The effect of pitavastatin calcium on endothelial dysfunction induced by hypercholesterolemia

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Objective: To investigate whether endothelial function can be improved by the treatment of pitavastatin calcium via its antioxidant properties in hypercholesteremia patients.

Methods: Forty patients with hypercholesteremia were randomized to receive pitavastatin calcium 1 or 2 mg/day for 8 weeks. Among them, four people were lost in the follow-up period. Before and after treatment, clinical and biochemical characteristics, markers of oxidative stress (plasma 8-iso-prostaglandin F2α and serum gp91phox) were determined and concomitantly endothelium-dependent brachial artery flow-mediated dilation (FMD) was measured by ultrasound examination. Thirty healthy subjects were chosen as controls.

Results: For individuals with hypercholesteremia, total cholesterol, low-density lipoprotein cholesterol (LDL-C) and serum gp91phox were significantly increased (p < 0.001 for all) and plasma 8-iso-PGF2α (8-iso-PGF2α) was significantly higher (p < 0.05), while FMD was obviously impaired (p < 0.001). Total cholesterol, LDL-C and serum gp91phox were significantly reduced (p < 0.001 for all), plasma 8-iso-PGF2α was lower and FMD was significantly improved after pitavastatin calcium treatment compared with those before treatment in any group (p < 0.05 for both). However, there was no significant difference between the 1-mg and 2-mg pitavastatin calcium groups post-therapy.

Conclusions: Endothelial dysfunction induced by hypercholesterolemia can be ameliorated by pitavastatin calcium treatment, which occurs in part through its antioxidative properties.

Keywords: endothelial function, hypercholesterolemia, oxidative stress, pitavastatin calcium


1. Introduction

Atherosclerosis is a pathological, physiological mechanism of cardiovascular disease. Hypercholesterolemia, a fundamental risk factor of atherosclerosis (AS), is associated with impaired endothelial function [1]; and it is endothelial dysfunction, which is reflected by flow-mediated brachial artery vasodilation (FMD), that is an initiating factor and the central link of AS.

Reactive oxygen species (ROS) is proposed to contribute to the origin and development of cardiovascular diseases [2]. Increased oxidative stress or overproduction of ROS, which is likely to exacerbate endothelial damage and dysfunction caused by hypercholesterolemia, were found in the patients with hypercholesterolemia. NADPH oxidase, one of the most important cellular producers of ROS [3], has
gp91phox as a key subunit. And 8-iso-prostaglandin F\textsubscript{2\alpha} (8-iso-PGF\textsubscript{2\alpha}), a lipid peroxidation biomarker, is one of the most reliable indices for assessing oxidative stress status \cite{4}. To estimate the oxidative stress status, gp91phox and 8-iso-PGF\textsubscript{2\alpha} tests were used. Simultaneously dysfunctional endothelium also becomes the source of ROS, and promotes injury in vascular function. In addition to low-density lipoprotein (LDL) oxidation, reaction of ROS with cell membrane bound lipids can further a vicious cycle of continued oxidative damage, resulting in the development of atherosclerosis. It prompted us to consider improving endothelial dysfunction to reverse and delay the progression of atherosclerosis through some effective strategies.

In previous studies, statins have been demonstrated to retard the progression of atherosclerosis formation and reduce both cardiovascular morbidity and mortality \cite{5}.

These beneficial effects of statins are reasonably due to its pleiotropy involving reduction of oxidative stress, improvement of endothelial function and stabilization of atherosclerotic plaques \cite{6,7,8}. A growing number of researchers are focusing on statins to improve endothelial function in the anti-atherosclerosis field. The detailed mechanisms by which statins ameliorate endothelial function have not been fully elucidated; however, statins have been shown to improve endothelial function in patients with hypercholesterolemia and atherosclerosis \cite{8}. These findings have motivated our interests in investigating whether pitavastatin calcium – a newly developed statin, called a 'super statin' because it has a stronger reducing cholesterol effect – could reduce oxidative stress and ameliorate endothelial dysfunction induced by hypercholesterolemia. However, clinical studies on the pleiotropy of pitavastatin calcium in hypercholesterolemia groups are rare. Moreover, it has not yet been possible to explain the mechanisms improving endothelial function in hypercholesterolemia patients in China. Thus, the present study was undertaken to determine whether endothelial dysfunction was reversed by pitavastatin and whether it is mediated by its antioxidant properties in hypercholesterolemia.

2. Materials and methods

2.1 Study protocol

A randomized, double-blind study was carried out at the Qilu Hospital, Shandong University, China. Major inclusion criteria for this study were: age 18 – 70 years, total cholesterol 5.72 – 12.7 mmol/liter, triglycerides < 4.52 and/or low-density lipoprotein cholesterol (LDL-C) 3.64 – 6.5 mmol/liter. Patients with a history of coronary heart disease, renal failure or other significant diseases were excluded. Any lipid-lowering medication (e.g., statins or ezetimibe) was stopped at least 2 weeks before the study. Written informed consent was obtained from all subjects, and procedures were approved by the institutional ethics committees. The protocol was approved by the Human Research Committees of the Qilu Hospital.

2.2 Methods

2.2.1 Study design

According to the inclusion and exclusion criteria, all hypercholesterolemia patients were washed out for 2 weeks and then, according to the inclusion and exclusion criteria, again, the right patients (age and gender were matched with the controls) were randomly assigned to take pitavastatin calcium 1 or 2 mg/day (Jinan LiMin Pharmaceutical Co., Ltd) for 8 weeks. Among them, four people were lost in the follow-up period. At baseline and after 8 weeks of treatment, the following parameters were measured (Tables 1 and 2). Thirty healthy controls were enrolled after a careful history and clinical examination. Immediately after the acquisition of venous blood, plasma and serum were separated by centrifugation (3000 g at 4°C for 15 min), and then stored at -80°C until use. Plasma 8-iso-PGF\textsubscript{2\alpha} and serum gp91phox were measured with an enzyme immunoassay kit (ELIA, Cayman) and an enzyme-linked immunoassay kit (ELISA, BG), respectively.

2.2.2 Flow-mediated brachial artery vasodilation (FMD)

Endothelial function was evaluated by measuring the FMD of the brachial artery with a high-resolution ultrasound machine \cite{9,10} (Sequoia 512, Siemens). Participants were studied in an appropriate room after resting supine for a minimum of 5 min. Longitudinal images of the brachial artery were obtained at a marked point 5 – 10 cm proximal to the antecubital fossa. All brachial diameter images were recorded in diastole (ECG gated R-wave), and flow velocity was measured with a pulsed Doppler signal at a 60° angle to vessel, with the range gate (1.5 mm) in the center of the artery. In brief, after baseline measurements of the diameter (D\textsubscript{0}) and flow velocity (V\textsubscript{0}) in the brachial artery, a blood pressure cuff placed around the forearm was inflated with a pressure of 250 – 300 mmHg and released after 5 min. Velocity (V\textsubscript{1}) and diameter (D\textsubscript{1}) measurements for the reactive hyperemia were respectively taken 15 s and 45 – 90 s after cuff deflation. The diameters and velocities of three cardiac cycles were analyzed for each scan, and the measurements were averaged. Throughout the study, FMD was examined by the same skilled examiner, who used the same ultrasound apparatus and probe set. Woodman et al. \cite{11} examined data from two studies that reported intra-observer coefficients of variation of 1.8 – 2.3% when measuring FMD, but when expressed as a percentage change, the coefficients of variation increased to 28 – 33%. The formulas were as follows:

\[
\Delta D = D_1 - D_0, \text{ FMD} \% = \Delta D / D_0 \times 100\% ,
\]

where D\textsubscript{0} = baseline diameter, D\textsubscript{1} = maximum diameter after cuff deflation, and \Delta D = change in diameter. Absolute change in diameter divided by baseline diameter and percentage brachial FMD was computed.

\[
\Delta V = V_1 - V_0, \Delta V \% = \Delta V / V_0 \times 100\% ,
\]

where V\textsubscript{0} = baseline velocity, D\textsubscript{1} = maximum velocity after cuff deflation, \Delta V = change in velocity, and \Delta V \% = absolute...
change in velocity divided by baseline velocity, ΔV (%) was computed.

2.3 Statistical analysis
Normally distributed data are presented as means ± SD. Continuous variables were compared between groups by an independent t test or paired Student’s t test. An analysis of covariance (ANCOVA) was used to analyze the effects of pitavastatin calcium on endothelial function and oxidative stress, with other study variables taken into account as covariates. Multiple linear regression analysis was used to evaluate the contribution of risk factors. Partial correlation analysis was used to assess for possible relationships between FMD, oxidative stress, with other study variables taken into account as covariates. Multiple linear regression analysis was used to evaluate the contribution of risk factors. Partial correlation analysis was used to assess for possible relationships between FMD, oxidative stress, with other study variables taken into account as covariates. Multiple linear regression analysis was used to evaluate the contribution of risk factors. Partial correlation analysis

2.3.1 Basic clinical characteristics and ultrasound indexes of the studied population (Table 1)
Compared with the controls, the 1-mg pitavastatin calcium group pre-therapy showed significantly higher BMI, hip circumference, body mass index (BMI) and mean arterial pressure (MBP; p < 0.05 vs control). The 2-mg pitavastatin calcium group pre-therapy showed significantly lower 8-iso-PGF2α (p < 0.01 vs 1 mg pitavastatin calcium group pre-therapy).

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Compared with the controls, the 1-mg pitavastatin calcium group pre-therapy showed significantly lower 8-iso-PGF2α (p < 0.01 vs 1 mg pitavastatin calcium group pre-therapy). The results demonstrated that BMI was significantly high and the endothelial function was impaired in hypercholesterolemia patients. Basic clinical characteristics did not significantly differ after therapy; however, FMD was significantly increased (9.62 ± 0.93 vs 6.05 ± 0.82; 9.50 ± 0.96 vs 7.10 ± 0.61), which indicated that endothelial dysfunction was obviously ameliorated by the treatment of pitavastatin calcium. Moreover, there was no significance between the 1-mg and 2-mg pitavastatin calcium groups post-therapy (p > 0.05).

2.3.2 Basic biochemical characteristics and indexes of oxidative stress of the studied population (Table 2)
Compared with the controls, the 1-mg/2-mg pitavastatin calcium group pre-therapy showed significantly higher fasting blood glucose (FBG), total cholesterol (6.41 ± 0.51/6.75 ± 0.83 vs 4.78 ± 0.76), triglycerides, LDL-C 4.05 ± 0.68/4.13 ± 0.51 vs 2.51 ± 0.47), 8-iso-PGF2α (1.85 ± 0.19/1.90 ± 0.17 vs 1.47 ± 0.08) and gp91phox (p < 0.01 vs control).

Compared with the controls, the 1-mg/2-mg pitavastatin calcium group pre-therapy showed significantly higher fasting blood glucose (FBG), total cholesterol (6.41 ± 0.51/6.75 ± 0.83 vs 4.78 ± 0.76), triglycerides, LDL-C 4.05 ± 0.68/4.13 ± 0.51 vs 2.51 ± 0.47), 8-iso-PGF2α (1.85 ± 0.19/1.90 ± 0.17 vs 1.47 ± 0.08) and gp91phox (p < 0.01 vs control). The results demonstrated that cholesterol and oxidative stress levels were increased in hypercholesterolemia patients. Compared with the pre-therapy group, the 1-mg/2-mg pitavastatin calcium group post-therapy showed significantly lower 8-iso-PGF2α, triglycerides, total cholesterol, LDL-C and gp91phox (p < 0.01 for
both); however, there was no significance in AST, creatinine, creatine kinase and high-density lipoprotein cholesterol (HDL-C; \( p > 0.05 \)). The results indicated that cholesterol and oxidative stress levels were obviously decreased by the treatment of pitavastatin calcium in hypercholesterolemia patients, and also showed the safety and efficacy of pitavastatin calcium. There was no significance between the 1-mg and 2-mg pitavastatin calcium post-therapy groups (\( p > 0.05 \)).

### 2.3.3 Relationship between FMD, markers of oxidative stress and the risk factors (Table 3)

Stepwise multivariate regression analysis revealed the risk factors for FMD as being BMI (\( \beta = -1.210, p = 0.012 \)) and total cholesterol (\( \beta = -0.539, p = 0.004 \)); for gp91phox as being age (\( \beta = 0.473, p = 0.000 \)), BMI (\( \beta = 0.504, p = 0.000 \)), waist-to-hip ratio (WHR; \( \beta = 0.556, p = 0.001 \)), LDL-C (\( \beta = 0.464, p = 0.000 \)) and FBG (\( \beta = -0.539, p = 0.000 \)); and for 8-iso-PGF2\( \alpha \) as being age (\( \beta = 0.699, p = 0.000 \)), WHR (\( \beta = 0.704, p = 0.000 \)) and LDL-C (\( \beta = 0.714, p = 0.000 \)).

### 2.3.4 Correlations between FMD, markers of oxidative stress and lipid profile (Table 4)

Total cholesterol and LDL-C significantly correlated negatively with FMD (model 1: \( r = -0.228, p = 0.027 \) and \( r = -0.309, p = 0.002 \); model 2: \( r = -0.243, p = 0.018 \) and \( r = -0.292, p = 0.004 \)), which denoted that total cholesterol and LDL-C are risk factors for FMD. Total cholesterol, LDL-C and triglycerides significantly correlated positively with gp91phox (model 1: \( r = 0.386, p = 0.000, r = 0.423, p = 0.000 \) and \( r = 0.271, p = 0.008 \); model 2: \( r = 0.330, p = 0.001, r = 0.267, p = 0.009 \) and \( r = 0.232, p = 0.024 \)), which denoted that total cholesterol, LDL-C and triglycerides are risk factors for oxidative stress. LDL-C significantly correlated positively with 8-iso-PGF2\( \alpha \) (model 1: \( r = 0.236, p = 0.024 \); model 2: \( r = 0.237, p = 0.024 \)), which denoted that LDL-C is a risk factor for oxidative stress.

### 2.3.5 Effects of pitavastatin calcium on endothelial dysfunction and oxidative stress in hypercholesterolemia patients (Table 5)

Attenuated brachial artery FMD and increased oxidative stress levels have been associated with multiple cardiovascular risk factors in our results (Tables 3 and 4). To clarify the relations between lipid profile, FMD and oxidative stress, and, moreover, to document the effects of pitavastatin calcium on endothelial dysfunction and oxidative stress in hypercholesterolemia patients, an ANCOVA was used to control BMI or WHR, SBP, DBP, total cholesterol, triglycerides,

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**Table 2. Basic biochemical characteristics and indexes of oxidative stress of the studied population.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 30)</th>
<th>1 mg pitavastatin calcium group</th>
<th>2 mg pitavastatin calcium group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-therapy (n = 20)</td>
<td>Post-therapy (n = 18)</td>
<td>Pre-therapy (n = 20)</td>
</tr>
<tr>
<td>ALT (mmol/liter)</td>
<td>18.00 ± 8.08</td>
<td>21.85 ± 7.92</td>
<td>27.17 ± 14.35*</td>
</tr>
<tr>
<td>r-GGT (mmol/liter)</td>
<td>19.27 ± 9.05</td>
<td>23.70 ± 18.75</td>
<td>30.44 ± 25.51</td>
</tr>
<tr>
<td>AKP (mmol/liter)</td>
<td>70.27 ± 22.27</td>
<td>78.00 ± 25.21</td>
<td>79.17 ± 26.47</td>
</tr>
<tr>
<td>TP (mmol/liter)</td>
<td>70.11 ± 6.27</td>
<td>77.82 ± 4.66***</td>
<td>77.19 ± 41.11***</td>
</tr>
<tr>
<td>ALB (mmol/liter)</td>
<td>42.99 ± 3.62</td>
<td>46.77 ± 2.07**</td>
<td>46.32 ± 2.06**</td>
</tr>
<tr>
<td>GLB (mmol/liter)</td>
<td>27.24 ± 3.02</td>
<td>31.06 ± 4.32**</td>
<td>30.87 ± 3.18**</td>
</tr>
<tr>
<td>A/G</td>
<td>1.64 ± 0.21</td>
<td>1.53 ± 0.22</td>
<td>1.52 ± 0.15</td>
</tr>
<tr>
<td>Tbil (mmol/liter)</td>
<td>11.66 ± 3.29</td>
<td>12.38 ± 3.92</td>
<td>12.91 ± 4.22</td>
</tr>
<tr>
<td>TC (mmol/liter)</td>
<td>4.78 ± 0.76</td>
<td>6.41 ± 0.51***</td>
<td>5.09 ± 1.07***</td>
</tr>
<tr>
<td>TG (mmol/liter)</td>
<td>1.09 ± 0.26</td>
<td>2.07 ± 0.78***</td>
<td>1.43 ± 0.29***</td>
</tr>
<tr>
<td>HDL-C (mmol/liter)</td>
<td>1.40 ± 0.34</td>
<td>1.34 ± 0.26</td>
<td>1.44 ± 0.25</td>
</tr>
<tr>
<td>LDL-C (mmol/liter)</td>
<td>2.51 ± 0.47</td>
<td>4.05 ± 0.68***</td>
<td>2.85 ± 0.86***</td>
</tr>
<tr>
<td>FBG (mmol/liter)</td>
<td>5.06 ± 0.50</td>
<td>5.63 ± 0.63**</td>
<td>5.56 ± 0.60*</td>
</tr>
<tr>
<td>Cr (mmol/liter)</td>
<td>62.22 ± 8.47</td>
<td>66.15 ± 8.59</td>
<td>70.26 ± 1.79</td>
</tr>
<tr>
<td>BUN (mmol/liter)</td>
<td>5.84 ± 0.99</td>
<td>4.96 ± 1.05*</td>
<td>5.28 ± 0.76</td>
</tr>
<tr>
<td>AST (mmol/liter)</td>
<td>19.03 ± 8.06</td>
<td>20.55 ± 5.37</td>
<td>26.44 ± 14.96</td>
</tr>
<tr>
<td>CK (mmol/liter)</td>
<td>74.83 ± 35.93</td>
<td>87.80 ± 28.35</td>
<td>89.39 ± 26.27</td>
</tr>
<tr>
<td>gp91phox (ng/ml)</td>
<td>7.53 ± 2.41</td>
<td>9.77 ± 1.06**</td>
<td>7.31 ± 1.00***</td>
</tr>
<tr>
<td>8-iso-PGF2( \alpha ) (pg/ml)</td>
<td>1.47 ± 0.08</td>
<td>1.85 ± 0.19*</td>
<td>1.52 ± 0.14†</td>
</tr>
</tbody>
</table>

\* \( p < 0.05 \), \(** \( p < 0.01 \), \(**\( p < 0.001 \) vs control.

\( p < 0.05 \), \( \dagger \) \( p < 0.01 \), \( \dagger \) \( p < 0.001 \) vs 1 mg pitavastatin calcium group pre-therapy.

\( p < 0.05 \), \( \dagger \) \( p < 0.01 \), \( \dagger \) \( p < 0.001 \) vs 2 mg pitavastatin calcium group pre-therapy.

8-iso-PGF2\( \alpha \): 8-iso-prostaglandinF2\( \alpha \); ALB: Albumin; A/G: Albumin/globulin; BUN: Blood urea nitrogen; CK: Creatine kinase; Cr: Creatinine; FBG: Fasting blood glucose; GLB: Globulin; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TP: Total protein; Tbil: Total bilirubin; TC: Total cholesterol; TG: Triglycerides.
HDL-C, LDL-C, FBG, smoke and drink. FMD was significantly higher, gp91phox and 8-iso-PGF$_{2\alpha}$ were lower (p < 0.01 ~ 0.001 for all), even after these risk factors were controlled. If only BMI or WHR, SBP, DBP, FBG, smoke and drink were controlled, then FMD and gp91phox were not significant, but 8-iso-PGF$_{2\alpha}$ was lower (p < 0.05); If only lipid profiles were controlled, then FMD was significantly higher, and gp91phox and 8-iso-PGF$_{2\alpha}$ were lower (p < 0.05). There were no significance in BMI, WHR, SBP, DBP, FBG, smoke and drink after therapy. These results indicated that cholesterol was an important risk factor for endothelial dysfunction and lower enhanced oxidative stress; pitavastatin calcium can indeed ameliorate endothelial dysfunction and lower enhanced oxidative stress in hypercholesterolemia patients.

### 3. Discussion

The present study has several salient findings. Endothelial function was impaired in hypercholesterolemia subjects and significantly improved by pitavastatin calcium therapy (Table 1, Figure 1). The levels of oxidative stress (plasma 8-iso-PGF$_{2\alpha}$ and serum gp91phox) were significantly decreased with pitavastatin treatment (Table 2, Figures 2A, B). Endothelial dysfunction was ameliorated in hypercholesterolemia patients owing to the antioxidative property of pitavastatin calcium.

Hypercholesterolemia is widely accepted as a major risk factor for atherosclerosis [1]. Accumulating evidence has demonstrated that the presence of endothelial dysfunction is a major promoter for atherosclerosis and proposed as an independent prognostic predictor for the risk of cardiovascular events in several high-risk groups [12,13]. In hypercholesterolemia subjects, endothelial dysfunction is reported to be associated with excessive production of reactive oxygen species (ROS); moreover, ROS interact and inactivate nitric oxide, resulting in endothelial dysfunction and reduced vasodilation.

### Table 3. Relationships between flow-mediated brachial artery vasodilation, levels of oxidative stress and the risk factors.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Model</th>
<th>Risk factors</th>
<th>Standardized regression coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD</td>
<td>Model 1*</td>
<td>HC</td>
<td>2.109</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Model 2†</td>
<td>BMI</td>
<td>-1.210</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>Model 2‡</td>
<td>TP</td>
<td>1.420</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Model 2§</td>
<td>TC</td>
<td>-0.539</td>
<td>0.004</td>
</tr>
<tr>
<td>gp91phox</td>
<td>Model 1§</td>
<td>Age</td>
<td>0.473</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Model 2‡</td>
<td>BMI</td>
<td>0.504</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Model 2§</td>
<td>TC</td>
<td>0.418</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Model 3§</td>
<td>WHR</td>
<td>0.556</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Model 3¶</td>
<td>LDL-C</td>
<td>0.464</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Model 3∥</td>
<td>FBG</td>
<td>0.527</td>
<td>0.000</td>
</tr>
<tr>
<td>8-iso-PGF$_{2\alpha}$</td>
<td>Model 1∥</td>
<td>WHR</td>
<td>0.704</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Model 2∥</td>
<td>Age</td>
<td>0.699</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Model 3∥</td>
<td>LDL-C</td>
<td>0.714</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Model 1 included the following confounders: age, weight, BMI, WC, HC, smoke, drink and family history of disease (HP, DM and CHD).
†Model 2 included the following confounders: ALT, AST, TP, TC, TG, HDL-C, LDL-C, FBG, gp91phox and 8-iso-PGF$_{2\alpha}$.
‡Model 1 included the following confounders: age, sex, weight, BMI, SBP, DBP, smoke, drink and FMD.
§Model 2 included the following confounders: age, sex, WC, WHR, SBP, DBP, smoke, drink and FMD.
¶Model 3 included the following confounders: ALT, AST, AKP, γ-GGT, TC, TG, HDL-C, LDL-C and FBG.
∥AKP: Alkaline phosphatase; BMI: Body mass index; CHD: Coronary heart disease; DBP: Diastolic blood pressure; DM: Diabetes; FBG: Fasting blood glucose; FMD: Flow-mediated dilatation; HC: Hip circumference; HDL-C: High-density lipoprotein cholesterol; HP: Hypertension; LDL-C: Low-density lipoprotein cholesterol; SBP: Systolic blood pressure; TC: Total cholesterol; TG: Triglycerides; WC: Waist circumference; WHR: Waist-to-hip ratio; 8-iso-PGF$_{2\alpha}$: 8-iso-prostaglandinF$_{2\alpha}$.

### Table 4. Correlations between flow-mediated brachial artery vasodilation, levels of oxidative stress and lipid profile.

<table>
<thead>
<tr>
<th>Model</th>
<th>Risk factors</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD</td>
<td>TC</td>
<td>-0.228</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>LDL-C</td>
<td>-0.309</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>gp91phox</td>
<td>-0.292</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>0.386</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>LDL-C</td>
<td>0.423</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>0.271</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>8-iso-PGF$_{2\alpha}$</td>
<td>0.232</td>
<td>0.024</td>
</tr>
</tbody>
</table>

*Model 1: control age, BMI, SBP, DBP, FBG, smoke and drink.
†Model 2: control age, WHR, SBP, DBP, FBG, smoke, drink and statin.
‡Model 1: control age, BMI, SBP, DBP, FBG, smoke and drink.
§Model 2: control age, WHR, SBP, DBP, FBG, smoke and drink.
BMI: Body mass index; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; FMD: Flow-mediated dilatation; HC: Hip circumference; HDL-C: High-density lipoprotein cholesterol; HP: Hypertension; LDL-C: Low-density lipoprotein cholesterol; SBP: Systolic blood pressure; TC: Total cholesterol; TG: Triglycerides; WHR: Waist-to-hip ratio.
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Table 5. Effects of pitavastatin calcium on endothelial dysfunction and oxidative stress in hypercholesterolemia patients.

<table>
<thead>
<tr>
<th></th>
<th>Model</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD</td>
<td>Model 1*</td>
<td>7.037</td>
<td>0.001</td>
</tr>
<tr>
<td>gp91phox</td>
<td>Model 2†</td>
<td>5.760</td>
<td>0.004</td>
</tr>
<tr>
<td>8-iso-PGF2α</td>
<td>Model 1*</td>
<td>4.672</td>
<td>0.012</td>
</tr>
<tr>
<td>8-iso-PGF2α</td>
<td>Model 2†</td>
<td>3.894</td>
<td>0.024</td>
</tr>
</tbody>
</table>

*p < 0.001 vs control, †p < 0.05 vs control group.

Figure 1. Effect of pitavastatin calcium on endothelial dysfunction in hypercholesterolemia patients. FMD of brachial artery in each group: controls (n = 30), 1 mg pitavastatin calcium group pre-therapy (1 mg pre; n = 20), 1 mg pitavastatin calcium group post-therapy (1 mg post; n = 18), 2 mg pitavastatin calcium group pre-therapy (2 mg pre; n = 20) and 2 mg pitavastatin calcium group post-therapy (2 mg post; n = 18).

p < 0.001 vs control, †p < 0.05 vs 1 mg pre. §p < 0.05 vs 1 mg pre.

Oxidative stress, resulting from an imbalance between ROS and the antioxidant defense system, is a crucial mediator of endothelial dysfunction during hypercholesterolemia [19,20]. ROS include free radicals such as the super oxide (O2-·), the hydroxyl radical (HO·) and nonradical species such as hydrogen peroxide (H2O2), hyperchlorous acid (HClO) and peroxynitrite [21]. Several enzymes have been reported to produce ROS; however, it is now well satisfied that NAD(P)H oxidase(s) are a major source of ROS in vascular cells, gp91phox (NOX2) is one of its subunits and Nox2-based ROS production is of major importance in the endothelium [22]. 8-iso-PGF2α (a family of new prostaglandin isomers generated by free radical-mediated peroxidation of arachidonic acid) was used to estimate the oxidative stress status, because it has been proposed as a reliable marker for lipid peroxidation and oxidative stress [23]. Oxidative stress leads to endothelial dysfunction in the following ways (for review, see [24]): an excess of ROS may result in reduction of NO biosynthesis, diminishing of NO bioactivity, acceleration of NO degradation, decrease in NO and endothelial nitric oxide synthase (eNOS) bioavailability and so forth. Although cardiovascular risk factors are associated with endothelial dysfunction [25] (Table 3), the observation that patients with different degrees of endothelial dysfunction might have similar traditional risk factor profiles [26] supports the concept that this relationship is complex and may be affected by additional mediators, such as oxidative stress [27]. Consequently, increased oxidative stress is deemed to be a major mechanism involved in the pathogenesis of endothelial cell dysfunction in hypercholesterolemia patients and may serve as a common pathogenic mechanism of the effect of risk factors on the endothelium [28] (Table 4). So, we surmised that hypercholesterolemia-induced and ROS-mediated endothelial dysfunction was a major trigger of atherosclerosis.

The endothelium takes the role of the 'first line of defense' in physiological state. Endothelial dysfunction occurring on the endothelium is important in the onset and progression of atherosclerosis. All sorts of cardiovascular risk factors cause endothelial dysfunction by damaging endothelial cells. Hypercholesterolemia exerts an adverse effect on endothelial function and increases the accumulation of lipids in the vessel wall, which could be an early marker of endothelial injury [29]. In agreement with our data, Mugge et al. [30] have reported that the endothelium itself can be a source of ROS in hypercholesterolemia vessels, which is the ultimate reason for hypercholesterolemia-induced endothelial dysfunction, that is, eNOS per se produces ROS. Thereafter, enhanced...
oxidative stress seems to be essential for the induction of endothelial function during hypercholesterolemia [31]. LDL-C is preferentially deposited in the vascular wall at sites of dysfunction endothelium early in the course of the development of atherosclerotic lesions, where it is oxidized as a consequence of oxidative stress [32], and where there is an excess endothelial super oxide generation, which leads to augmented oxidation of LDL-C and inactivating NO, and as part of a vicious circle further promote endothelial cell membrane damage through generation of ROS [33]. Thus, in our opinion, it is thought that a vicious cycle of endothelial dysfunction, cholesterol and oxidative stress leads to development of atherosclerosis in hypercholesterolemia patients. This analysis would help us to gain additional, substantial insights into determining interventional measures to block the development of atherosclerosis in hypercholesterolemia patients.

Statins, inhibitors of cholesterol biosynthesis, are endowed with pleiotropic effects which include improvement of endothelial function, stability of atherosclerotic plaques and decrease of oxidative stress and inflammation [5,34-35]. It has been reported that the other statin could protect the endothelial function in hypercholesterolemic patients [36]. A more recent study has been shown that the antioxidant effect of pitavastatin could improve NO bioavailability [37], thus contributing to angiotensin II-induced cardiovascular remodeling and renal insufficiency. Nevertheless, so far, no research on the effect of pitavastatin calcium on vascular damage has been reported in Chinese hypercholesterolemia subjects. To the best of our knowledge, the data from our study provide for the first time evidence that pitavastatin calcium ameliorates endothelial function and attenuates oxidative stress (Table 5). The mechanism through which hypercholesterolemia-induced endothelial dysfunction is ameliorated with pitavastatin calcium treatment has been proposed to be the production of an oxidative stress.

The limitation of this study was that we did not detect the detailed mechanisms of pitavastatin calcium improving endothelial function; but its antioxidative property could be one vital of mechanisms to protect endothelial function. Although supporting data from large clinical studies are needed to substantiate the effects of pitavastatin calcium on restoring endothelial function, decreasing enhanced oxidative stress and lowering cholesterol before its therapy for hypercholesterolemia patients can be recommended, our results indicated a potential role for pitavastatin calcium therapy in the atherosclerosis diseases.

In conclusion, our study is the first to demonstrate that pitavastatin calcium improves endothelial dysfunction in patients with hypercholesterolemia, which is mediated partly by antioxidant mechanisms.

Declaration of interest

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