Coronary artery disease

Coronary endothelial dysfunction in patients with early coronary artery disease is associated with the increase in intravascular lipid core plaque†

Byoung-Joo Choi1,2, Abhiram Prasad1, Rajiv Gulati1, Patricia J. Best1, Ryan J. Lennon3, Gregory W. Barsness1, Lilach O. Lerman4, and Amir Lerman1*

1Division of Cardiovascular Disease, Department of Internal Medicine, Mayo Clinic Rochester, Mayo Clinic and College of Medicine, 200 First ST. SW, Rochester, MN 55905, USA; 2Department of Cardiology, Ajou University School of Medicine, Suwon, Korea; 3Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, USA; and 4Division of Nephrology, Department of Internal Medicine, Mayo Clinic and College of Medicine, Rochester, USA

Received 15 November 2012; revised 4 March 2013; accepted 26 March 2013; online publish-ahead-of-print 7 April 2013

See page 2025 for the editorial comment on this article (doi:10.1093/eurheartj/eht199)

Aims

Endothelial dysfunction is considered to play a key role in the development of atherosclerosis. However, only a limited number of human imaging studies have been available to demonstrate this hypothesis. The present study used near-infrared spectroscopy (NIRS) to investigate whether coronary endothelial dysfunction is associated with the lipid core plaque (LCP) in patients with early coronary artery disease.

Methods and results

A total of 32 patients with chest pain who had diameter stenosis <30% were enrolled. All patients underwent coronary endothelial function assessment using intracoronary acetylcholine infusion and NIRS of the proximal left anterior descending artery. The lipid core burden index (LCBI), LCBI/L (LCBI divided by the length of scanned artery), maxLCBI4mm (maximum value of LCBI for any of the 4-mm segment) and block chemogram (yellow: probability of LCP presence [0.98, tan: 0.84 [P] ≤ 0.98, orange: 0.57 [P] ≤ 0.84, red: P < 0.57) were measured. The mean percentage of yellow, tan, and orange colour blocks in patients with epicardial endothelial dysfunction was significantly higher than in those with normal epicardial endothelial function (9.5 ± 11.4 vs. 3.1 ± 6.5%, P = 0.042). There was a significant correlation between LCBI (r = -0.460, P = 0.008), LCBI/L (r = -0.453, P = 0.009), and maxLCBI4mm (r = -0.431, P = 0.014) and the degree of epicardial endothelial function. However, there was no significant correlation between LCBI (r = -0.101, P = 0.58), LCBI/L (r = -0.099, P = 0.59), and maxLCBI4mm (r = -0.063, P = 0.73) and the degree of microvascular endothelial function.

Conclusion

Patients with early coronary artery disease and endothelial dysfunction had a higher lipid content in the vascular wall than patients with normal endothelial function. The result of the present study supports the hypothesis that endothelial dysfunction is associated with pathogenesis of early atherosclerosis.

Keywords

Coronary endothelial dysfunction • Lipid core plaque • Near-infrared spectroscopy

Introduction

The endothelium, which is the monolayer of endothelial cells lining the lumen of vascular beds separating the vascular wall from the circulation, regulates vascular tone and maintains vascular homeostasis.1,2 Several animal1,4 and in vitro experiments5–9 have suggested that alterations in endothelial function, also referred to as endothelial dysfunction, limit the production of various vascular protective molecules, which exacerbates the atherosclerotic process. Furthermore, endothelial dysfunction has been also considered one of the earliest manifestations of the atherosclerosis, preceding angiographic or ultrasonic evidence of atherosclerotic

†This work has been performed in Mayo Clinic, Rochester, USA.
* Corresponding author. Tel: +1 507 255 4152, Fax: +1 507 255 2550, Email: lerman.amir@mayo.edu
Published on behalf of the European Society of Cardiology. All rights reserved. © The Author 2013. For permissions please email: journals.permissions@oup.com

European Heart Journal (2013) 34, 2047–2054
doi:10.1093/eurheartj/eht132
plaque. However, although many animal and in vitro evidences strongly suggest that there is an association between the presence of endothelial dysfunction and the pathogenesis of atherosclerosis, only a limited number of human studies using imaging modality are available to demonstrate this association and most of them have failed to substantiate the hypothesis.\(^{11,12}\)

Near-infrared spectroscopy (NIRS) has been widely used to characterize chemical composition of tissue. Recently, a novel intracoronary catheter system employing NIRS has been rigorously validated against autopsy specimens and is now established as a method to accurately identify a lipid core plaque (LCP) \textit{in vivo}.\(^{13,14}\) Moreover, demonstration of lipid deposition with NIRS may represent active atherosclerosis in the vascular wall, which is an advantage of new imaging modality compared with conventional grey scale intravascular ultrasound (IVUS). Therefore, the present study used NIRS to investigate whether coronary endothelial dysfunction is associated with detectable morphologic changes in the vascular wall in patients with early coronary artery disease. The result of the present study supports the hypothesis that endothelial dysfunction is associated with pathogenesis of early atherosclerosis.

**Methods**

**Patient population**

Between 1 March 2010 and 31 May 2012, patients who were referred for the elective coronary angiography and endothelial function assessment for the evaluation of chest pain were screened. Among them patients with early coronary artery disease, defined as diameter stenosis < 30% throughout entire coronary arteries on diagnostic coronary angiography were consecutively enrolled. Exclusion criteria included the presence of angiographic stenosis $\geq 30\%$, ejection fraction < 45\%, acute coronary syndrome, angioplasty or bypass surgery within 6 months prior to study, uncontrolled hypertension, valvular heart disease, significant endocrine, renal disorder, or pregnancy.

**Endothelial function assessment**

Coronary angiography was performed according to standard techniques using the femoral approach. Endothelium-dependent coronary vascular function was assessed as previously described.\(^{15-17}\) A 0.014-inch Doppler guidewire (FloWire, Volcano Therapeutics, Rancho Cordova, CA, USA) within a 2.2Fr coronary infusion catheter (Transit Microcatheter, DePuy, Warsaw, IN, USA) was advanced and positioned into the proximal portion of the left anterior descending artery (LAD). Intracoronary bolus injection of an incremental dose (24–60 $\mu$g) of adenosine, an endothelium-independent vasodilator primarily of the microcirculation, was administered into the guiding catheter to achieve maximal hyperaemia. Subsequently, endothelium-dependent coronary vascular function was assessed by a selective infusion of increasing concentrations of intracoronary acetylcholine for 3 min at each (10\(^{-6}\), 10\(^{-5}\), and 10\(^{-4}\) mol/L) into the LAD. Haemodynamic data (heart rate and mean blood pressure), Doppler parameter (average peak velocity (APV)), and coronary angiography were obtained after each infusion. The infusion was terminated when the highest molar concentration of acetylcholine (10\(^{-4}\) mol/L) was reached. The luminal diameter (LD) at the corresponding segment of NIRS scan between the proximal and middle LAD was measured using quantitative coronary angiography (QCA, Medis Corporation). QCA was performed by a technician who was blinded to the clinical and NIRS data. The coronary blood flow (CBF) was calculated as $\pi(LD/2)^2(APV/2)$. After the assessment of endothelium-dependent function, 200 $\mu$g of nitroglycerine was injected as an intracoronary bolus for the assessment of endothelium-independent function. The CBF was calculated for each patient at baseline and after each infusion of acetylcholine.\(^{18}\) The maximal effect of acetylcholine was expressed as per cent change in LD (represents epicardial endothelial function) and per cent change in the CBF (represents microvascular endothelial function) relative to baseline. Microvascular endothelial dysfunction was defined as an increase in the CBF of $<$ 50% in response to the maximum dose of acetylcholine.\(^{15,16}\) Since all enrolled patients, who had been referred for the evaluation of chest pain were not included in normal population, the median value of LD change of all enrolled patients was calculated for cut-off of epicardial endothelial dysfunction. Epicardial endothelial dysfunction was defined as decrease in LD below the median value in response to the maximum dose of acetylcholine.

**Intravascular ultrasound**

The methods of the IVUS examination have been described previously.\(^{19}\) The IVUS examination was performed after intracoronary administration of 100–200 $\mu$g nitroglycerine. A 20-MHz, 2.9F monorail, electronic Eagle Eye Gold IVUS catheter (Volcano Therapeutics) was advanced into the proximal LAD and automatic pullback at 0.5 mm/s was performed.

**Near-infrared spectroscopy**

At first, a coronary artery segment with vasoconstriction or vasodilation in response to acetylcholine was identified between the proximal and middle LAD using distance from anatomical landmarks such as side branches that are seen on angiography. Then, for each identified segment NIRS examination was performed to correlate with epicardial endothelial function of the same segment. The NIRS system consists of 3.2 Fr, 160 cm rapid exchange catheter (Lipiscan, InfraReDx, Burlington, MA, USA), a motorized pullback device and a console.\(^{13}\) Near-infrared spectroscopy catheter was introduced into the proximal LAD over a 0.014-inch coronary guidewire and pulled back using an automated mechanical pullback and rotation device at a speed of 0.5 cm/s and 240 rotation/m until the NIRS catheter was withdrawn into the guiding catheter. The NIRS system acquires $\approx$ 1000 NIRS measurement/12.5 cm of artery scanned and determines the presence of LCP at each interrogated location in the artery using a predictive algorithm. The calculated data are displayed in a two-dimensional map of the vessel (chemogram) (Figure 1). The $x$-axis of the chemogram represents the pullback position in millimetre scale and the $y$-axis represents the circumferential position in degrees (0–360\(^\circ\)); a colour scale from red to yellow indicates increasing algorithm probability that an LCP is present.\(^{13}\) From the chemogram, a summary metric of the probability that an LCP is present in a 2-mm interval of pullback is computed and displayed in a colour map called a ‘block chemogram’ (Figure 1). The block chemogram is mapped to the same colour scale as the chemogram, but the display is binned to four discrete colours to aid visual interpretation (red: $P < 0.57$, orange: 0.57 $\leq P < 0.84$, tan: 0.84 $\leq P < 0.98$, yellow: $P > 0.98$; algorithm probability that an LCP is present in that 2 mm block). To provide a quantitative summary metric of the LCP presence in the entire scanned segment, the lipid core burden index (LCBI) was calculated, which is the fraction of valid pixel in the chemogram that exceeds an LCP probability of 0.6, multiplied by 1000. Because LCBI is dependent on the length of the artery scanned, LCBI per length of the scanned artery (LCBL/L) was also analysed in the present study. The maxLCBL/L was defined as the maximum value of the LCBI for any of the 4 mm segment in the
interrogated region and used as the index representing the size of the LCP. The NIRS images and the block chemograms were recorded to a compact disk for offline quantitative analysis. The analysis was performed using the LipiScan analyzer software (LipiScan, InfraReDx, Burlington, MA, USA).

### Statistical analysis
Statistical analysis was performed using the JMP 8.0 Software and SAS 9.3 (SAS Institute, Cary, NC, USA). Continuous variables were presented as mean ± standard deviation if normally distributed and were compared with the unpaired Student’s t test. Variables not normally distributed were presented as median (first, third quartile) and compared with the Mann–Whitney rank-sum test. Categorical variables were presented as frequency (percentage) and compared with Pearson’s χ² test. Since distribution of NIRS data was skewed, log transformation was applied to LCBI, LCBI/L, and maxLCBI4mm for correlation analysis. Pearson’s correlation coefficient was calculated to summarize the linear association between endothelial function and NIRS variables. For the analysis of colour block, percentages of colour blocks within each patient were calculated and compared with the Mann–Whitney rank-sum test. Lin’s concordance coefficient was used to describe intra- and inter-observer reproducibility. All statistical tests were two-sided and a P-value <0.05 was considered to be statistically significant.

### Results

#### Patient characteristics
A total of 32 patients were enrolled in the present study and found to have abnormal (n = 15) or normal (n = 17) epicardial endothelial function. There was no statistical difference in baseline characteristics between the two groups (Table 1). The median value of LD change in response to acetylcholine was −8.6%, which was used for cut-off of epicardial endothelial dysfunction. The mean length of the LAD interrogated by NIRS pullback was 60.8 ± 23.8 mm overall and was 61.5 ± 17.9 mm and 58.6 ± 25.9 mm, respectively, in patients with epicardial endothelial dysfunction and with normal epicardial endothelial function (P = 0.641). In the analysis of chemogram, overall LCBI and overall maxLCBI4mm were 3.5 (1.0, 21.75) and 29.0 (6.0, 158.3), respectively. The mean percentage of yellow, tan, orange, and red colour blocks were 0.6 ± 1.6, 2.2 ± 4.3, 3.2 ± 5.6, and 93.9 ± 9.4%, respectively. The per cent plaque burden measured by IVUS was similar in both groups (28.0 ± 4.4 vs. 25.5 ± 4.1%, P = 0.94, Table 2). The mean lesion length of the plaque measured by QCA was also similar between both groups (11.1 ± 1.7 vs. 11.7 ± 1.8 mm, P = 0.39). Endothelium-independent function of the epicardial artery as assessed by per cent change of LD in response to nitroglycerine was similar between two groups (13.8 ± 13.0 vs. 18.3 ± 12.7%, P = 0.34) (Figure 2A). Endothelium-independent function of microvasculature as assessed by CFR was also similar between two groups (2.8 ± 0.7 vs. 3.2 ± 0.6, P = 0.07) (Figure 2B). There was no clinical major complication attributable to NIRS or IVUS procedures.

### Block chemogram analysis
The mean percentage of the yellow, tan, and orange colour blocks (the probability of the LCP presence is >57%) in patients with epicardial endothelial dysfunction was significantly higher (9.5 ± 11.4 vs. 3.1 ± 6.5%, P = 0.042) (Figure 3A). When 0% LD change was used for cut-off of epicardial endothelial dysfunction instead of the median value, mean percentage of the yellow, tan, and orange colour blocks in patients with epicardial endothelial dysfunction (n = 26) was still significantly higher than in those with normal (n = 6) epicardial endothelial function (7.4 ± 10.0 vs. 0%, P = 0.032).

However, when the patients were grouped on the basis of their microvascular endothelial function, mean percentages of yellow, tan, and orange colour blocks in both groups were similar (7.5 ± 11.2 vs. 4.4 ± 7.2%, P = 0.49) (Figure 3B).

### Correlation analysis between endothelial function and near-infrared spectroscopy
There was a significant correlation between LCBI (r = −0.460, P = 0.008) (Figure 4A), LCBI/L (r = −0.453, P = 0.009) (Figure 4B), maxLCBI4mm (r = −0.431, P = 0.014) (Figure 4C), and per cent change of LD in response to intracoronary acetylcholine infusion.

However, there was no significant correlation between LCBI (r = −0.101, P = 0.58) (Figure 4D), LCBI/L (r = −0.099, P = 0.59) (Figure 4E), maxLCBI4mm (r = −0.063, P = 0.73) (Figure 4F) and per cent change of the CBF in response to acetylcholine infusion.

### Intra- and inter-observer reproducibility
The intra- and inter-observer reproducibility for the LCBI and maxLCBI4mm were high. The concordance coefficient for intra/ LCBI, inter/LCBI, intra/ maxLCBI4mm, and inter/maxLCBI4mm was 0.9986 (95% CI: 0.9977–0.9996), 0.9992 (95% CI: 0.9987–0.9997),
0.9997 (95% CI: 0.9994–0.9999), and 0.9996 (95% CI: 0.9994–0.9999), respectively.

**Discussion**

**Endothelial dysfunction and atherosclerosis**

The current study demonstrates that patients with early coronary atherosclerosis and endothelial dysfunction had a significantly higher probability of the presence of an LCP in the vascular wall as detected by the higher percentage of yellow, tan, and orange colour blocks in block chemogram analysis. Moreover, those patients had significantly greater LCP burden (LCBI) and the larger LCP (maxLCBI4mm) with increasing severity of epicardial endothelial dysfunction. Thus, the present study demonstrates for the first time that patients with early stage coronary artery disease and epicardial endothelial dysfunction have evidence of more lipid deposition in the vascular wall.

However, we also found a discrepancy between the IVUS-determined plaque burden and the NIRS-determined LCP burden. While NIRS scan demonstrated clear association between endothelial dysfunction and lipid deposition in the vascular wall, grey scale IVUS was not able to show the difference in the plaque burden according to endothelial function. The fundamental difference between NIRS and IVUS is that, whereas grey scale IVUS shows only atherosclerosis burden, NIRS can demonstrate lipid deposition in the vascular wall, which may represent activity of atherosclerosis. Therefore, this discrepancy could be interpreted that although these patients have the comparable amount of the atherosclerosis burden, as determined with IVUS, those with endothelial dysfunction may have more ‘active’ atherosclerotic plaque as they have more LCP. The current observations may provide a rationale why endothelial dysfunction is closely associated with rapid progression of coronary atherosclerosis and cardiovascular events due to plaque rupture.20 Furthermore, these data may also explain

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epicardial endothelial dysfunction (n = 15)</td>
</tr>
<tr>
<td>Age</td>
<td>58.0 ± 9.4</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>10 (66.7)</td>
</tr>
<tr>
<td>Hypercholesterolaemia (%)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30.1 ± 7.9</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>193.7 ± 39.9</td>
</tr>
<tr>
<td>Low-density lipoprotein cholesterol (mg/dL)</td>
<td>102.3 ± 37.4</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol (mg/dL)</td>
<td>66.9 ± 20.2</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>122.4 ± 70.0</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>95.2 ± 9.1</td>
</tr>
<tr>
<td>Haemoglobin A1c (%)</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>4.8 ± 6.6</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>8.1 ± 2.6</td>
</tr>
<tr>
<td>Medications (%)</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitors/ARB’s</td>
<td>2 (13.3)</td>
</tr>
<tr>
<td>Statins</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>6 (40.0)</td>
</tr>
</tbody>
</table>

Values are mean ± SD or n (%). 
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Comparison of intravascular ultrasound findings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epicardial endothelial dysfunction (n = 15)</td>
</tr>
<tr>
<td>Vessel area (mm²)</td>
<td>10.8 ± 1.4</td>
</tr>
<tr>
<td>Lumen area (mm²)</td>
<td>7.8 ± 1.3</td>
</tr>
<tr>
<td>Plaque area (mm²)</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>Plaque burden (%)</td>
<td>28.0 ± 4.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
in part why some individuals are more prone to develop more coronary artery plaque burden than others despite a similar cardiovascular risk profile.

In addition, it is worth noting that while epicardial endothelial function was spatially associated with the LCP, there was a lack of association between endothelial dysfunction of microcirculation and the presence of the LCP in the same epicardial coronary artery. This dissociation can be interpreted in a way that the impact of endothelial dysfunction on the pathogenesis of atherosclerosis may be regionally different despite endothelial dysfunction being a systemic process. It is certainly a compatible finding with a previous report that endothelial dysfunction enhances local vascular inflammation by the loss of protective and physiological function. Another possible explanation is that endothelial dysfunction may play a key role in the process of atherosclerosis, not just one of the earliest manifestations of atherosclerosis. The spatial association between the LCP and endothelial dysfunction of the epicardial artery, i.e. they share the same anatomic location, but not with microvascular endothelial dysfunction may represent that atherosclerosis develops just on the dysfunctional endothelium, which suggests causality between endothelial dysfunction and atherosclerosis.

The mechanisms of exacerbating atherosclerosis by endothelial dysfunction have been extensively investigated through in vitro and animal studies. Decreased bioavailability of nitric oxide and subsequent loss of vascular protective function have been thought of as main mechanism for the progression of atherosclerosis. However, in vivo human studies using imaging modalities have failed to definitely substantiate this association. Nishimura et al. reported that coronary endothelial dysfunction was not predicted by coronary atherosclerosis as assessed by grey scale IVUS, which is in line with our data. On the contrary, Zeiher et al. reported that the coronary vasomotor response to endothelium-dependent dilator acetylcholine was correlated with

**Figure 2** Endothelium-dependent and -independent function assessment for the epicardial coronary artery and coronary microvasculature. (A) Segmental response to acetylcholine and nitroglycerine as determined by per cent change of luminal diameter. (B) Per cent change of the coronary blood flow in response to acetylcholine and coronary flow reserve.

**Figure 3** The mean percentages of colour blocks in near-infrared spectroscopy block chemogram analysis. (A) The mean percentages of colour blocks in patients with epicardial endothelial dysfunction and with normal endothelial function. (B) The mean percentages of colour blocks in patients with microvascular endothelial dysfunction and with normal endothelial function. These colours blocks indicate the probability of the presence of the lipid core plaque as described in the method (yellow: $P > 0.98$, tan: $0.84 \leq P \leq 0.98$, orange: $0.57 \leq P \leq 0.84$).
local atherosclerotic wall thickening measured by IVUS. There are several possible speculations for these discrepancies between studies. The population enrolled in Zeiher et al.’s study had more advanced coronary artery disease (37.5 ± 11.1 and 39.0 ± 10.9% plaque burden for each group) compared with our population (25.5 ± 4.1 and 28.0 ± 4.4% plaque burden for each group). Since the more plaque burden tends to produce the less vasoreactivity to endothelium-dependent or -independent vasodilators,
with advanced plaque endothelial dysfunction could be overestimated. Further, endothelial dysfunction may precede the structural atherosclerotic change which is apparent enough to be identified by IVUS. Celemajer et al.\(^{10}\) and Reddy et al.\(^{12}\) have reported that selective endothelial dysfunction can be detected at both conduit and microvascular vessels in patients with coronary risk factors but no angiographic or ultrasound evidence of structural coronary artery disease. The current observation that a substantial number of patients with epicardial endothelial dysfunction had normal or near normal NIRS scores supports these previous studies.\(^{10,12}\) Another potential explanation may be a limited ability of grey scale IVUS to identify the component of the atheromatous plaque. It is conceivable that in patients with early atherosclerosis, the difference of each patient’s plaque burden is so small that it cannot be differentiated without tissue characterization. Thus, the current study underscores the need for more assessment of plaque components by imaging modalities and the use of these novel-imaging technologies is more likely to detect these differences.

### Acetylcholine

In the present study, we investigated the relationship between endothelial function and atherosclerosis using acetylcholine. Acetylcholine, although it is a standard probe for the assessment of endothelial function may raise an issue. Recently, Tracey and co-workers\(^{23,24}\) have reported that efferent activity of vagal nerve termed ‘cholinergic anti-inflammatory pathway’ releases acetylcholine in the vicinity of macrophage and inhibits active inflammation by down-regulating the expression of pro-inflammatory cytokines. Potential effects that acetylcholine has on active inflammatory process of atherosclerosis should be paid attention to. It may at least in part underlie the relationship of lipid core characteristics to the integrity of the endothelium.

### Near-infrared spectroscopy

NIRS is capable of determining chemical compositions on the basis that the amount of NIR light reflected or absorbed from a substance varies according to its chemical components at different wavelengths, providing unique spectral signatures that can be used as ‘chemical thumbprints’. The NIRS system showed excellent ability in discriminating tissue type difference based on chemical composition in coronary autopsy specimen and in vivo validation study.\(^{13,25}\) Thus, NIRS has been proved to be the only available tool capable of reliably detecting the LCP. We used NIRS to investigate the relationship between endothelial function and coronary atherosclerosis. As demonstrated in the current study, NIRS was able to identify greater lipid deposition in the vascular wall of patients with epicardial endothelial dysfunction than those with normal function. Identification of plaque components by NIRS may have potential clinical implication that it can predict the presence of early stage of epicardial atherosclerosis. Furthermore, the ability to correlate the images with these emerging technologies to early functional changes of the vascular wall may serve as an important tool to assess the effect and potential mechanism of novel therapy for coronary atherosclerosis in humans.

### Study limitation

First, although NIRS provides identification and accurate assessment of the LCP, there were several limitations to consider that may influence on the interpretation of the results. Gardner et al.\(^{13}\) comparing the chemogram signals and histological findings, reported that false-positive reading of NIRS could be caused by fibroatheromas too small or with caps too thick to meet criteria for the LCP of interest or by lesions containing significant lipid, but not having the necrotic core (intimal xanthoma and pathologic intimal thickening). Since the patients enrolled in the present study have early coronary atherosclerosis, there is a possibility of false positive readings. However, the lipid content in atherosclerosis generates near-infrared signals and subsequently is measured by NIRS, which is consistent with our study purpose. Secondly, whereas endothelial dysfunction is typically considered as a systemic phenomenon, we studied only LAD which may not have fully represented the extent of systemic endothelial dysfunction. Furthermore, several factors, such as mental stress\(^{26}\) and anxiety,\(^{27}\) have been known to be the regulators of the microvascular endothelial function, which may have affected the results of the current study. Thirdly, although we showed excellent results regarding intra- and inter-observer variability, we did not provide the data regarding inter-scan variability, which may raise bias issue regarding measurement. Fourthly, the characteristics of our study population are not typical for general patients with coronary artery disease. The symptomatic patients without flow-limiting stenosis of the epicardial coronary artery may represent a selected group of patients, i.e. they may represent patients with microvascular disease. Thus, caution should be taken in interpreting and generalizing the results to all patient population. Further studies are warranted to confirm the results.

### Conclusion

In summary, the current study demonstrates that patients with early coronary atherosclerosis and endothelial dysfunction exhibit evidence of more lipid deposition in the vascular wall compared with similar patients with normal coronary endothelial function. The result of the present study supports the hypothesis that endothelial dysfunction is associated with pathogenesis of early atherosclerosis.

### Funding

This work was supported by National Institute of Health grants HL92954, G31750, HL77131 and HL085307 and the Mayo Foundation.

### Conflict of interest

None declared.

### References


