic markers of cholesterol had low diagnostic value (AUC=0.728, sensitivity=77.94%, specificity=62.50%). Details are shown in **Table 4** and **Figure 2**.

### Results of the ROC Curve for the Dyslipidemia and FH Groups

We performed statistical analysis of the diagnostic value of the 6 synthetic and absorption markers of cholesterol in the dyslipidemia and FH groups. Except for campesterol (%) and stigmasterol (%), the results showed above-moderate diagnostic

levels for the other 4 indicators (**Table 5**). Model 3, which was constructed with the 6 markers, had a near-perfect diagnostic value (AUC=1.000, sensitivity=100.00%, specificity=100.00%) (**Figure 3**).

### Discussion

The Scandinavian Simvastatin Survival Study [4] showed a reduction in major coronary events in one-third of patients treated with simvastatin. The reduction could not be explained by baseline lipid values but was found to come from the cholesterol/cholesterol ratio; patients with high cholesterol absorption markers and low cholesterol synthesis markers do not benefit from simvastatin therapy alone. Therefore, it is crucial to understand the processes of cholesterol absorption and synthesis and the different homeostatic patterns of cholesterol in different populations.

The detection of cholesterol absorption and synthesis markers is a new method to detect cholesterol metabolism in vivo and evaluate the efficacy of drugs [9], which has been extensively studied elsewhere but has been rarely seen in China. In our study, the 6 sterol indicators of cholesterol absorption and synthesis had a dynamic course in all study participants and were an indicator of dyslipidemia.

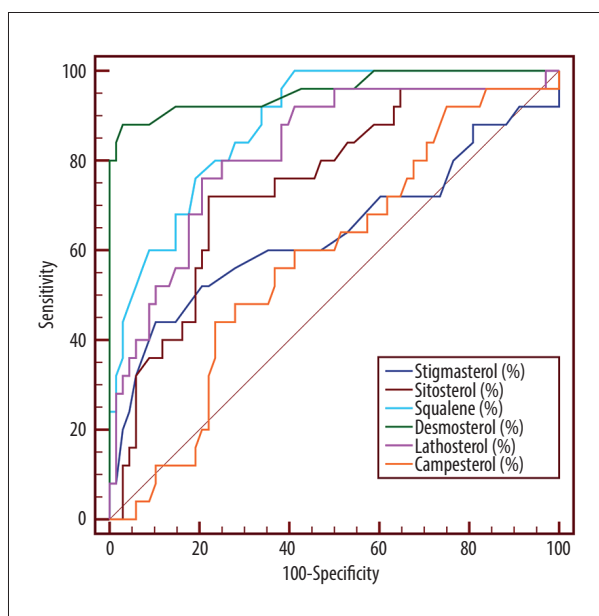
### Distribution of Markers of Cholesterol Absorption and Synthesis and Individual Differences in Cholesterol Metabolism

Cholestanol, a marker of cholesterol absorption, exists in small amounts with cholesterol in the organism and is reduced by cholesterol contact. Like cholesterol, phytosterols are absorbed in the small intestine but are difficult to esterify. The phytosterols absorbed by intestinal mucosal cells are secreted back into the intestinal cavity, and their absorption rate is much lower than

**Table 5.** Results of receiver operating characteristic curve for dyslipidemia group (n=69) and familial hypercholesterolemia group (n=25).

Item	AUC	SE	95% CI	z	P	Youden Index	Associated criterion	Sensitivity (%)	Specificity (%)
Squalene	0.883	0.035	0.799-0.940	1.386	<0.001	0.588	≤0.76	100.00	58.82
Desmosterol	0.956	0.027	0.892-0.988	16.618	<0.001	0.851	>0.24	88.00	97.06
Lathosterol	0.822	0.051	0.729-0.893	6.352	<0.001	0.554	≤1.23	76.00	79.41
Campesterol	0.581	0.065	0.475-0.683	1.249	0.212	0.205	≤0.86	44.00	76.47
Stigmasterol	0.640	0.076	0.534-0.737	1.853	0.064	0.337	≤0.19	44.00	89.71
Sitosterol	0.746	0.059	0.645-0.831	4.184	<0.001	0.499	≤1.53	72.00	77.94
Model 3	1.000	<0.001	0.961-1.000	–	<0.001	1.000	>-14.51	100.00	100.00

Model 3: squalene+desmosterol+lathosterol+campesterol+stigmasterol+sitosterol. AUC – area under the ROC curve; SE – standard error; TC – total cholesterol; TG – triglycerides.



**Figure 3.** Receiver operating characteristic curve comparing familial hypercholesterolemia group and dyslipidemia group.

that of cholesterol [10,11]. The rate of bile excretion is faster than that of cholesterol, so the concentration of phyosterols in the body is much lower than that of cholesterol. The cholesterol synthesis markers are the precursors of cholesterol biosynthesis, which is constantly biotransformed, and the concentration is also low. Therefore, the structure of non-cholesterol sterol in serum is similar to that of cholesterol, but the content of non-cholesterol sterol is much lower than that of cholesterol. The results of the present study are consistent with the report by Baila-Rueda et al [12], in which the sum average value of 6 non-cholesterol sterols was 0.995 mg/dL (0.814, 1.171),

which is about 0.672% (0.549%, 0.800%) of the total cholesterol in healthy individuals. Only Hamilton et al [13] reported that the high and low values of lathosterol were measured according to circadian rhythm ( $5.50 \pm 1.52$  [SD]  $\mu\text{mol/L}$ ,  $6.18 \pm 1.80$  [SD]  $\mu\text{mol/L}$ ), and the values were much higher in infants. The value of lathosterol ( $5.6 \pm 2.2$  [SD]  $\mu\text{mol/L}$ ) in the present experiment was within this range. In addition, the value of lathosterol/cholesterol in the present experiment was  $153 \pm 0.58$   $\mu\text{mol/mmol}$  in ages 30 to 65 years, and in recent foreign studies was  $107 \pm 58$   $\mu\text{mol/mmol}$  in ages 12 to 18 years. A rise in cholesterol synthesis could explain the difference in the decrease of cholesterol absorption with the increase of age. The mean sitosterol values of all non-cholesterol were consistent with those reported by Baila-Rueda et al [12] while the others were significantly different. This can be partly explained by environmental genetics. Studies have shown that the absorption of dietary cholesterol is highly individualized, and different countries, periods, and individuals have different dietary habits [14-16]. In addition, in the present study, the non-cholesterol steroid content was minimal, and experimental errors were partly to blame. Some people had high absorption and some people had high synthesis. Other studies have shown that patients with type 2 diabetes and metabolic syndrome have high cholesterol synthesis [17-19], while patients with type 1 diabetes and familial hypercholesterolemia have high cholesterol absorption [20-22], further explaining the characteristics of individual differences in cholesterol absorption and synthesis.

**Homeostasis of Cholesterol Metabolism and Correlation Between Markers of Cholesterol Absorption and Synthesis**

The overall cholesterol metabolic balance is regulated by dietary cholesterol absorption, endogenous cholesterol synthesis, and bile acid cholesterol secretion [5,23-24], and when any link is



disturbed, this balanced relationship is destroyed. That is, in the healthy human body, cholesterol absorption and synthesis maintain a dynamic balance, when absorption increases, synthesis decreases. It is clear that absorption and synthesis change in opposite directions, maintaining the homeostasis of the internal environment. However, this self-regulation will disappear after reaching a certain high value [25]. In the present study, this was confirmed in healthy controls and in patients with hyperlipidemia and familial hypercholesterolemia. In the healthy control group, campesterol, an absorptive marker of cholesterol, was positively correlated with sitosterol, another absorptive factor; and negatively correlated with lathosterol, a synthesis factor. The synthesis factor desmosterol was positively correlated with another synthesis factor, lathosterol, which was negatively correlated with sitosterol. The various absorption and synthesis factors work together to maintain the dynamic equilibrium of cholesterol. In patients with dyslipidemia, the synthesis factor lathosterol was negatively correlated with the absorption factors campesterol and sitosterol and positively correlated with the synthesis factor desmosterol. Campesterol was negatively correlated with desmosterol and positively correlated with sitosterol. The above results show that in patients with dyslipidemia, the synthesis factor and absorption factor of cholesterol still have mutual adjustment effects. More encouragingly, this relationship disappeared in patients with familial hypercholesterolemia. In patients with FH, the results showed that the absorption factor campesterol was not only positively correlated with stigmasterol and sitosterol but was also positively correlated with the synthesis factor desmosterol ( $P < 0.05$ ). This showed that there was a disorder between cholesterol absorption and synthesis factor in patients with FH. The contents of squalene and the 5 non-cholesterol sterols in the hyperlipidemia group were higher than those in the healthy control group. In familial hypercholesterolemia group, the contents of the 5 non-cholesterol sterols were higher than those in the dyslipidemia group. There were statistically significant differences, except for squalene ( $P < 0.05$ ). The reason may be that squalene is the sterol in the metabolic production is the final metabolite of cholesterol synthesis, but is far less effective than the precursor sterol, so that in the squalene/cholesterol ratio, the synthetic inhibitor in the sterol ester does not change its high content [26-27] in type 2 diabetes and metabolic syndrome and in patients with obesity [18,28-30]. One reason for high cholesterol synthesis, in this case, is that there is no direct cholesterol consumption, but rather the excess of the remaining fatty acids initiates a large amount of cholesterol synthesis. This is possible because of the accumulation of early cholesterol metabolites such as squalene in the serum. However, this is completely different from the actual cholesterol consumption when cholesterol absorption is maladjusted. When cholesterol absorption is maladjusted, precursor sterols like lathosterol are elevated, but squalene is not. In the Scandinavian Simvastatin Survival

Study, low levels of sitosterol, cholestanol, oat sterols, campesterol squalene, and squalene/cholesterol did not change even with a high dose of anti-statin therapy [31]. Synthetic markers and absorption markers of cholesterol are more sensitive to cholesterol metabolism than each marker, which has been pointed out in previous studies on cholesterol synthesis [32]. Moreover, recent studies have shown that increased cholesterol synthesis and absorption is a major cause of coronary heart disease [33].

In this study, we also found that in healthy people and patients with hyperlipidemia, in addition to triglycerides (mmol/L) and total cholesterol (mmol/L), desmosterol (%) was an independent factor affecting the risk of hyperlipidemia in patients. Also, in patients with hyperlipidemia and familial hypercholesterolemia, the model constructed by the 6 factors of cholesterol can be used for the differential diagnosis of these 2 groups of patients. This is consistent with recent studies which have reported that it suggests the existence of different lipoprotein abnormalities according to various patterns of cholesterol homeostasis. The desmosterol/ $\beta$ -sitosterol ratio could be used for estimating an individual propensity toward dyslipidemia development and direct future treatment [2].

This study has some limitations, including (1) the small sample size of this experiment limits the conclusions of this study to some extent; (2) the study needs to be further developed in different disease populations; (3) the participants in this study were from a single center, and a multicenter study is needed to make the conclusions more reliable; and (4) GC-FID is much more complex than routine lipid testing and needs to be simplified so that it can be carried out in large numbers.

## Conclusions

There are individual differences between absorption and synthesis of cholesterol and diverse characteristics in different individuals and patients. In the healthy population and in patients with hyperlipidemia, absorption synthesis changes in the opposite direction up to a point where this relationship disappears, as in healthy individuals with hyperlipidemia, whereas in FCH, this relationship disappears. Different people have different characteristics of cholesterol absorption and synthesis; therefore, lipid-lowering drugs can be selected according to the characteristics of absorption and synthesis to achieve a therapeutic effect. In this study of 158 Chinese individuals with normolipidemia, hyperlipidemia, and familial hypercholesterolemia, the 6 sterol indicators of cholesterol absorption and synthesis had a dynamic course in all study participants. Desmosterol was an indicator of dyslipidemia. The combined use of the 6 sterol indicators differentiated between healthy individuals and patients with dyslipidemia and FH.

The results of this study may aid understanding of the characteristics of cholesterol absorption and synthesis in various populations and patients with various diseases, and aid in selecting cholesterol absorption or synthesis inhibitors to achieve therapeutic effect according to the situation, thereby providing a new vision for the treatment of lipid-regulating diseases.

### Data Availability

All data associated with this study are presented in the paper, and all figures and tables have associated raw data. Any materials that can be shared will be released via a material transfer agreement. The images are original with no duplication and have not been previously published in whole or in part. Written informed consent was obtained from each patient. This study was approved by the Ethics Committee of Anzhen Hospital.

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### Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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