# Effect of vitamin D therapy on endothelial function in ischemic heart disease female patients with vitamin D deficiency or insufficiency: A primary report

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**Short Communication**

## Abstract

**BACKGROUND:** Vitamin D deficiency is associated with vascular endothelial dysfunction. We evaluated endothelial function in ischemic heart disease (IHD) patients with vitamin D deficiency or insufficiency before and after vitamin D therapy.

**METHODS:** An uncontrolled before-after study was conducted in Isfahan, Iran on consecutive sample of female IHD patients who had undergone percutaneous coronary intervention in the preceding 6 months and/or referred with chronic stable angina. Forty patients with vitamin D deficiency or insufficiency (serum 25-hydroxy vitamin D < 20 or 20-30 ng/ml, respectively) were included and received two intramuscular injections of 300,000 IU cholecalciferol with 1 month interval. Endothelial function, assessed by measuring flow-mediated dilatation (FMD), and serum 25-hydroxy vitamin D level were measured at baseline and 1 month after the second dose of cholecalciferol.

**RESULTS:** A total of 30 patients completed the study, age = 59.4 ± 8.7 years; serum 25-hydroxy vitamin D = 19.0 ± 6.5 ng/ml. After treatment, serum 25-hydroxy vitamin D was reached to > 30 ng/ml in all patients. Brachial artery diameter (mm) after ischemia increased significantly, statistically but not clinically (4.55 ± 0.37 to 4.67 ± 0.38, P < 0.001). Furthermore, FMD (%) was increased from 1.96 ± 1.65 to 4.65 ± 1.27 (P < 0.001). The amount of change in FMD was not significantly correlated with serum 25-hydroxy vitamin D (r = 0.038, P = 0.858).

**CONCLUSION:** Endothelial function was improved after vitamin D therapy in IHD patients with low serum vitamin D. Controlled studies with larger sample size are required to confirm if vitamin D therapy has effects on endothelial function.

**Keywords:** Cardiovascular Diseases, Coronary Artery Disease, Endothelium, Vitamin D Deficiency

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## Introduction

Cardiovascular diseases (CVDs) are the most common causes of morbidity and mortality in developed and developing countries.[1] Coronary artery disease (CAD) is a common form of CVD, and it is reported that more than 4.5 million deaths occur in developing countries due to CAD.[2] According to estimations, mortality rate of CAD will double from 1990 to 2020.[3] Different risk factors are mentioned to be associated with CVDs. It is reported that vitamin D deficiency plays a role in developing of CVDs and CAD risk factors such as hypertension, diabetes, and metabolic syndrome.[4,5]

Vitamin D deficiency is a common health problem, and it is estimated that about 1 billion people are suffering from vitamin D insufficiency or deficiency.[6] Vitamin D deficiency is associated with endothelial vascular dysfunction and will increase the risk of CVD.[7] Also, peripheral arterial disorders are reported to be associated with low serum vitamin D levels.[8]

It is not well-known whether vitamin D therapy

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in patients with vitamin D deficiency causes a reduction in CVD.[9] Some studies have reported that vitamin D supplementation will improve vascular health markers such as endothelial function in type 2 diabetes mellitus patients and asymptomatic vitamin D deficient subjects.[10,11] Considering the limited data on this subject, especially in patients with CADs, the aim of this study was to evaluate the effect of vitamin D therapy on the endothelial function in CAD patients with vitamin D deficiency/insufficiency.

## Materials and Methods

This was an uncontrolled before-after study conducted on a consecutive sample of ischemic heart disease (IHD) patients referred to the cardiology clinic of the Sina Hospital in Isfahan, Iran, in 2013. The Sina Hospital is a general private medical center with a specialized unit for care of heart disease patients including interventional and cardiac care units. Inclusion criteria were as follow; (a) female subjects, (b), having chronic stable angina or had performed percutaneous coronary intervention during preceding 6 months, and (c) having vitamin D deficiency (serum 25-hydroxy vitamin D level < 20 ng/ml) or insufficiency (serum 25-hydroxy vitamin D level = 20-30 ng/ml).[4] Patients with uncontrolled hypertension, osteomalacia, and taking of vitamin D supplements were not included. Those who developed unstable angina or myocardial infarction and undergone coronary artery bypass grafting after the beginning of the study were excluded from the study. Considering Type I error (alpha) = 0.05, study power = 0.8, and expecting at least 5% increase in flow-mediated dilatation (FMD) after intervention, the required sample size was calculated as 30 cases. The Ethics Committee of the Isfahan University of Medical Sciences approved the study protocol and informed consent was taken from all enrolled patients.

Age was asked, weight and height were measured, and body mass index was calculated as weight divided by height squared. Medical history of hypertension, diabetes mellitus, hyperlipidemia and smoking and drug history for the treatment of the mentioned diseases were recorded. All participants were examined for systolic and diastolic blood pressure. All measurements were performed with calibrated equipment. Examinations and interviews were done by a single cardiologist.

Serum vitamin D concentration: Five ml of venous blood was taken from the patients before and 1 month after the intervention. The samples were centrifuged to separate the serum and were kept in −70 °C until measurement. Samples were measured for plasma levels of 25-hydroxy vitamin D using a chemiluminescent immunoassay kit (“25 OH vitamin D total assay, DiaSorin LIAISON) by LIAISON analyzer.

FMD analysis: The FMD was measured to assess the vascular endothelial function. Participants rested for 10 min on a plain surface to reach a stable status of the heart rate and blood pressure. Participants were asked not to use caffeine and fat-rich meals, do exercise or smoke 4-6 h before the measurements. Brachial artery diameter was assessed using a high-resolution B-mode sonogram (Vivid 3, General Electric, 7.5 MHz transducer) by placing the probe at 5 cm above the anterior Cubital cavity of the non-dominant arm. Forearm ischemia was induced by inflating a sphygmomanometer cuff to 50-100 mmHg more than systolic blood pressure for 5 min. Brachial artery diameter before ischemia was assessed as baseline brachial artery diameter. Sixty seconds after deflation the same assessment was done to measure the brachial artery diameter after ischemia. Measurement of arteries was performed during the diastolic phase, measuring the distance between outermost limit of one side of the artery to the other. The FMD% was calculated according to the following formula;[12] FMD% = [(maximum diameter−baseline diameter)/baseline diameter] × 100. FMD was assessed before the intervention and also 1 month after the final dose of vitamin D injection by a single cardiologist.

All participants who had the criteria to be enrolled in the study received two doses of intramuscular injection of 300,000 IU of cholecalciferol (vitamin D3) with an interval of 1 month (the second dose was administrated 1 month after the first injection).[4] The Study Protocol has shown in figure 1.

Statistical analysis was performed using SPSS software for windows (version 16.0, SPSS Inc., Chicago, IL, USA). Quantitative data are presented as mean ± standard deviation and qualitative data are presented as number (%). Quantitative data were checked for being normally distributed with the Kolmogorov–Smirnov test, all were normally distributed. A paired t-test was used to compare variables before and after the study. Pearson correlation coefficient was applied for evaluating the correlation between variables. Statistical significance was assessed at the 0.05 probability level in all analyses.

## Results

A total of 73 IHD female patients were evaluated during the study period. There were 40 patients with low serum vitamin D levels (< 30 ng/ml), from them 10 patients left the study, and final study sample consisted of 30 patients. Baseline characteristics of the patients are reported in table 1.

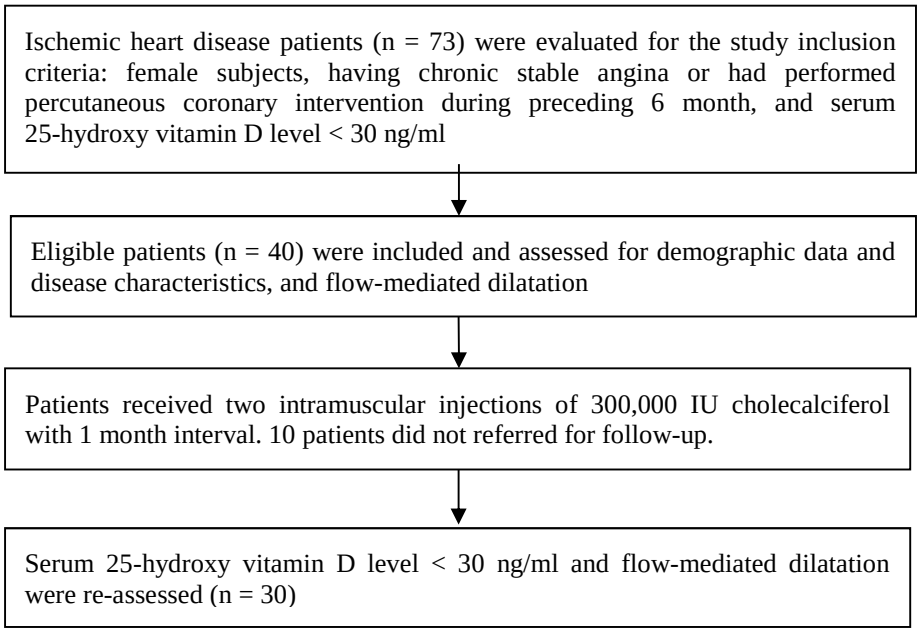


**Figure 1.** Study protocol

**Table 1.** Baseline characteristics of the participants (n = 30)

| Variables | Mean ± SD | n (%) |
|---|---|---|
| Age (year) | 59.4 ± 8.7 | - |
| Height (cm) | 161.4 ± 3.6 | - |
| Weight (Kg) | 66.7 ± 6.2 | - |
| BMI (Kg/m$^2$) | 25.5 ± 1.9 | - |
| SBP (mmHg) | 131.2 ± 17.5 | - |
| DBP (mmHg) | 80.0 ± 8.6 | - |
| Serum vitamin D level (ng/ml) | 19.0 ± 6.5 | - |
| Serum vitamin D level | | |
| < 20 ng/ml | - | 18 (60.0) |
| 20-30 ng/ml | - | 12 (40.0) |
| Comorbidities | | |
| Previous MI | - | 5 (16.7) |
| Hypertension | - | 20 (66.7) |
| Heart failure | - | 4 (13.3) |
| Diabetes mellitus | - | 17 (56.7) |
| Dyslipidemia | - | 22 (73.3) |
| Drug history | | |
| Aspirin | - | 30 (100) |
| Beta blocker | - | 22 (73.3) |
| Statins | - | 28 (93.3) |
| ACE inhibitor | - | 18 (60.0) |
| Plavix | - | 11 (36.7) |
| Angiography results | | |
| SVD | - | 12 (40.0) |
| 2VD | - | 12 (40.0) |
| 3VD | - | 6 (20.0) |
| History of PCI | - | 21 (70.0) |

Data are presented as mean ± SD or numbers (%)

SD: Standard deviation; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MI: Myocardial infarction; ACE: Angiotensin-converting-enzyme; SVD: Single vessel disease; 2VD: Two vessel disease; 3VD: Three vessel disease; PCI: Percutaneous coronary intervention

**Table 2.** Comparison of endothelial function parameters before and after receiving vitamin D supplements in patients with vitamin D deficiency and stable angina

| Endothelial function parameters | Before intervention | After intervention | P* |
|---|---|---|---|
| Serum vitamin D level (ng/ml) | 19.00 ± 6.50 | 53.20 ± 17.50 | < 0.001 |
| Baseline brachial artery diameter (mm) | 4.47 ± 0.37 | 4.47 ± 0.38 | > 0.999 |
| Brachial artery diameter after ischemia (mm) | 4.55 ± 0.37 | 4.67 ± 0.38 | < 0.001 |
| FMD (%) | 1.96 ± 1.65 | 4.65 ± 1.27 | < 0.001 |

Data are presented as mean ± SD; FMD: Flow-mediated dilatation; SD: Standard deviation; * Paired t-test

There was no significant correlation between baseline vitamin D level and brachial artery diameter before (r = 0.333, P = 0.078) or after ischemia (r = 0.286, P = 0.132) or with baseline FMD (r = 0.193, P = 0.355). Serum vitamin D level was increased by 34.0 ± 15.0 ng/ml and reached to > 30 ng/ml in all patients after the intervention (table 2). Analysis of brachial artery diameters showed that the mean of baseline diameters did not statistically change after the intervention (P > 0.999). Brachial artery diameter after ischemia increased significantly, statistically but not clinically, after the intervention (P < 0.001). FMD analysis showed that after vitamin D injection, FMD was significantly increased (P < 0.001) (table 2). Split analysis of patients with vitamin D deficiency and those with insufficiency provided the same results; FMD was improved in both groups (P < 0.001). There was no significant correlation between the amount of change in FMD and vitamin D after the intervention (r = 0.038, P = 0.858).

## Discussion

The aim of this study was to evaluate the effect of vitamin D therapy on vascular endothelial function in IHD patients with vitamin D deficiency/insufficiency. Our results revealed that two single intramuscular injection of vitamin D (300,000 IU) with 1-month interval can correct vitamin D deficiency in these patients. The increase in vitamin D level (though with no significant correlation) was accompanied with improvement of FMD as a marker of endothelial function in these patients, although, due to the uncontrolled design of the study, we cannot confirm that such improvement was exactly the result of vitamin D therapy.

Vitamin D deficiency is associated with a higher incidence of cardiovascular events and is treatable by vitamin D supplements that are inexpensive and available.[13] According to previous studies, low vitamin D level is associated with endothelial dysfunction.[14] Chitalia et al. have reported that patients with lower vitamin D levels had lower FMD. They have reported an independent association between low serum vitamin D level and endothelial dysfunction.[13] Similar to that study, Yiu et al. has reported that serum vitamin D status was significantly associated with brachial artery FMD and vitamin D deficiency might contribute to endothelial dysfunction.[15] Another study revealed that vitamin D insufficiency is associated with arterial stiffness and endothelial dysfunction.[16] Ertek et al. showed that serum vitamin D level is associated with better endothelial function by comparison of normal and vitamin D deficient subjects.[17] In contrast, we found no clear relationship in this regard that might be related to the small sample of our patients. The exact mechanisms in which vitamin D can influence the CVDs are not completely elucidated. However, there are some mechanisms that can be the explanation for the effect of vitamin D on the endothelial function. Vitamin D can decrease blood pressure and improve endothelial function by suppressing rennin system, decreasing vascular resistance, and by its effect on vascular calcifications.[10,18,19]

The effect of vitamin D supplements on CVDs is not well demonstrated. It has been reported that vitamin D therapy is associated with better survival of patients with CVD, especially in those with documented vitamin D deficiency.[20] Matias et al. have reported that 6 months oral cholecalciferol (a form of vitamin D) improves cardiac function in patients with chronic kidney disease.[21] There are limited studies on the effect of vitamin D supplements on endothelial function. Sugden et al. showed that a single large dose of vitamin D improves endothelial function in type 2 diabetes mellitus and vitamin D insufficient patients.[10] In contrast to Sugden et al.[10] study and also in contrast to our study, Yiu et al. has reported that 12 weeks oral supplementation of vitamin D does not significantly affect vascular function in patients with diabetes mellitus and suboptimal vitamin D levels.[22] Another study conducted by Tarcin et al. on asymptomatic vitamin D deficient subjects showed that mean FMD in deficient patients was significantly lower than normal controls, and also vitamin D supplements causes improvement of

FMD.[11] Similar to these studies, Stricker et al. showed that most of the patients with peripheral artery disease are vitamin D deficient. However, these investigators coul not find any association between vitamin D supplementation and improvement of endothelial function.[23] Witham et al. in a study on stroke patients showed that although high-dose oral vitamin D supplementation does not improved blood pressure status, but it causes an improvement of endothelial function in a short-term period.[24] We found no relationship between the amount of increase in vitamin D level and change in FMD after intervention. The change in serum level of vitamin D measured shortly after treatment may not exactly be correlated with its clinical consequences, and longer follow-up measurement is required in this regard.

The most important limitation of our study was its uncontrolled design accordingly we cannot confirm that the observed improvement in endothelial function was exactly the result of vitamin D therapy. It was unethical to not to treat vitamin D deficiency in IHD patients and consider them as controls. However, comparison with a control group of IHD patients without vitamin D deficiency would provide more reliable results by controlling for the clinical course of the disease. Furthermore, the study sample was selected consecutively from a single center and the sample size was small.

## Conclusion

Our results showed that vitamin D therapy (with a total dose of 600,000 IU intramuscular injection of cholecalciferol) is associated with improvement in endothelial function in IHD patients with vitamin D deficiency or insufficiency. We cannot confirm that the observed improvement in endothelial function was exactly the result of vitamin D therapy due to uncontrolled design of the study. According to these findings and the fact that vitamin D supplements are available and are inexpensive, vitamin D may be useful for prevention of more cardiovascular events in IHD patients especially in vitamin D deficient subjects. Controlled studies with larger sample of patients are required to confirm this study results.

## Conflict of Interests

Authors have no conflict of interests.

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