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Correction of plasma fat-soluble vitamin levels by blood lipids in elderly patients with coronary heart disease

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ABSTRACT

This study aims to investigate the correlation between plasma fat-soluble vitamin levels and blood lipid in elderly patients with coronary heart disease (CHD). A total of 120 participants were enrolled, including 60 CHD patients and 60 controls without CHD. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to quantify plasma levels of vitamins A, D_3, E, and K. Data analysis was conducted using the statistical analysis system module of MetaboAnalyst 5.0. The CHD group showed significantly higher levels of plasma total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) but not high-density lipoprotein cholesterol (HDL-C) compared to controls. The CHD group exhibited significantly higher plasma levels of VA and VE, positively correlating with TC, TG, and LDL-C. After adjusted by TG levels, the CHD group had significantly lower plasma levels of VA and VE, negatively correlating with TC, TG, and LDL-C. The CHD group also had significantly lower concentrations of VD_3, independent of TG modification, compared to controls. VD_3 negatively correlated with TC, TG, and LDL-C. Elderly individuals with CHD display abnormal blood lipid metabolism, and fat-soluble vitamins adjusted by TG levels can more accurately and timely response to implicit fat-soluble vitamins deficiency in CHD patients.

1. Background

Coronary heart disease (CHD) is primarily attributed to atherosclerosis of the coronary artery, resulting in luminal stenosis or occlusion. Its clinical manifestations mainly include myocardial ischemia, chest pain, and chest tightness [1]. According to statistical data, China has approximately 330 million individuals diagnosed with cardiovascular disease, with 11 million suffering from CHD. Furthermore, cardiovascular disease-related fatalities rank the highest among all causes of death, making it a prevalent ailment within the elderly population. Extensive epidemiological research has consistently identified dyslipidemia as the primary risk factor for CHD [2,3]. It plays a crucial role in the development and progression of coronary atherosclerosis, thereby exacerbating the condition [4].

Vitamins are essential micronutrients that participate in various vital bodily functions. Research has established an association between multiple fat-soluble vitamins and the development of coronary atherosclerosis, making them novel risk factors for CHD [5]. While individual studies have explored the impact of vitamin A (VA), vitamin D (VD), vitamin E (VE), and vitamin K (VK) on

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cardiovascular health, no comprehensive investigation has examined their collective influence. Given their anti-inflammatory and antioxidant properties, as well as their potential role as early disease indicators, it is important to analyze the correlation between plasma fat-soluble vitamins levels of and lipid profiles in elderly patients with CHD. This study aims to provide a theoretical foundation for the prevention and evaluation of CHD among elderly patients in clinical settings.

2. Materials and methods

2.1. CHD patients and controls

Clinical data were obtained from individuals aged 60 and above who underwent physical examinations at Hebei Yanda Hospital. The CHD group consisted of 60 elderly individuals who were all diagnosed with CHD. The inclusion criteria encompassed patients with standard CHD or those diagnosed with CHD following previous coronary angiography. Among the participants, there were 36 men and 24 women, with a mean age of (63 ± 3) years. Additionally, the control group comprised 60 elderly patients who were part of the same physical examination. Among them, 47 were men and 13 were women, with a mean age of (64 ± 2) years. The study excluded individuals under the age of 60, as well as those with a history of cardiac surgery, other cardiac organ lesions, infections, malignancies,

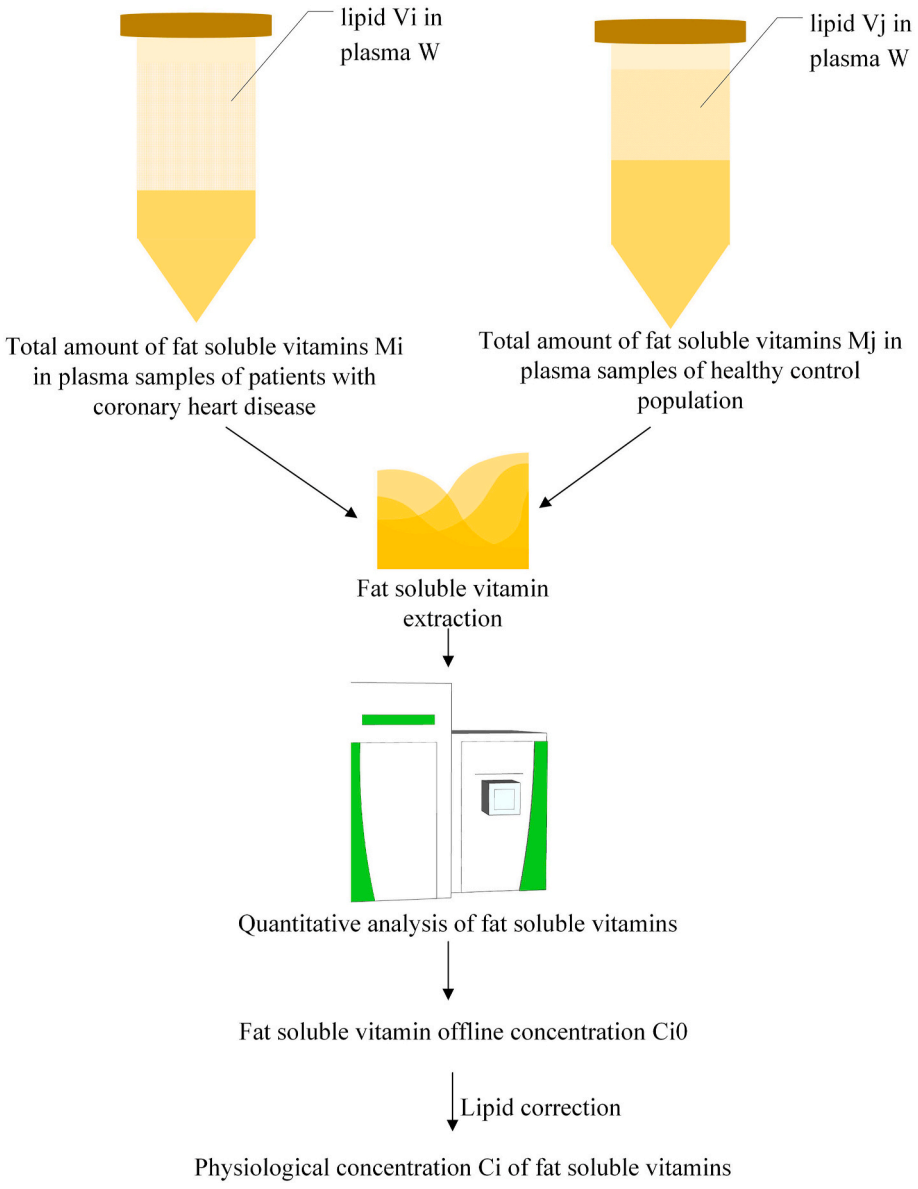

Fig. 1. Schematic diagram for correcting fat-soluble vitamin data in plasma.

tumors, and severe hepatic or renal insufficiency. All patients have duly completed and signed the informed consent forms (Protocol Number: 2020–006), which have undergone thorough review and approval by the Ethical Board of Hebei Yanda Hospital, China.

2.2. Blood collection and plasma isolation

Peripheral venous blood samples were obtained from all participants after an overnight fast. The samples were collected into spray-coated EDTA blood collection tubes (BD Biosciences; Beijing, China; #367861). Subsequently, the whole blood samples were subjected to centrifugation at 3000*g* for 5 min to facilitate the separation of plasma and plasma.

2.3. Quantification of fat-soluble vitamins by Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS)

Plasma samples were subjected to protein precipitation using a release agent (Beijing Huada Jibiai Biological Co., Ltd; #20202401355) and treatment solution (Beijing Huada Jibiai Biological Co., Ltd; #20222400603). The targeted analytes were extracted from the samples utilizing the principle of intermolecular phase dissolution, where the material of interest is solubilized within a similar phase. The detection of fat-soluble vitamins was subsequently performed using LC-MS/MS, following a previously established protocol.

2.4. Quantification of blood lipids

The enzymatic measurement of total cholesterol (TC) in plasma was conducted using the Cholesterol Quantitation Kit (Siemens Healthcare Diagnostics Inc, Shanghai, China; #566312), following the manufacturer's guidelines. Plasma triglycerides (TG) were determined using the glycerol phosphate oxidase (GPO)-PAP method, employing a Triglyceride Colorimetric Assay Kit (Siemens Healthcare Diagnostics Inc, Shanghai, China; #573003). High-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were determined utilizing colorimetric assay kits (Siemens Healthcare Diagnostics Inc, #572639 for HDL-C and #568171 for LDL-C), following the protocols provided by the manufacturer.

2.5. Correction of fat-soluble vitamin results

Due to the fact that fat soluble vitamins are mainly soluble in blood lipids, people with high blood lipids may experience implicit nutritional hunger of fat soluble vitamins. The correction of fat-soluble vitamin data was illustrated in Fig. 1. During the assessment of VA, VE, 25-hydroxyvitamin D, and VK levels in plasma using LC-MS/MS, certain limitations were encountered in the sample pre-treatment method. Even when extracting plasma samples of uniform quality for fat-soluble vitamin analysis, significant variations in TG levels were observed. Consequently, samples with lower fat-soluble vitamin levels exhibited higher absolute contents compared to samples with lower TG levels but higher lipid-soluble vitamin levels. Thus, in order to accurately portray the physiological levels of lipid-soluble vitamins, it is imperative to rectify the concentration data obtained through LC-MS/MS using TG data. The correction formula is presented as follows.

$$C_{i0} = \frac{M_i}{W} = \frac{W \times V_i \times C_i}{W} = V_i \times C_i$$

$$C_i = \frac{C_{i0}}{V_i}$$

In this formula, C_{i0} represents the concentration of fat-soluble vitamins obtained through mass spectrometry. M_i signifies the absolute content of fat-soluble vitamins in the sample. W refers to the weight of the sample. V_i indicates the concentration of blood lipid levels in the samples. C_i denotes the physiological concentration of lipid-soluble vitamins, corrected using triglyceride data.

2.6. Statistical analysis

Statistical analysis and data visualization were performed using the MetaboAnalyst 5.0 [6] online platform and R version 4.1.3. Prior to analysis, the original data underwent format standardization. Initially, the data was directly analyzed without standardization or conversion. The analysis comprised various methods, including *t*-test, correlation analysis, and orthogonal partial least squares orthogonal discriminant analysis. Subsequently, the plasma levels of VA, VD2, VD3, VE, and VK were normalized by their corresponding TG concentrations. These normalized datasets were then uploaded to the analysis system for further analysis using the aforementioned methods. The analysis results were determined to be statistically significant at a significance level of $P < 0.05$ and highly significant at a significance level of $P < 0.01$.

3. Results and analysis

3.1. Comparative analysis of TG, TC, HDL and LDL levels in plasma samples from the two groups

To assess the potential correlation between plasma fat-soluble vitamin levels and blood lipid profiles in elderly patients with CHD,

we initially conducted measurements of plasma TG, TC, HDL, and LDL levels. Upon comparing the plasma lipid profiles of the CHD group with those of the control group, noteworthy findings emerged. Specifically, we observed that the plasma TC, TG, and LDL-C levels were significantly higher in the CHD group compared to the control group ($P < 0.01$) (Fig. 2A–C). However, the plasma HDL-C levels exhibited similar values between the control and CHD groups, with no statistically significant difference detected ($P > 0.05$) (Fig. 2D).

3.2. Comparative analysis of plasma concentrations of VA, VE, and VK

We measured plasma concentrations of VA, VE, and VK in both CHD and control groups using LC-MS/MS, followed by a comparative analysis. The LC-MS/MS results revealed notable differences in plasma concentrations of VA, VE, and VK between the two groups. Specifically, the CHD group exhibited significantly higher levels of VA and VE compared to the control group ($P < 0.01$) (Fig. 3A and B). Moreover, the VK concentration in the CHD group was also significantly elevated compared to the control group ($P < 0.05$) (Fig. 3C). When we adjusted the results based on TG concentration, it became evident that the VA/TG, VE/TG and VK/TG ratios in the control group were considerably higher than those in the CHD group, demonstrating a highly significant difference ($P < 0.01$) (Fig. 3D–F). These findings emphasize the significant variations in plasma levels of VA, VE, and VK between the CHD and control groups, both before and after TG correction.

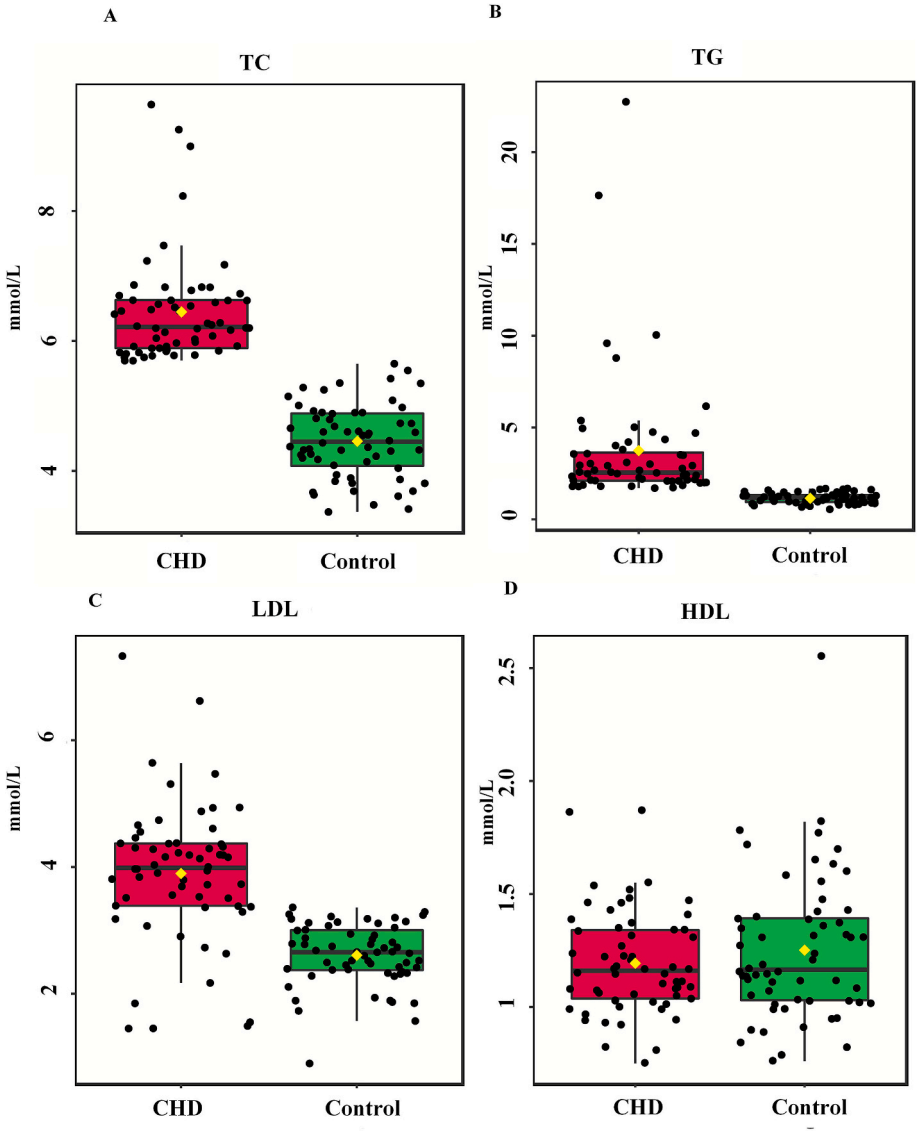

Fig. 2. Comparison of TG, TC, HDL and LDL levels in plasma samples from CHD and control groups
A: TC; B: TG; C: LDL; D: HDL.

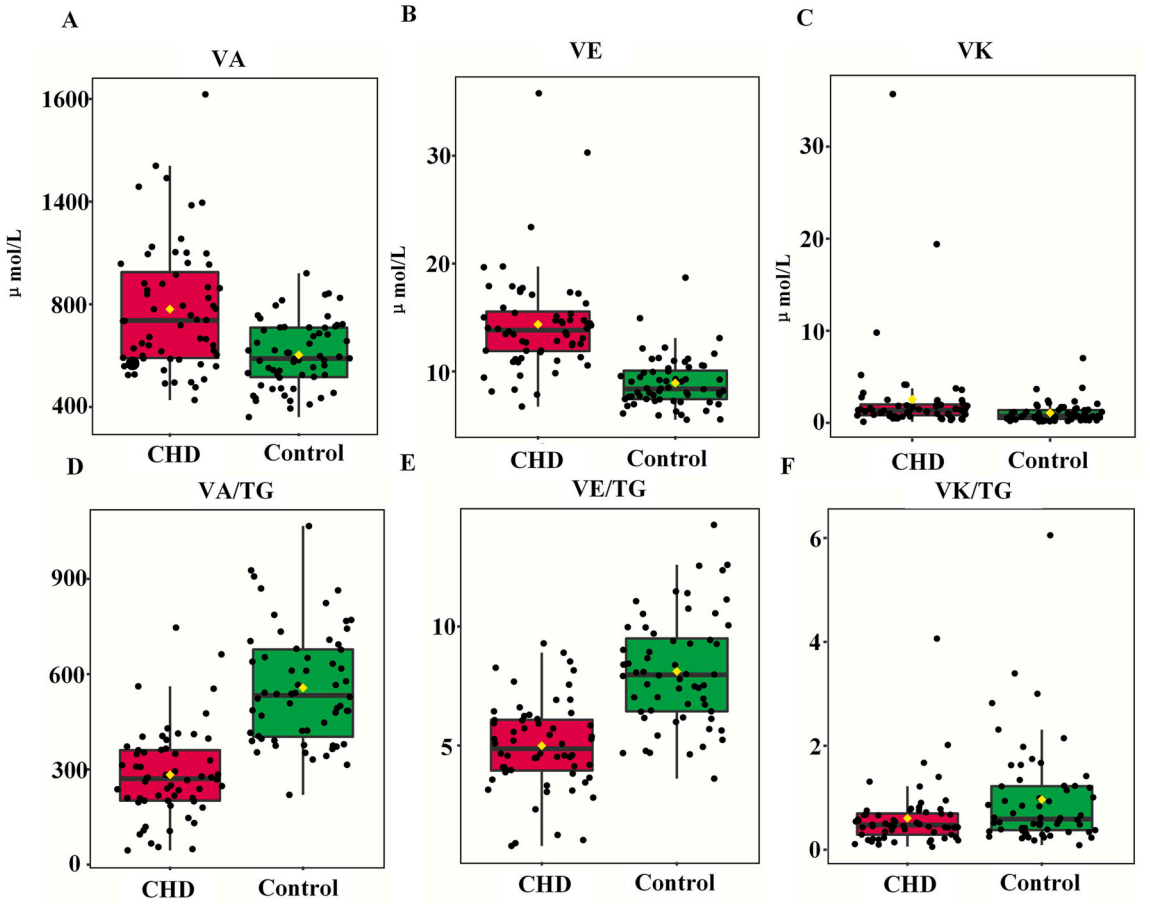

Fig. 3. Comparison of VA, VE, VK levels and their corresponding corrected values in plasma samples from CHD and control groups
A: VA; B: VE; C: VK; D: VA/TG; E: VE/TG; F: VK/TG.

3.3. Differential analysis of plasma VD levels

In addition to measuring the concentrations of VA, VE, and VK, we also conducted measurements of VD2 and VD3 concentrations using LC-MS/MS. The concentrations of VD2 in the plasma samples from both the control and CHD groups did not show a significant difference ($P > 0.05$) (Fig. 4A). However, the concentrations of VD3 were found to be decreased in the CHD group compared to the control group (Fig. 4B). Furthermore, upon correction with TG concentration, both VD2/TG ($P < 0.05$) and VD3/TG ($P < 0.01$) exhibited significant decreases in the CHD group.

3.4. Correlation analysis between fat-soluble vitamins and blood lipids

The aforementioned results have demonstrated significant alterations in fat-soluble vitamins and blood lipids within the CHD group. Consequently, our subsequent objective was to assess the associations between these fat-soluble vitamins and blood lipids. Our mass spectrometry findings revealed a positive correlation coefficient of 0.18 between VA and TG, as well as correlation coefficients of 0.29 and 0.30 between TG and TC, respectively (Fig. 5A). Additionally, concentration data of VD3 exhibited a notable negative correlation with LDL and TC, with correlation coefficients of −0.30 and −0.34, respectively (Fig. 5A). Notably, VE concentration determined by mass spectrometry exhibited a significant positive correlation with LDL, TC, and TG, with correlation coefficients of 0.36, 0.71, and 0.49, respectively (Fig. 5A). Furthermore, VK concentration data displayed a significant positive correlation with TC and TG, with correlation coefficients of 0.24 and 0.48, respectively (Fig. 5A).

Further correlation analysis was conducted between blood lipids and fat-soluble vitamins corrected by TG. The concentration of VA/TG revealed a significant negative correlation with TG, TC, and LDL, with correlation coefficients of −0.56, −0.60, and −0.31, respectively (Fig. 5B). Similarly, the concentration of VD2/TG also exhibited a significant negative correlation of −0.23 with TG, with a correlation coefficient of −0.20 (Fig. 5B). Moreover, the concentration of VD3/TG showed a highly significant negative correlation with TG, TC, and LDL, with correlation coefficients of −0.48, −0.66, and −0.45, respectively (Fig. 5B). Furthermore, it displayed a significant positive correlation with HDL, with a correlation coefficient of 0.21. Additionally, the VE/TG values indicated a significant

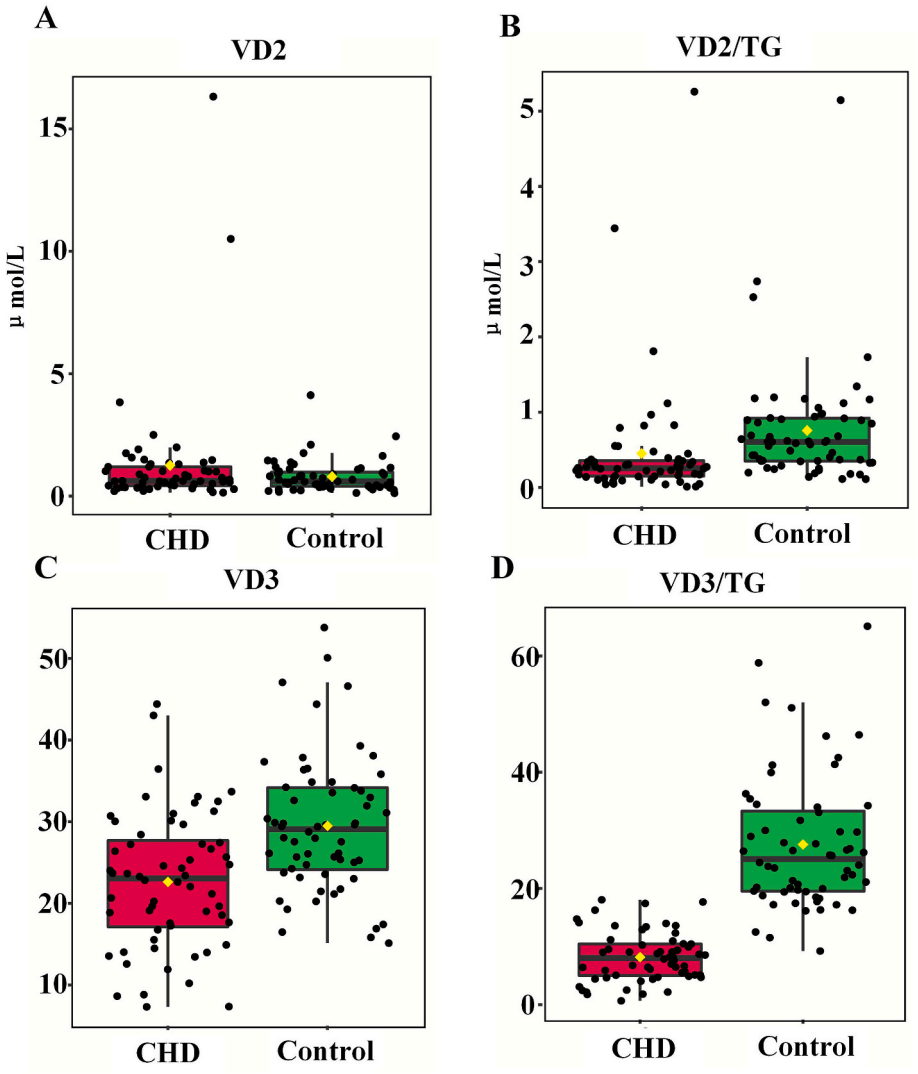

Fig. 4. Comparison of VD2, VD3 levels and their corresponding corrected values in plasma samples from CHD and control groups
A: VD2; B: VD2/TG; C: VD3; D: VD3/TG.

negative correlation with TC and TG, with correlation coefficients of −0.39 and −0.59, respectively, and a significant positive correlation with HDL, with a correlation coefficient of 0.28 (Fig. 5B).

3.5. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA)

We conducted OPLS-DA to assess the diagnostic differences between fat-soluble vitamins and blood lipids in the CHD and control groups. The results, presented in Fig. 6A, demonstrate the successful discrimination of the control group from the CHD group, with a T score of 38.2 % and an orthogonal T score of 18 %. Furthermore, an orthogonal bias least squares discriminant analysis was performed, incorporating the plasma concentration data of VA/TG, VE/TG, VD2/TG, VD3/TG, and VK/TG, along with plasma levels of TC, TG, HDL, and LDL. Fig. 6B illustrates the effective classification between the control group and the CHD group, with a T score of 29.1 % and an orthogonal T score of 15.7 %. The established OPLS-DA model, based on the combination of plasma lipid profiles and fat-soluble vitamin data, accurately distinguishes individuals with CHD from the healthy population.

4. Discussion

CHD is characterized by inflammation-induced damage to the vascular endothelium, leading to remodeling of the coronary artery wall. Inflammatory cytokines activate the fibrinolytic system, resulting in platelet adhesion, thrombosis, and deposition of fat, connective tissue, and calcium carbonate in blood vessels, ultimately forming atherosclerotic plaques [7,8]. This leads to stenosis or

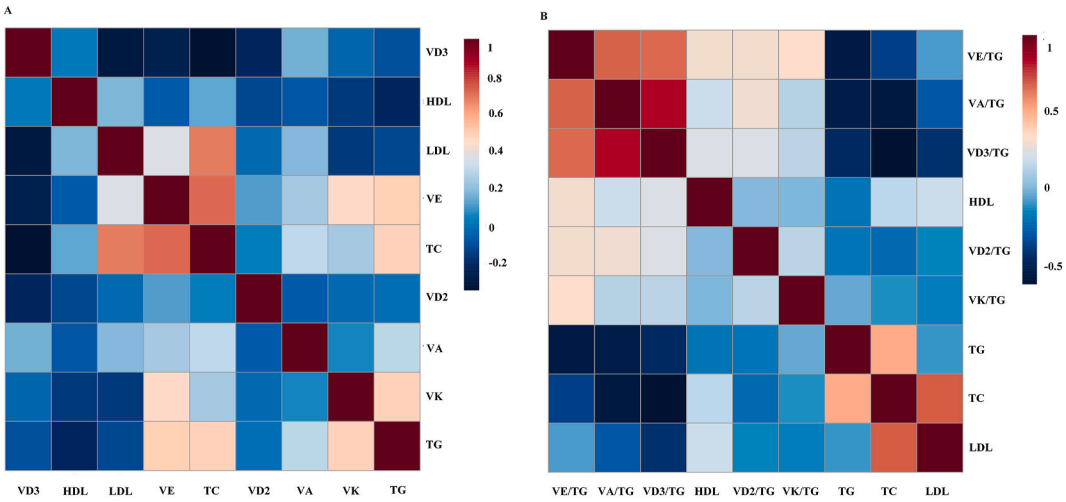

Fig. 5. Results oof correlation analysis between fat-soluble vitamins and blood lipids.

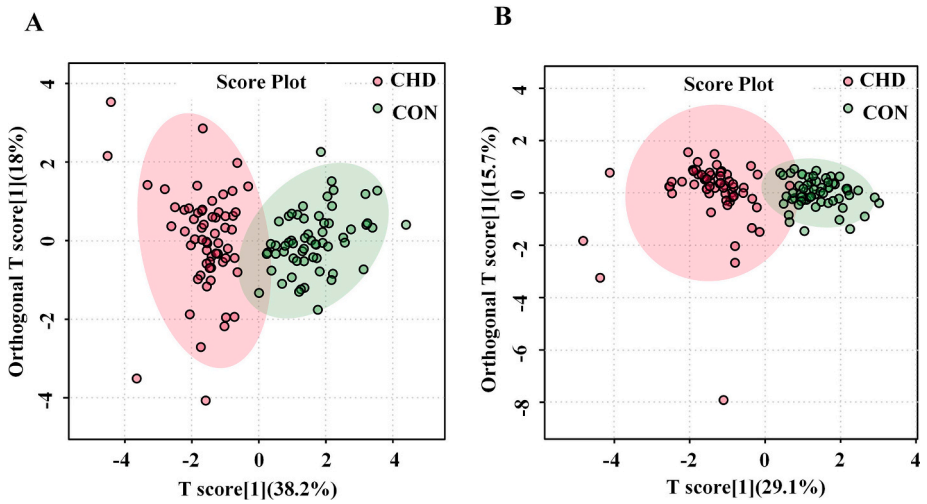

Fig. 6. Results of OPLS-DA based on plasma lipids and fat-soluble vitamin levels.

occlusion of the vessels [7,8]. Abnormal blood lipid metabolism, causing stenosis of the vascular lumen, is the main cause of myocardial ischemia [9]. While significant progress has been made in the diagnosis and treatment of CHD and the understanding of the relationship between dyslipidemia, atherosclerosis, and their mechanisms, there is still a need for more effective early screening mechanisms to prevent and treat atherosclerosis [10]. VA and VE have been identified as potential biochemical parameters for the preclinical assessment of "high-risk populations" and for the evaluation and monitoring of CHD cases [11]. This study aims to analyze the correlation between lipid-soluble vitamin levels and blood lipid levels in the plasma of elderly patients with coronary heart disease. The findings of this research provide novel insights and methodologies for the prevention and treatment of CHD.

Both VA and VE possess antioxidant effects [12]. VA, with its diene conjugated bond in the side chain, can bind to peroxidized free radicals, preventing damage to the body caused by free radicals and lipid peroxidation [13]. Atherosclerosis, a chronic inflammatory disease associated with the blood vessel wall, involves an imbalance between oxidants and antioxidants, leading to oxidative stress and the oxidation of LDL-C [14]. Oxidized LDL-C (oxLDL) damages the endothelium and is internalized by monocytes and macrophages, transforming them into foam cells and intensifying the inflammatory response, thus accelerating vascular atherosclerosis [15]. VE acts as a scavenger of free radicals, inhibiting lipid peroxidation [16]. One of its hydrolysis products, Laccopheroll, exhibits potent anti-oxidant and anti-inflammatory properties [17]. The present study found higher VA/TG and VE/TG levels in the CHD group compared to the control group, indicating a significant difference and suggesting that reduced levels of VA and VE increase the risk of CHD. VA plays a crucial role in lipid metabolism, promoting the breakdown and metabolism of fat in the body and reducing the occurrence of arteriosclerosis [18,19]. Additionally, it possesses anti-inflammatory properties, mediated by the retinoid receptor-related orphan receptor-α (RORα), which regulates macrophage polarization, intraplaque inflammation, and plaque stability, ultimately protecting

blood vessels and alleviating inflammatory diseases [20,21]. VE is believed to delay arterial wall narrowing by inhibiting smooth muscle cell proliferation [22]. It also prevents atherosclerotic disease by inhibiting platelet adhesion, aggregation, and release response [23].

VD exists primarily in two active forms, VD2 and VD3. The most reliable indicator of VD levels is the measurement of its main form in the body, 25-hydroxy vitamin D [25 (OH) D], specifically VD3 [24]. In this study, plasma levels VD3/TG were found to be significantly negatively correlated with TC, TG, and LDL levels in the lipid profile, as well as with HDL levels in plasma. These findings are consistent with the results reported by Zhang et al. [25]. Another study by Hu et al. demonstrated that for every 10 ng/mL decrease in plasma VD3 levels, there was a 0.84-fold increase in the risk of dyslipidemia [26]. Increased dyslipidemia can lead to vascular endothelial cell damage, thereby promoting the development of CHD [26]. Su et al. also reported that VD3 regulates the number of LDL receptors on hepatocyte surfaces, enhancing their operation and reducing blood LDL concentrations [27]. Furthermore, Zhao et al. found that VD3 participates in the regulation of lipid synthesis and breakdown [28]. In cases of abnormal lipid metabolism, it promotes timely lipid breakdown and suppresses synthesis, thus restoring lipid levels to normal [28]. These conclusions align with the findings of our study.

Numerous studies have provided evidence that vitamin D can serve as a predictive marker for atherosclerosis risk [29]. Plasma VD levels have been inversely associated with the risk of developing coronary artery disease (CAD) [30]. VD has been shown to inhibit angiotensin production by acting on the renin-angiotensin-aldosterone system (Dibaba, 2019). Deficiency in VD can lead to increased activity of this system, including elevated angiotensin levels, resulting in vasoconstriction, increased blood pressure, greater myocardial load, and an increased likelihood of cardiovascular disease [31]. Endothelial dysfunction, vascular smooth muscle cell activation, and vascular inflammation are key factors in the development and progression of cardiovascular diseases [32]. VD possesses antioxidant properties, reducing oxidative stress, promoting nitric oxide synthesis, and protecting endothelial cells [33]. It has also been found to inhibit smooth muscle cell proliferation, thereby slowing down the progression rate of atherosclerosis [34]. Studies have indicated that vitamin D can attenuate the inflammatory response, inhibit the transcription and secretion of inflammatory cells and factors, enhance the production of anti-inflammatory factors, and thereby reduce inflammatory damage to the blood vessel wall, playing a protective role in vascular health [35]. In our study, we observed significantly lower VD levels in the CHD group compared to the control group, and these levels were associated with the occurrence of CHD. These findings are consistent with the conclusions of the aforementioned studies.

VK exists in two natural forms: VK1 and VK2. VK1 is involved in the body's coagulation process, while VK2 is primarily found in arterial tissues and cells, where it plays a role in inhibiting vascular calcification [36]. Vascular calcification is known to increase the risk of atherosclerotic plaque rupture and raise the morbidity and mortality associated with cardiovascular disease [37]. Vascular calcification is a regulated pathophysiological process, wherein matrix γ-carboxyglutamate protein (MGP), a VK-dependent protein, acts as an effective inhibitor of calcification by binding with calcium. VK serves as a necessary cofactor in the carboxylation process of MGP. When there is a deficiency of VK, inactive, uncarboxylated MGP (dp-ucMGP) levels increase, leading to reduced inhibition of vascular calcification and diminished GMP activity ([38]; Aoun et al., 2017). VK has emerged as a potential and significant risk factor for vascular calcification [39]. Several studies have demonstrated that increased intake of VK can prevent cardiovascular diseases such as atherosclerosis [40]. VK2, in particular, has been shown to attenuate the progression of atherosclerotic plaque by increasing HDL levels and reducing total cholesterol levels, thus preventing or delaying the advancement of atherosclerosis [41]. On the other hand, VK1 is primarily involved in the synthesis of coagulation factors and coagulase γ-carboxylase, but its utilization outside the liver is limited [42]. A study found that higher intake of VK2 reduced the risk of coronary heart disease, while no association was found between VK1 intake and CHD [43]. The study by Asmar et al. revealed that individuals receiving moderate to high doses of VK2 experienced reduced mortality from CHD and a lower risk of severe aortic calcification compared to those receiving low doses of VK2 [44]. VK1 intake did not show any association with CHD, and the relative risk in the highest intake group was not statistically significant. However, this study contradicts the aforementioned findings, potentially due to its limited sample size and regional scope. Further investigations with larger sample sizes are necessary to provide more comprehensive evidence. Additionally, it is important to consider the impact of other influencing factors on the determination of detection indicators. Some studies suggest that dp-ucMGP levels above 500 pmol/L indicate VK insufficiency, making assessing MGP activity a useful method for determining VK status [44,45].

5. Conclusion

In summary, elderly patients with CHD exhibit abnormal lipid metabolism, with significantly higher plasma levels of VA and VE compared to the control group. These elevated levels showed a significant positive correlation with TC, TG, and LDL-C. However, after adjusting for TG levels, plasma VA and VE were significantly lower than in the control group, and showed a significant negative correlation with TC, TG, and LDL-C. Additionally, the concentration of VD3 was significantly lower in the CHD group compared to the control group and exhibited a negative correlation with TC, TG, and LDL-C. By utilizing four parameters, including plasma lipid levels and fat-soluble vitamin data, an OPLS-DA model was successfully established. This model accurately classified individuals with CHD and those in the healthy population. It may serve as an early indicator and evaluation tool for the prevention and treatment of CHD in elderly patients.

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CRediT authorship contribution statement

Xin-Yu Wang: Writing – original draft, Funding acquisition, Conceptualization. **Xiangzhi Liu:** Methodology, Writing – original draft. **Chengliang Zhen:** Investigation, Methodology, Writing – original draft. **Nannan Tian:** Writing – review & editing, Visualization. **Haina Ma:** Software. **Menghan Wang:** Methodology. **Li Wang:** Investigation, Project administration, Writing – review & editing.

Declaration of competing interest

No competing financial interests or personal interests that could have appeared to influence the work reported in this manuscript.

Data availability

Data will be made available on request.

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