

Original Article

Novel findings in relation to multiple anti-atherosclerotic effects of XueZhiKang in humans

Rui-Xia Xu^a, Yan Zhang^a, Yuan-Lin Guo, Chun-Yan Ma, Yu-Hong Yao, Sha Li, Xiao-Lin Li, Ping Qing, Ying Gao, Na-Qiong Wu, Cheng-Gang Zhu, Geng Liu, Qian Dong, Jing Sun, Jian-Jun Li**Division of Dyslipidemia, State Key Laboratory of Cardiovascular Disease, Fu Wai Hospital, National Center for Cardiovascular Disease, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, China*

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Abstract

Background: Previous studies have clearly demonstrated that XueZhiKang (XZK), an extract of cholestin, can decrease low-density lipoprotein cholesterol (LDL-C) and cardiovascular events. However, the mechanism of the effects of XZK on atherosclerosis (AS) in humans has been reported less frequently. In the present study, we investigated the impact of XZK on lipoprotein subfractions, oxidized LDL (oxLDL), and interleukin-6 (IL-6).

Methods: From October 2015 to July 2016, 40 subjects were enrolled in this study. Of them, 20 subjects with dyslipidemia received XZK 1200 mg/day for 8 weeks (XZK group); 20 additional healthy subjects who did not receive therapy acted as controls. The plasma lipoprotein subfractions, oxLDL, and IL-6 were examined at baseline and again at 8 weeks.

Results: Data showed that XZK could significantly decrease not only plasma LDL-C levels (87.26 ± 24.45 vs. 123.34 ± 23.99 , $P < 0.001$), total cholesterol (4.14 ± 0.87 vs. 5.08 ± 1.03 , $P < 0.001$), triglycerides (0.95 ± 0.38 vs. 1.55 ± 0.61 , $P < 0.05$), and apolipoprotein B (1.70 ± 0.35 vs. 1.81 ± 0.72 , $P < 0.05$), but also oxLDL (36.36 ± 5.31 vs. 49.20 ± 15.01 , $P < 0.05$) and IL-6 (8.50 ± 7.40 vs. 10.40 ± 9.49 , $P < 0.05$). At the same time, XZK reduced the concentration of small LDL-C (1.78 ± 2.17 vs. 6.33 ± 7.78 , $P < 0.05$) and the percentage of the small LDL subfraction (1.09 ± 1.12 vs. 3.07 ± 3.09 , $P < 0.05$).

Conclusions: Treatment with 1200 mg/day XZK for 8 weeks significantly decreased the atherogenic small LDL subfraction and reduced oxidative stress and inflammatory markers, in addition to affecting the lipid profile, suggesting multiple beneficial effects in coronary artery disease.

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Keywords: XueZhiKang; Hyperlipidemia; Low-density lipoprotein cholesterol subfraction; Oxidized LDL; Interleukin-6

* Corresponding author.

E-mail address: lijianjun938@126.com (J.-J. Li).

^a The first two authors contributed equally to this study.

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Introduction

Atherosclerotic cardiovascular disease (ASCVD) is a well-known life-threatening multi-factorial disease that has become the primary cause of morbidity and mortality worldwide, including in China.¹ Previous

studies have confirmed that dyslipidemia, especially increased low-density lipoprotein (LDL) cholesterol (LDL-C) levels, is the most important risk factor for ASCVD, including coronary artery disease (CAD).^{2,3} ASCVD is a well-known inflammatory disease that is also related to oxidative stress.⁴ Previous data have shown that inflammation is an important feature of atherosclerotic lesions.^{5,6} Increased levels of inflammatory markers have been documented in various settings of coronary artery disease.^{7,8} Moreover, oxidized LDL (oxLDL) as an oxidized stress biomarker plays a critical role in atherogenesis.^{9,10} Ox-LDL can induce endothelial cell apoptosis and reduce antioxidant capabilities through changes in the secretory activities of the endothelium.¹¹ Hence, the mechanism, which can modify multiple atherosclerotic pathways, may be important for clinical practice.

In fact, LDL and high-density lipoprotein (HDL) both consist of a heterogeneous group of particles differing not only in size and density, but also in chemical composition and physiological function.^{12–14} Recent studies have suggested that lipoprotein subfractions or particles may be more promising markers for the prediction of future cardiovascular events (CVE). Our previous observations indicated that the changes in lipoprotein subfractions are associated with inflammatory markers, diabetes, hypertension, and clinical outcomes, suggesting that a drug that can modify the lipoprotein subfractions may be more promising for the prevention and treatment of ASCVD.

XueZhiKang (XZK) is an extract of cholestin, which contains a combination of plant sterols, isoflavones, and lovastatin; each 1200 mg XZK capsule contains approximately 10 mg lovastatin. It has shown lipid-lowering effects comparable to those induced by statins.^{15,16} Previous studies have demonstrated that XZK is safe and effective for the prevention and treatment of coronary heart disease in elderly patients.^{17,18} However, no data are currently available concerning the impact of XZK on multiple anti-atherosclerotic pathways. The aim of the present study was to investigate the potential effects of XZK on lipoprotein subfractions in humans as a novel lipid marker, on oxLDL as an oxidative stress parameter, and on interleukin-6 (IL-6) as a pro-inflammatory factor.

Methods

Study design and population

The study protocol was reviewed and approved by the Ethics Committee of Fu Wai Hospital and Cardiovascular Institute, Beijing, China, and informed consent

was obtained from all patients. This prospective study was conducted from October 2015 to July 2016. The XZK group of 20 patients with dyslipidemia received XZK 1200 mg/day for 8 weeks; 20 healthy subjects who had not received any drug treatment previously were enrolled as the control group. Serum lipid profiles and HDL and LDL subfractions were measured at baseline and again after 8 weeks of treatment.

The inclusion criteria of the present study were as follows: (1) patients with dyslipidemia (fasting total cholesterol (TC) \geq 5.18 mmol/L and/or triglyceride (TG) \geq 1.70 mmol/L), without diagnostic imaging evidence of atherosclerotic lesions detected by arterial ultrasound, coronary chest tomography, or coronary angiography; (2) no history of treatment with statins or other drugs known to affect blood lipids within the previous 4 weeks; and (3) aged 18–70 years. Patients with previous acute coronary syndrome within 1 month, serious heart failure or arrhythmia, infectious disease within 1 month, serious liver or renal dysfunction, autoimmune disease, malignant disease, pregnancy or lactation, or psychiatric disorders, were excluded from this study. In addition, patients with laboratory values >3 times the upper limit of normal (ULN) for aspartate aminotransferase or alanine aminotransferase, or >5 times the ULN for creatine phosphokinase, were also excluded.

Laboratory examinations

Blood samples were obtained from the cubital vein at both baseline and 8 weeks after fasting overnight. All samples were subsequently stored at -80°C and analyzed immediately after thawing. The concentrations of plasma TC, TG, HDL-C, LDL-C, apolipoprotein A-I (ApoA-I), and apolipoprotein B (ApoB) were measured using an automatic biochemistry analyzer (Hitachi 7150, Tokyo, Japan). TC, TG, HDL-C, and LDL-C levels were measured using enzymatic assays. ApoA-I and ApoB levels were measured using turbidimetric immunoassays. Plasma oxLDL levels were detected by a sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Merckodia, Uppsala, Sweden). The detection limit was 0.6 mU/L. Plasma IL-6 concentrations were measured using a high-sensitivity quantitative sandwich ELISA (Quantikine ELISA kit, R&D Systems, Inc., Minneapolis, MN, USA). The mean minimum detectable dose of IL-6 was 0.070 pg/ml.

HDL and LDL subfraction analysis

Blood samples were also used for subfraction analysis. HDL subclass analysis was performed

electrophoretically using high-resolution 3% polyacrylamide gel tubes and the Lipoprint HDL/LDL System (Quantimetrix Corp., Redondo Beach, CA, USA) according to the manufacturer's instructions.^{19,20} HDL was divided into 10 subfractions for this analysis. Subfractions 1–3 included large HDL particles, subfractions 4–7 included intermediate HDL particles, and subfractions 8–10 included small HDL particles. The cholesterol concentration (mg/dl) of each HDL subfraction and the percentage of the cholesterol concentration of each HDL subfraction over the HDL-C concentration were subsequently determined.

LDL was divided in 7 subfractions for this analysis. Subfraction 1 included large LDL particles; subfraction 2 included intermediate LDL particles, and subfractions 3–7 included small LDL particles. The mass (mg/dl) of each subfraction and the percentage of the concentration of each LDL subfraction in the total LDL-C concentration were subsequently determined.²¹

Statistical analysis

Data are expressed as mean ± standard deviation for continuous variables and number (percentage) for categorical variables. Both the Student's *t*-test and the Mann–Whitney *U* test were used to compare clinical parameters between the two groups. Categorical variables were compared with a χ^2 test. Differences between the baseline and post-treatment values of the parameters studied were evaluated using a paired samples *t*-test. Statistical significance was defined as *P* < 0.05. Statistical analysis was performed using SPSS Statistics for Windows, Version 19.0 (IBM Corp., Armonk, NY, USA).

Results

Clinical characteristics

The baseline clinical characteristics of the study subjects are summarized in Table 1. All baseline characteristics were well matched between the two groups.

Effects of XZK on lipid parameters

As shown in Table 2, an 8-week treatment of 1200 mg/day XZK decreased the serum levels of TC, TG, ApoB, and LDL-C compared to the baseline (*P* < 0.05). Meanwhile, there were no significant changes in the serum levels of ApoA-I or HDL-C after XZK treatment. There were no changes in any serum lipid parameters in the control group after 8 weeks.

Table 1
Baseline clinical characteristics of the study subjects.

Group	Demographic		Clinical profiles				Laboratory values							
	Age (years)	Men	BMI (kg/m ²)	Hypertension	Diabetes	Family history of CHD	Smoker	WBC count (× 10 ⁹ /L)	Neutrophils (× 10 ⁹ /L)	ESR (mm/h)	ALT (U/L)	AST (U/L)	Cr (μmol/L)	BUN (mmol/L)
Control (n = 20)	54.7 ± 7.0	8 (40.0)	25.1 ± 3.0	5 (25.0)	2 (10.0)	1 (5.0)	6 (30.0)	6.4 ± 1.6	3.6 ± 1.0	9.9 ± 8.2	23.7 ± 11.7	18.8 ± 8.3	68.4 ± 8.8	5.2 ± 1.5
XueZhiKang (n = 20)	56.0 ± 6.8	7 (35.0)	24.9 ± 3.1	6 (30.0)	2 (10.0)	1 (5.0)	8 (35.0)	6.0 ± 1.3	3.5 ± 0.9	10.1 ± 8.3	22.3 ± 9.7	18.9 ± 6.7	66.6 ± 5.4	5.0 ± 1.9

Data are presented as mean ± standard deviation or n(%).

BMI: body mass index; CHD: coronary heart disease; WBC: white blood cell; ESR: erythrocyte sedimentation rate; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Cr: creatinine; BUN: blood urea nitrogen.

Table 2

The effects of XueZhiKang on lipid parameters, lipoprotein subfraction, oxLDL, and plasma IL-6 levels.

Variable	Control (n = 20)		XueZhiKang (n = 20)	
	Baseline	8 weeks	Baseline	8 weeks
TC (mmol/L)	4.86 ± 1.11	4.89 ± 0.97	5.08 ± 1.03	4.14 ± 0.87 ^a
TG (mmol/L)	1.46 ± 0.82	1.51 ± 0.93	1.55 ± 0.61	0.95 ± 0.38 ^b
ApoA-I (g/L)	1.42 ± 0.35	1.38 ± 0.29	1.81 ± 0.72	1.70 ± 0.35
ApoB (g/L)	1.08 ± 0.19	1.11 ± 0.26	1.03 ± 0.16	0.83 ± 0.17 ^b
HDL-C (mg/dl)	47.56 ± 8.84	46.93 ± 9.11	61.56 ± 20.30	64.26 ± 21.50
Large HDL-C (mg/dl)	19.84 ± 8.56	19.68 ± 7.95	23.33 ± 13.30	24.78 ± 13.89
Intermediate HDL-C (mg/dl)	20.75 ± 4.71	20.21 ± 5.13	29.22 ± 7.76	29.78 ± 7.82
Small HDL-C (mg/dl)	6.69 ± 2.18	7.11 ± 2.14	8.89 ± 2.93	10.00 ± 2.96
Large HDL (%)	41.07 ± 10.25	40.38 ± 9.69	35.67 ± 10.57	36.38 ± 9.45
Intermediate HDL (%)	43.68 ± 8.69	43.21 ± 7.53	48.46 ± 4.93	47.32 ± 5.20
Small HDL (%)	15.71 ± 4.21	16.51 ± 5.14	15.78 ± 6.52	16.23 ± 5.12
LDL-C (mg/dl)	101.48 ± 20.75	102.46 ± 21.48	123.34 ± 23.99	87.26 ± 24.45 ^a
Large LDL-C (mg/dl)	30.50 ± 7.65	30.00 ± 7.81	21.56 ± 5.57	18.78 ± 3.87
Intermediate LDL-C (mg/dl)	14.08 ± 8.25	14.09 ± 8.65	12.67 ± 7.71	9.11 ± 4.81
Small LDL-C (mg/dl)	2.50 ± 2.47	2.55 ± 2.58	6.33 ± 7.78	1.78 ± 2.17 ^b
Large LDL (%)	15.84 ± 2.93	15.67 ± 3.01	10.61 ± 1.94	11.97 ± 2.27
Intermediate LDL (%)	7.26 ± 3.95	7.30 ± 4.14	6.51 ± 3.63	5.72 ± 2.58
Small LDL (%)	1.34 ± 1.10	1.35 ± 1.16	3.07 ± 3.09	1.09 ± 1.12 ^b
oxLDL (U/L)	47.34 ± 11.00	47.43 ± 11.08	49.20 ± 15.01	36.36 ± 5.31 ^b
IL-6 (pg/ml)	4.82 ± 3.91	4.69 ± 4.15	10.40 ± 9.49	8.50 ± 7.40 ^b

^a $P < 0.001$, ^b $P < 0.05$, compared with baseline data.

Data are presented as mean ± standard deviation.

TC: total cholesterol; TG: triglycerides; ApoA-I: apoprotein AI; ApoB: apoprotein B; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; oxLDL: oxidized low-density lipoprotein cholesterol; IL-6: interleukin-6.

Changes in HDL and LDL subfractions after XZK treatment

The effects of 8 weeks of treatment with 1200 mg/day XZK on the cholesterol concentration of lipoprotein subfractions, oxLDL, and IL-6 are summarized in Table 2. There were no significant changes in the cholesterol concentration of any of the HDL subfractions or the percentage of each HDL subfraction (Fig. 1B and D; all, $P > 0.05$) after 8 weeks. There were also no significant differences in the concentrations of the HDL subfractions between the baseline and 8 weeks in the control group (Fig. 1A and C; all, $P > 0.05$). In contrast, there was a significant decrease in both the concentration and the percentage of small LDL-C (Fig. 2B and D; both, $P < 0.05$). However, there was no change in the cholesterol level or the percentage of each LDL subfraction between the baseline and the end of the study in the control group (Fig. 2A and C; both, $P > 0.05$).

Changes in oxLDL and IL-6 levels after XZK treatment

The changes in the plasma oxLDL and IL-6 levels in patients following XZK treatment, and in control

subjects that did not receive therapy, are provided in Table 2 and Figs. 3 and 4. The data show that there was no change in the oxLDL levels of the control group between the baseline and 8 weeks. In contrast, there was a significant decrease in the oxLDL level of the XZK group after 8 weeks ($P = 0.013$; Table 2, Fig. 3).

Similarly, treatment with 1200 mg/day XZK for 8 weeks significantly decreased the plasma IL-6 concentration in patients with dyslipidemia, while there was no change in the control group after 8 weeks ($P = 0.017$; Table 2, Fig. 4).

Correlations of plasma IL-6 levels with lipid profile and lipoprotein subfractions

The Pearson correlation analysis revealed that plasma IL-6 correlated positively with small LDL-C and the percentage of small LDL-C, both at baseline ($r = 0.865$, $P = 0.006$; $r = 0.716$, $P = 0.046$, respectively) and after 8 weeks of XZK treatment ($r = 0.905$, $P = 0.002$; $r = 0.849$, $P = 0.008$, respectively). IL-6 levels after 8 weeks of XZK treatment were also determined to be positively associated with LDL-C ($r = 0.833$, $P = 0.010$) (Table 3). However, there were no significant associations between IL-6 levels and any HDL subfraction.

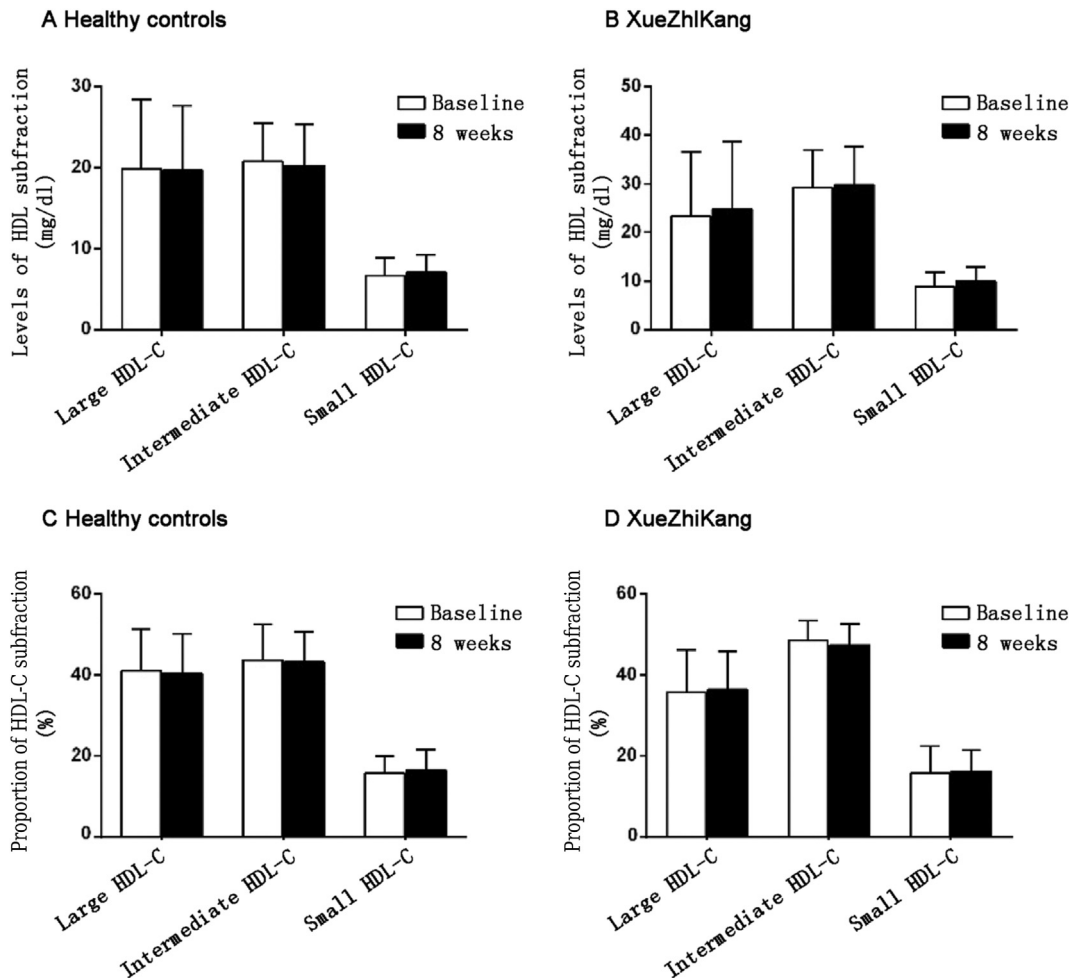


Fig. 1. Effects of XueZhiKang on the HDL-C level and HDL subfractions. There was no significant difference in the cholesterol concentration of any HDL subfraction between the baseline and 8-week follow-up in the control group (A) or the XueZhiKang group (B) (all, $P > 0.05$). There was no significant difference in the percentage of any HDL subfraction between the baseline and the 8-week follow-up in the control group (C) or the XueZhiKang group (D) (all, $P > 0.05$).

Discussion

XZK treatment can decrease the occurrence of cardiovascular events in CAD patients with or without dyslipidemia by significantly reducing blood lipid levels, and fewer adverse reactions have been noted.^{22,23} The effects of XZK on the plasma lipid profile have been reported previously, but the impacts of XZK on the distribution of HDL and LDL subfractions have not been investigated, which indicates that a different mechanism may underlie the effect of XZK on the reduction of cardiovascular events. In the present study, our data indicated for the first time that a treatment of 1200 mg/day for 8 weeks significantly decreased the concentration of small LDL-C and the percentage of the small LDL subfraction, with the

exception of favorable effects on LDL-C. It had no impact on the cholesterol concentration of any HDL subfraction in patients with hyperlipidemia. Additionally, XZK significantly reduced plasma oxLDL and IL-6 levels in patients with dyslipidemia. Moreover, IL-6 levels were correlated with low LDL-C and the percentage of the small LDL subfraction both at baseline and after XZK treatment.

XZK, an extract of red yeast rice, is a traditional Chinese medicine with multiple cardio-protective effects.^{24,25} It is composed of a family of natural statins, unsaturated fatty acids, and other substances and has been demonstrated to be a natural lipid-modulating polyphill.²⁶ Chen et al found that 1200 mg/day XZK treatment for 4 weeks significantly decreased the levels of TC, LDL-C, and non-HDL-C in older patients with

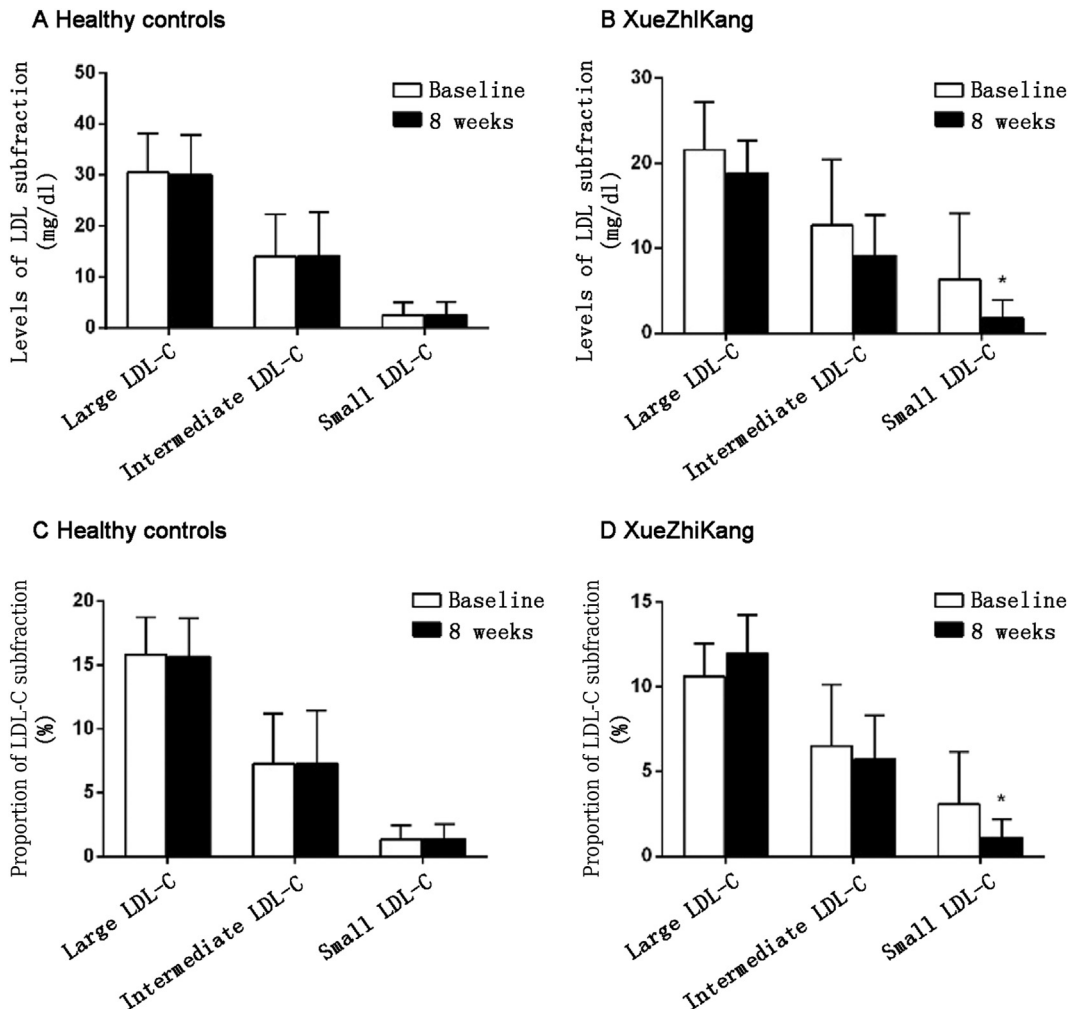


Fig. 2. Effects of XueZhiKang on LDL-C level and LDL subfractions. There was no significant difference in the cholesterol concentration of any LDL subfraction between the baseline and 8-week follow-up in the control group (A) ($P > 0.05$). XueZhiKang treatment for 8 weeks significantly decreased the concentration of small LDL-C (B) ($P < 0.05$). In the control group, there was no significant difference between the percentage of any LDL subfraction at the baseline and after 8 weeks (C) ($P > 0.05$). XueZhiKang treatment for 8 weeks significantly decreased the percentage of small LDL-C (D) ($P < 0.05$).

CAD.²⁷ Another study by Moriarty et al demonstrated that 1200 or 2400 mg/day XZK treatment for 8–12 weeks significantly decreased the levels of non-HDL-C, LDL-C, and ApoB in patients with dyslipidemia but no coronary heart disease, and the tolerance was better than that of statin in therapy groups.²³ Recently, clinical studies indicated that XZK could be used as an alternative therapy for patients intolerant of statins, or could be combined with statins to improve the lipid-lowering effects.^{28,29} In our study, XZK significantly decreased plasma LDL-C, TC, TG, and ApoB concentrations, although there were no significant differences in HDL-C or ApoA-I levels, which was consistent with previous studies.

Recently, the failure of several HDL-C-raising drugs in clinical studies has greatly challenged the traditional concept of the inverse relationship between the HDL-C and cardiovascular outcomes.^{30,31} The emerging opinion is that the quality of the HDL particles may be more important than the quantity of HDL.³² In a recent study, Goliash et al demonstrated an inverse association of the large HDL subfraction and a positive association of intermediate and small HDL subfractions with premature CAD.¹⁹ Data from our group revealed that higher small HDL-C, but not large, medium, or total HDL-C, is associated with higher cardiovascular risk, highlighting the potential benefit of the HDL subfraction in patients with stable

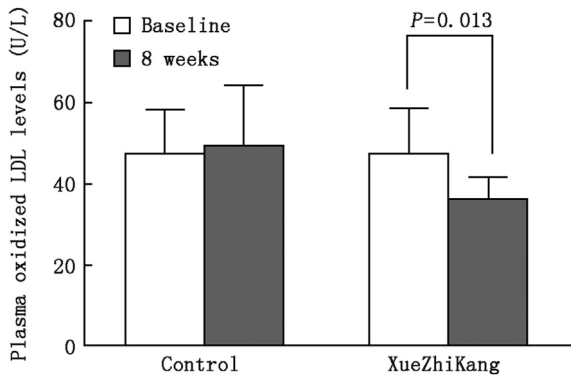


Fig. 3. Effects of XueZhiKang on plasma oxidized LDL levels in patients with dyslipidemia and control subjects. XueZhiKang treatment for 8 weeks significantly decreased oxidized LDL levels ($P = 0.013$).

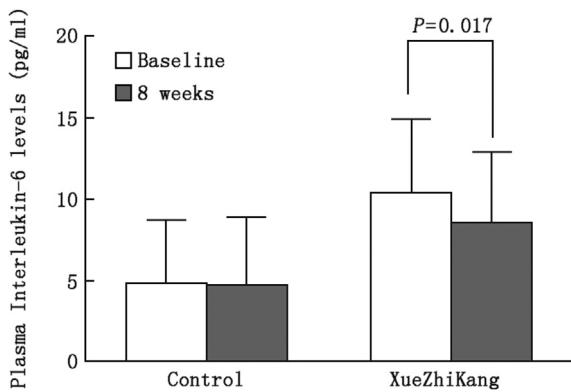


Fig. 4. Effects of XueZhiKang on plasma interleukin-6 levels in patients with dyslipidemia and control subjects. XueZhiKang treatment for 8 weeks significantly decreased interleukin-6 levels ($P = 0.017$).

CAD.³³ Until now, there have been no reports available on the impact of XZK on the HDL subfraction. In the present study, although the effect of XZK on the HDL subfraction was first examined, no significant change was found. Although the reason cannot be determined from the present study, the short term and low dose of XZK, in addition to the small sample size, may be the explanations, suggesting further study may be needed.

Lowering the LDL-C has convincingly been shown to reduce major adverse cardiovascular events.³⁴ Nevertheless, recent studies have demonstrated that the content of LDL particles in LDL-C exhibits considerable individual variation, and patients with the same LDL-C levels but different amounts of LDL particles differ in terms of their absolute risk of CAD.³⁵ The differences in LDL particles lead to the recognition of two distinct phenotypes: phenotype A,

associated with large, buoyant LDL particles (Ib-LDL), and phenotype B, in which small, dense LDL particles (sd-LDL) predominate.³⁶ Presently, the small LDL particles have been recognized as more atherogenic than the large ones, and can predict atherosclerosis progression and incident coronary heart disease.³⁷ We previously indicated that the levels of intermediate and small LDL-C and LDL subfraction percentages are closely associated with CAD risk.³³ Jia et al demonstrated that 1200 mg/day XZK treatment significantly increased the concentration of proprotein convertase subtilisin/kexin type 9 (PCSK9) in patients with dyslipidemia, accompanied by a marked decrease in total cholesterol and LDL-C.¹⁶ Moreover, data from our previous study showed that plasma PCSK9 levels were significantly and independently associated with the cholesterol concentrations of the intermediate and small LDL subfractions, as well as mean LDL particle size, in patients with stable CAD.^{38,39} In the present study, our data showed that XZK treatment of 1200 mg/day for 8 weeks significantly decreased the concentration of small LDL-C and the percentage of the small LDL subfraction, which confers a favorable effect in terms of plasma LDL subfraction distribution; these findings provide novel information with regard to the clinical implications of XZK.

The oxidation of LDL is a key process in the early progression of atherosclerotic diseases.⁴⁰ oxLDL, derived from LDL-C under oxidative stress, encompasses many atherogenic properties. oxLDL levels may be used to differentiate patients with CAD from healthy cohorts⁴¹ and serve as a predictor of future myocardial infarction in patients with unstable CAD.⁴² The effect of XZK on oxLDL levels in patients with CAD has rarely been reported. A study by Yao et al showed that the circulating ox-LDL concentration in patients with unstable angina pectoris was reduced after treatment with XZK for 2 months at a dose of 1200 mg/day. In the present study, our data demonstrated that XZK treatment of 1200 mg/day for 8 weeks significantly decreased the plasma oxLDL levels in patients with dyslipidemia. Therefore, XZK may achieve its anti-atherosclerotic effect by decreasing the oxLDL levels and inhibiting the oxidative stress process, which are involved in the initiation of atherosclerosis.⁴³

In addition to its impact on blood lipids and lipoprotein subfractions, XZK can attenuate inflammatory factors, as demonstrated by previous studies. A study suggested that 1200 mg/day XZK treatment for 8 weeks significantly decreased the serum levels of

Table 3

The relationship between plasma IL-6 levels and lipoprotein subfractions in patients with dyslipidemia.

Variable	Baseline		XueZhiKang for 8 weeks	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
HDL-C (mg/dl)	−0.173	0.682	−0.306	0.460
Large HDL-C (mg/dl)	−0.300	0.470	−0.345	0.403
Intermediate HDL-C (mg/dl)	0.011	0.979	−0.162	0.701
Small HDL-C (mg/dl)	0.069	0.871	0.023	0.957
Large HDL (%)	−0.373	0.363	−0.367	0.371
Intermediate HDL (%)	0.626	0.097	0.444	0.271
Small HDL (%)	0.136	0.748	0.243	0.563
LDL-C (mg/dl)	0.556	0.153	0.833	0.010
Large LDL-C (mg/dl)	−0.079	0.853	0.248	0.553
Intermediate LDL-C (mg/dl)	0.683	0.062	0.669	0.070
Small LDL-C (mg/dl)	0.865	0.006	0.905	0.002
Large LDL (%)	−0.432	0.285	−0.085	0.842
Intermediate LDL (%)	0.414	0.308	0.505	0.202
Small LDL (%)	0.716	0.046	0.849	0.008

The results of univariate correlation analysis are shown.

IL-6: interleukin-6; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

high-sensitivity C-reactive protein (Hs-CRP) and matrix metalloproteinase-9 (MMP-9), as well as the LDL-C concentration in patients with acute coronary syndrome.⁴⁴ Another study showed that XZK treatment for 2 weeks rapidly reduced the levels of Hs-CRP and MMP-9 without changing the lipid levels, suggesting that the anti-inflammatory action of XZK occurs prior to its effect on lipids. This action may play an important role in the early-stage treatment of ACS patients.⁴⁵ In the present study, we found that 1200 mg/day XZK treatment for 8 weeks notably decreased the IL-6 levels in patients with atherosclerosis, which was similar to the study performed in patients with cardiac syndrome X.⁴⁶ In addition, our previous study demonstrated that systemic inflammatory markers were positively correlated with small LDL cholesterol and LDL scores, indicating the potential interactive contribution of these factors to increased cardiovascular risk.⁴⁷ Based on this information, we further analyzed the association of plasma IL-6 levels and lipoprotein subfractions, and found that IL-6 levels were positively correlated with small LDL-C and the percentage of the small LDL subfraction in patients with CAD both at baseline and after XZK treatment, while no such changes were found in the control group. Briefly, our findings showed that XZK reduced the plasma IL-6 concentration and had a beneficial impact on small LDL-C and the percentage of the small LDL subfraction, indicating that some parallel interaction exists between the IL-6 and LDL subfractions.

The present study had several limitations. First, the study included a relatively small sample size

from a single center; however, the number of subjects may be enough to explore the changes in lipoprotein subfractions. Second, the study duration was short, which may have been insufficient to fully determine the impacts of XZK on lipid parameters such as HDL-C and ApoA-I. Moreover, the effect of high-dose XZK therapy on lipids and lipoproteins was not evaluated; however, the dosage of XZK used in this study is commonly prescribed in clinical practice, which may reflect the impact of XZK therapy in “real world” practice. Finally, the impact of XZK-induced changes in the small LDL subfraction on clinical outcomes was not investigated in our study.

In summary, our findings indicated that 8 weeks of therapy with a clinically conventional dose of 1200 mg/day XZK resulted in a favorable modification of the LDL subfraction distribution, in addition to the effect on serum LDL-C concentration, and also significantly decreased circulating oxLDL and IL-6 levels in patients with dyslipidemia. Our data suggest multiple beneficial impacts of XZK on ASCVD.

Conflicts of interest

The authors declare that they have no competing interests.

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References

1. Wang JC, Bennett M. Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. *Circ Res*. 2012;111:245–259.
2. Acharjee S, Boden WE, Hartigan PM, et al. Low levels of high-density lipoprotein cholesterol and increased risk of cardiovascular events in stable ischemic heart disease patients: a post-hoc analysis from the courage trial (clinical outcomes utilizing revascularization and aggressive drug evaluation). *J Am Coll Cardiol*. 2013;62:1826–1833.
3. Lyons JG, O'Dea K, Walker KZ. Evidence for low high-density lipoprotein cholesterol levels in Australian indigenous peoples: a systematic review. *BMC Public Health*. 2014;14:545.
4. Li JJ. Inflammation in coronary artery diseases. *Chin Med J Engl*. 2011;124:3568–3575.
5. Li JJ, Zhang YP, Yang P, et al. Increased peripheral circulating inflammatory cells and plasma inflammatory markers in patients with variant angina. *Coron Artery Dis*. 2008;19:293–297.
6. Li JJ, Zhu CG, Yu B, Liu YX, Yu MY. The role of inflammation in coronary artery calcification. *Ageing Res Rev*. 2007;6:263–270.
7. Wu M, Gu X, Li X, et al. C-reactive protein and inflammatory cytokines during percutaneous coronary intervention. *J Vasc Res*. 2016;53:39–48.
8. Guaricci AI, Pontone G, Fusini L, et al. Additional value of inflammatory biomarkers and carotid artery disease in prediction of significant coronary artery disease as assessed by coronary computed tomography angiography. *Eur Heart J Cardiovasc Imaging*. 2017;18:1049–1056.
9. Huang YQ, Cai AP, Chen JY, Huang C, Li J, Feng YQ. The relationship of plasma miR-29a and oxidized low density lipoprotein with atherosclerosis. *Cell Physiol Biochem*. 2016;40:1521–1528.
10. Wang A, Yang Y, Su Z, et al. Association of oxidized low-density lipoprotein with prognosis of stroke and stroke subtypes. *Stroke*. 2017;48:91–97.
11. Zhang L, Jia YH, Zhao XS, et al. Trichosanatine alleviates oxidized low-density lipoprotein induced endothelial cells injury via inhibiting the LOX-1/p38 MAPK pathway. *Am J Transl Res*. 2016;8:5455–5464.
12. Hirayama S, Miida T. Small dense LDL: an emerging risk factor for cardiovascular disease. *Clin Chim Acta*. 2012;414:215–224.
13. Superko HR, Pendyala L, Williams PT, Momary KM, King SB, Garrett BC. High-density lipoprotein subclasses and their relationship to cardiovascular disease. *J Clin Lipidol*. 2012;6:496–523.
14. Rosenson RS, Brewer HB, Chapman MJ, et al. HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardiovascular events. *Clin Chem*. 2011;57:392–410.
15. Li JJ, Lu ZL, Kou WR, et al. Beneficial impact of Xuezhikang on cardiovascular events and mortality in elderly hypertensive patients with previous myocardial infarction from the China Coronary Secondary Prevention Study (CCSPS). *J Clin Pharmacol*. 2009;49:947–956.
16. Jia YJ, Zhang Y, Liu J, Guo YL, Xu RX, Li JJ. Short- and long-term effects of xuezhikang, an extract of cholestin, on serum proprotein convertase subtilisin/kexin type 9 levels. *Chin J Integr Med*. 2016;22:96–100.
17. Ye P, Lu ZL, Du BM, et al. Effect of xuezhikang on cardiovascular events and mortality in elderly patients with a history of myocardial infarction: a subgroup analysis of elderly subjects from the China coronary secondary prevention study. *J Am Geriatr Soc*. 2007;55:1015–1022.
18. Li JJ, Lu ZL, Kou WR, et al. Beneficial impact of Xuezhikang on cardiovascular events and mortality in elderly hypertensive patients with previous myocardial infarction from the China Coronary Secondary Prevention Study (CCSPS). *J Clin Pharmacol*. 2009;49:947–956.
19. Goliash G, Oravec S, Blessberger H, et al. Relative importance of different lipid risk factors for the development of myocardial infarction at a very young age (≤ 40 years of age). *Eur J Clin Invest*. 2012;42(6):631–636.
20. Christogiannis LG, Kostapanos MS, Tellis CC, Milionis HJ, Tselepis AD, Elisaf MS. Distinct effects of fixed combinations of valsartan with either amlodipine or hydrochlorothiazide on lipoprotein subfraction profile in patients with hypertension. *J Hum Hypertens*. 2013;27:44–50.
21. Xu RX, Guo YL, Li XL, Li S, Li JJ. Impact of short-term low-dose atorvastatin on low-density lipoprotein and high-density lipoprotein subfraction phenotype. *Clin Exp Pharmacol Physiol*. 2014;41:475–481.
22. Wang Y, Chen ZH, Liu GH, et al. Adjunctive therapy of xuezhikang capsule for coronary heart disease: a systematic review and meta-analysis of randomized controlled trials [in Chinese]. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 2014;34:1182–1191.
23. Moriarty PM, Roth EM, Karns A, et al. Effects of Xuezhikang in patients with dyslipidemia: a multicenter, randomized, placebo-controlled study. *J Clin Lipidol*. 2014;8:568–575.
24. Lu Z, Kou W, Du B, et al. Effect of Xuezhikang, an extract from red yeast Chinese rice, on coronary events in a Chinese population with previous myocardial infarction. *Am J Cardiol*. 2008;101:1689–1693.
25. Li JJ, Hu SS, Fang CH, et al. Effects of xuezhikang, an extract of cholestin, on lipid profile and C-reactive protein: a short-term time course study in patients with stable angina. *Clin Chim Acta*. 2005;352:217–224.
26. Cao R, Bai Y, Sun L, et al. Xuezhikang therapy increases miR-33 expression in patients with low HDL-C levels. *Dis Markers*. 2014;2014, 781780.
27. Chen Y, Jiang C, Liu M, Liu F, Fan Y. Efficacy and safety comparison of different statins in elderly patients [in Chinese]. *Zhonghua Xin Xue Guan Bing Za Zhi*. 2014;42:910–915.
28. Ni L, Wang RB, Guo HJ, et al. Multi-centered, randomized controlled clinical study on Chinese medicine formula particles for hyperlipidaemia associated with highly active antiretroviral therapy [in Chinese]. *Zhongguo Zhong Yao Za Zhi*. 2013;38:2443–2447.
29. Li Y, Jiang L, Jia Z, et al. A meta-analysis of red yeast rice: an effective and relatively safe alternative approach for dyslipidemia. *PLoS One*. 2014;9, e98611.
30. Kingwell BA, Chapman MJ, Kontush A, Miller NE. HDL-targeted therapies: progress, failures and future. *Nat Rev Drug Discov*. 2014;13:445–464.

31. Otvos JD, Collins D, Freedman DS, et al. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the veterans affairs high-density lipoprotein intervention trial. *Circulation*. 2006;113:1556–1563.
32. Barylski M, Toth PP, Nikolic D, Banach M, Rizzo M, Montalto G. Emerging therapies for raising high-density lipoprotein cholesterol (HDL-C) and augmenting HDL particle functionality. *Best Pract Res Clin Endocrinol Metab*. 2014;28:453–461.
33. Xu RX, Zhang Y, Ye P, et al. Analysis of lipoprotein subfractions in Chinese han patients with stable coronary artery disease. *Heart Lung Circ*. 2015;24:1203–1210.
34. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. *Circulation*. 2002;106:3143–3421.
35. Irving BA, Nair KS, Srinivasan M. Effects of insulin sensitivity, body composition, and fitness on lipoprotein particle sizes and concentrations determined by nuclear magnetic resonance. *J Clin Endocrinol Metab*. 2011;96:E713–E718.
36. Gentile M, Panico S, Jossa F, et al. Small dense LDL particles and metabolic syndrome in a sample of middle-aged women. Findings from progetto atena. *Clin Chim Acta*. 2008;388:179–183.
37. Williams PT, Zhao XQ, Marcovina SM, Brown BG, Krauss RM. Levels of cholesterol in small LDL particles predict atherosclerosis progression and incident CHD in the HDL-Atherosclerosis Treatment Study (HATS). *PLoS One*. 2013;8, e56782.
38. Xu RX, Li S, Zhang Y, et al. Relation of plasma PCSK9 levels to lipoprotein subfractions in patients with stable coronary artery disease. *Lipids Health Dis*. 2014;13:188.
39. Zhang Y, Xu RX, Li S, et al. Association of plasma small dense LDL cholesterol with PCSK9 levels in patients with angiographically proven coronary artery disease. *Nutr Metab Cardiovasc Dis*. 2015;25:426–433.
40. Mäkelä KM, Traylor M, Oksala N, et al. Association of the novel single-nucleotide polymorphism which increases oxidized low-density lipoprotein levels with cerebrovascular disease events. *Atherosclerosis*. 2014;234:214–217.
41. Huang H, Mai W, Liu D, Hao Y, Tao J, Dong Y. The oxidation ratio of LDL: a predictor for coronary artery disease. *Dis Markers*. 2008;24:341–349.
42. Johnston N, Jernberg T, Lagerqvist B, Siegbahn A, Wallentin L. Oxidized low-density lipoprotein as a predictor of outcome in patients with unstable coronary artery disease. *Int J Cardiol*. 2006;113:167–173.
43. Aguilar EC, Santos LC, Leonel AJ, et al. Oral butyrate reduces oxidative stress in atherosclerotic lesion sites by a mechanism involving NADPH oxidase down-regulation in endothelial cells. *J Nutr Biochem*. 2016;34:99–105.
44. Jiang FY, Sun LP, Yang J. Effects of xuezhikang at different doses on patients suffering from acute coronary syndrome after percutaneous coronary intervention [in Chinese]. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 2011;31:1607–1610.
45. Huang J, Wang MH, Peng XP, Wei C, Lu J. Effects of Xuezhikang on serum levels of high sensitive-C reactive protein, matrix metalloproteinase-9 and lipoprotein in patients with acute coronary syndrome [in Chinese]. *Zhongguo Zhong Xi Yi Jie He Za Zhi*. 2006;26:221–223.
46. Li JJ, Wang Y, Nie SP, et al. Xuezhikang, an extract of cholestin, decreases plasma inflammatory markers and endothelin-1, improve exercise-induced ischemia and subjective feelings in patients with cardiac syndrome X. *Int J Cardiol*. 2007;122:82–84.
47. Xu RX, Zhang Y, Li XL, et al. Relationship between plasma phospholipase A2 concentrations and lipoprotein subfractions in patients with stable coronary artery disease. *Clin Chim Acta*. 2015;446:195–200.

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