Impact of Oxidative Stress on Arterial Elasticity in Patients with Atherosclerosis

Jaak Kals, Priit Kampus, Mart Kals, Kersti Zilmer, Tiitu Kullisaar, Rein Teesalu, Andres Pulges, and Mihkel Zilmer

Background: Alterations in the elastic behavior of arteries is an early sign of vascular damage in atherosclerosis and may be promoted by oxidative stress (OxS). However, studies designed for simultaneous assessment of arterial elasticity and OxS status in patients with peripheral arterial disease (PAD) are absent. The purpose of this study was to assess large (C1) and small artery elasticity (C2) and indices of OxS in patients with PAD as well as to investigate possible relationships between these parameters.

Methods: Arterial elasticity was assessed noninvasively by pulse wave analysis (PWA) and biochemical measurements were taken from 38 patients with PAD and from 28 matched control subjects. The elasticity indices of the arteries were derived from PWA based on the modified Windkessel model and the OxS status was measured using urinary 8-iso-prostaglandin F$_2$alpha (F$_2$-IsoPs) and plasma baseline diene conjugates of low-density lipoproteins (LDL-BDC).

Results: Patients with PAD showed significantly reduced C1 and C2 and increased values of F$_2$-IsoPs and LDL-BDC. There was an inverse association between C1 and F$_2$-IsoPs, as well as between C2 and F$_2$-IsoPs ($R = -0.3, P = .04; R = -0.49, P = .002$, respectively) in the patient group, but not in the controls. After controlling for potential confounders in a multiple regression model, the associations between C2 and F$_2$-IsoPs remained significant in the patient group ($P < .001$).

Conclusions: The possible link between arterial elasticity and F$_2$-IsoPs in patients with PAD suggests that oxidative modifications may be involved in alterations of arterial elastic properties in atherosclerosis.

Key Words: Arterial elasticity, atherosclerosis, oxidative stress, pulse wave analysis.

Atherosclerosis is a progressive process that begins in the arterial wall and affects vascular elastic properties. Reduced arterial elasticity, measured also in terms of vascular compliance, stiffness, or distensibility, is an early sign of vascular damage in atherosclerosis and can be used as a surrogate marker of arterial function. Assessment of arterial elastic properties has an impact on identification of subclinical cardiovascular pathologies, enabling prevention and targeted early treatment, and may also allow to evaluate the effects of therapeutic interventions.

Pulse wave analysis (PWA), based on the modified Windkessel model of the vasculature, is a noninvasive, convenient, and reproducible method for assessing large and small artery compliance. Measures of PWA are useful in estimating vascular abnormalities in cardiovascular disorders, associated with functional as well as structural properties of the vasculature and independently predict cardiovascular risk.

There is consensus that the common mechanism underlying vascular damage relates to increased production of reactive oxygen species (ROS) and consequent high-grade oxidative stress (OxS). Studies directed to assessment of OxS suggest that 8-iso-prostaglandin F$_2$alpha (F$_2$-IsoPs), generated as a result of the free radical-mediated peroxidation of arachidonic acid, is a relevant marker for quantifying OxS in humans. In addition, oxidized LDL (oxLDL) is an indicator of OxS in atherogenesis and measurement of baseline diene conjugates of LDL (LDL-BDC) is a reliable method to monitor oxidative modifications of LDL.

However, there are no studies designed for simultaneous assessment of arterial elasticity, F$_2$-IsoPs, and LDL-BDC in patients with PAD. Therefore, the aims of this study were to assess arterial elasticity, using PWA,
and the status of Oxs in patients with PAD as well as to
determine possible associations between altered arterial
elasticity and Oxs.

Methods

Study Population

The study group consisted of 38 patients with PAD having
stages II–III as defined by Fontaine: stage II, intermittent
claudication and stage III, leg pain at rest. All patients
were recruited from the Clinic of Cardiovascular and Tho-
racic Surgery, University Clinics of Tartu, Estonia. The
subjects were all men with angiographically proven PAD
(i.e., with stenosis or occlusion of the arteries of the lower
extremities). Ankle brachial pressure index (ABPI) was
less than 0.8 (range 0.22 to 0.79) in the patients with PAD.
The patients’ exclusion criteria were the following: any con-
comitant acute or chronic inflammatory disease, myocardial
infarction, coronary revascularization, or cerebrovascular
events during the past 6 months, earlier revascularization
procedures at the lower limb, upper limb occlusive arterial
disease, hypertension (blood pressure [BP] ≥140/90 mm
Hg), cardiac arrhythmias, or valve pathologies, diabetes
mellitus (fasting serum glucose level >6 mmol/L), malig-
nancies, and renal failure. Ten (26.3 %) patients with
coronary artery disease (CAD) as comorbidity were re-
cruited in the study.

The control group of men (N = 28) was recruited from
the general population and the exclusion criteria were the
following: any acute or chronic inflammatory disease,
CAD, cardiac arrhythmias, or valve pathologies, hyperten-
sion (BP ≥140/90 mm Hg), cerebral or peripheral athero-
sclerotic disease, diabetes mellitus (fasting serum glucose
level >6 mmol/L), malignancies, and renal failure. Ten (26.3 
%) patients with coronary artery disease (CAD) as comorbidity were re-
cruited in the study.

Study Protocol

The subjects were studied and the plasma samples were
collected between 8:00 and 10:00 AM, after an overnight
fast and abstinence from any medications, tobacco, alco-
hol, and tea or coffee. After 15 min of rest in a quiet,
temperature-controlled room, ABPI and BP were mea-
sured and PWA was performed. Thereafter, venous blood
samples were drawn from the antecubital fossa, and urine
samples were collected. Height and weight were recorded,
and body mass index (BMI) was calculated.

Biochemical Analyses

Blood samples were centrifuged and plasma for LDL-BDC
as well as urine for F2-IsoPs and creatinine were divided into
aliquots and stored at −70°C until analysis. The urinary
content of F2-IsoPs was analyzed by competitive enzyme-
linked immunoassay (BIOXYTECH 8-Isoprostane Assay,
OxisResearch, Portland, OR). Briefly, F2-IsoPs in the sam-
ple competed for binding (to the antibody coated on the
plate) with F2-IsoPs conjugated to horseradish (Amoracia
rusticana) peroxidase. Peroxidase activity resulted in color
development when the substrate was added. The intensity
of the color was proportional to the amount of bound
F2-IsoPs–horseradish peroxidase (HRP) and inversely
proportional to the amount of F2-IsoPs-HRP in the sam-
ple or standards. The urinary concentrations of F2-IsoPs
were corrected by urinary creatinine concentrations to
account for the differences in renal function.

Baseline diene conjugates of LDL as markers of ox-
LDL were measured by determining the level of LDL
diene conjugation using a method that has been recently
validated and reported in detail.14 In brief, serum LDL was
isolated by precipitation with buffered heparin citrate. The
amount of peroxidized lipids in the samples was deter-
mined by the degree of conjugated diene double bonds.
Lipids were extracted from the samples by a mixture of
chloroform and methanol (2:1), dried under nitrogen, re-
dissolved in cyclohexane, and analyzed spectrophoto-
metrically at 234 nm. For LDL-BDC, the coefficient of
variance for within-assay and between-assay precision
was 4.4% and 4.5%, respectively.

Plasma glucose, total cholesterol, LDL-cholesterol, HDL-
cholesterol, triglyceride levels, and urinary creatinine concen-
trations were determined by standard laboratory methods
using certified assays in a local clinical laboratory.

Assessment of Arterial Elasticity

The arterial waveform was measured in the dominant arm by
a Cardiovascular Profiling Instrument (HDI/Pulse Wave CR-
2000, Hypertension Diagnostics Inc, Eagan, MN). Briefly,
the tonometer was applied to the patient’s radial artery at the
wrist overlying the radial bony prominence. The subject’s
arm was supported by a wrist stabilizer for optimal position-
ing and minimal movement during the measurements. The
cuff for BP measurement was placed on the contralateral arm
and inflated concurrently with pulse waveform recording
for calibration. The elasticity indices of the arteries (C1 and
C2) were quantified during the diastolic portion of the
cardiac cycle (mean of 30-sec recording). According to the
modified Windkessel model of circulation, C1 is a marker
for large artery elasticity and C2 is a marker for small
artery elasticity. Heart rate, mean arterial pressure (MAP),
and stroke volume were also calculated from the radial
pressure waveform using the HDI/Pulse Wave CR-2000
software. These hemodynamic parameters (i.e., MAP and
stroke volume) are used in the multivariate algorithms for
determining both C1 and C2. The full method has been
validated and also described in detail previously.6,7

Measurement of ABPI

The ABPI was measured using Mini Dopplex D900 (Hun-
tleigh Healthcare Ltd., Cardiff, UK). The recordings were
taken from the (more) symptomatic lower limb in the patients. The value of ABPI was calculated as an average of two resting measurements.

Statistical Analysis

All data were tested for normality using the Kolmogorov-Smirnov test. The continuous data are expressed as the means ± SD if distributed normally, or otherwise by medians with 25% and 75% percentiles. The dichotomous variables are given as prevalence in number and percentage. The skewed data were log-transformed to obtain a normal distribution and then analyzed. The comparisons between the patients and the controls were assessed using unpaired two-tailed Student \( t \) test (for the means) and Mann-Whitney U test (for the medians). The correlations between the variables were examined using multiple linear regression analysis (free software R, version 1.9.0 for Windows). Significance was defined as \( P < .05 \).

Results

Subject Characteristics

The clinical characteristics of the 38 patients and the 28 matched controls are summarized in Table 1. There was no significant difference between the groups in age, height, systolic and diastolic BP, MAP, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glucose, and urinary creatinine level. However, there occurred a significant difference in ABPI, C1, C2, LDL-BDC, F2-IsoPs (also in creatinine indexed F2-IsoPs) between the groups. After adjustment for LDL-BDC and F2-IsoPs, the differences between the groups in small artery elasticity remained significant (\( P < .001 \)), but the differences in large artery elasticity did not achieve significance (\( P = .16 \)).

Relationships Between Arterial Elasticity and Oxidative Stress

Linear regression analysis was used to establish whether arterial elasticity correlated with the OxS-related indices. There was a significant inverse association between C1 and F2-IsoPs, as well as between C2 and F2-IsoPs only in the patient group, but not in the controls (Figs. 1 and 2). The results of correlation analysis and clinical data encouraged elucidation of the relationships between arterial elasticity and F2-IsoPs also in multivariate models, adjusted for age and BMI, separately for both groups. The final model with these covariates (Table 2) revealed that C1 was not significantly related to F2-IsoPs level in the controls but was inversely associated with age and positively with BMI in the healthy subjects. In the patient group C1 clearly tended to inversely correlate with F2-IsoPs after such adjustment.

The data for either group were used separately in multivariate models to determine also correlations between C2 and F2-IsoPs. Table 3 shows that logC2 is determined inversely by age and positively by BMI in the healthy

### Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>PAD patients ( N = 38 )</th>
<th>Controls ( N = 28 )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>58.6 ± 7.8</td>
<td>55.6 ± 6.7</td>
<td>.11</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.06</td>
<td>1.75 ± 0.04</td>
<td>.15</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 ± 3.5</td>
<td>25.3 ± 2.8</td>
<td>.02</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>125.3 ± 8.7</td>
<td>121 ± 11.6</td>
<td>.09</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>76.9 ± 7.6</td>
<td>74 ± 7.4</td>
<td>.14</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>92.9 ± 6.4</td>
<td>90.7 ± 9.3</td>
<td>.27</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>64.9 ± 8.6</td>
<td>58.5 ± 7.1</td>
<td>.002</td>
</tr>
<tr>
<td>ABPI</td>
<td>0.41 ± 0.12</td>
<td>1.34 ± 0.29</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.59 ± 0.87</td>
<td>5.21 ± 0.82</td>
<td>.07</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.28 ± 0.33</td>
<td>1.36 ± 0.33</td>
<td>.34</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.93 ± 1.13</td>
<td>3.59 ± 0.76</td>
<td>.17</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.33 ± 0.31</td>
<td>1.25 ± 0.5</td>
<td>.46</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.13 ± 0.59</td>
<td>5.25 ± 0.48</td>
<td>.36</td>
</tr>
<tr>
<td>LDL-BDC (µmol/L)</td>
<td>23.7 ± 8.13</td>
<td>18.34 ± 5.74</td>
<td>.004</td>
</tr>
<tr>
<td>Urinary creatinine (mmol/L)</td>
<td>12.72 ± 4.43</td>
<td>14.59 ± 5.83</td>
<td>.2</td>
</tr>
<tr>
<td>F2-IsoPs (ng/mL)</td>
<td>5.34 ± 1.95</td>
<td>2.34 ± 0.79</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>F2-IsoPs (ng/mg creatinine)</td>
<td>9.47 ± 5.71</td>
<td>4.29 ± 2.58</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Stroke volume (mL/beat)</td>
<td>72.5 ± 12.7</td>
<td>89.6 ± 12.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>C1 (mL/mm Hg*10)</td>
<td>14.5 ± 4.3</td>
<td>18.6 ± 4.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>C2 (mL/mm Hg*100)</td>
<td>2.7 (1.9–3.4)</td>
<td>7.5 (5–9.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Medication, N (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentoxyfylline</td>
<td>38 (100)</td>
<td>0 (0)</td>
<td>—</td>
</tr>
<tr>
<td>Aspirin</td>
<td>30 (78.9)</td>
<td>0 (0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>10 (26.3)</td>
<td>0 (0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Current smoking, N (%)</td>
<td>31 (81.6)</td>
<td>6 (21.4)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Values are expressed as means (± SD), medians (with 25% and 75% percentiles).
controls. There was no relationship between small artery elasticity and isoprostanes in the controls. However, logC2 was significantly inversely associated with F2-IsoPs and age in the patient group.

In addition, there occurred a significant linear correlation between F2-IsoPs and LDL-BDC in all study subjects (Fig. 3).

Discussion
The current study compared for the first time C1 and C2, measured by diastolic PWA, and the direct indices of OxS in patients with PAD. We detected reduced arterial elasticity and high-grade OxS in patients with atherosclerosis. The significant linear associations between C1 and F2-IsoPs as well as between C2 and F2-IsoPs indicate that impaired vascular vasodilatory function is related to the degree of OxS in patients with PAD. The strong correlation between C2 and F2-IsoPs after adjustment for potential confounders in the patient group suggests that the small arteries rather than the large conduit arteries could be affected by OxS in atherosclerosis. The significant relationship between F2-IsoPs and LDL-BDC may exhibit their synergistic impact on the expression of the OxS status in humans.

The atherosclerotic process1 as well as the risk factors of atherosclerosis15,16 can alter arterial wall elasticity. Subclinical dysfunction of arteries is important because stiffer vessels produce an earlier return of the reflected wave from vascular branching points to the ascending aorta.

Table 2. Multiple regression model for controls and patients with C1 as the dependent variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls*</td>
<td>Age (y)</td>
<td>-0.361</td>
<td>0.118</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>0.682</td>
<td>0.269</td>
</tr>
<tr>
<td></td>
<td>F2-IsoPs (ng/mL)</td>
<td>-0.424</td>
<td>0.956</td>
</tr>
<tr>
<td>Patients†</td>
<td>Age (y)</td>
<td>-0.137</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>0.186</td>
<td>0.197</td>
</tr>
<tr>
<td></td>
<td>F2-IsoPs (ng/mL)</td>
<td>-0.662</td>
<td>0.341</td>
</tr>
</tbody>
</table>

* R² = .43, P < .004; † R² = .2, P < .057.

Table 3. Multiple regression model for controls and patients with logC2 as the dependent variable

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient</th>
<th>Standard error</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls*</td>
<td>Age (y)</td>
<td>-0.14</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>0.038</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>F2-IsoPs (ng/mL)</td>
<td>0.004</td>
<td>0.04</td>
</tr>
<tr>
<td>Patients†</td>
<td>Age (y)</td>
<td>-0.009</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>0.009</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>F2-IsoPs (ng/mL)</td>
<td>-0.046</td>
<td>0.012</td>
</tr>
</tbody>
</table>

* R² = .47, P < .001; † R² = .45, P < .0001.
arterial elasticity and oxidative stress

There was no significant association between C1 and F2-IsoPs in either group after adjustment for age and BMI, and no significant differences occurred in C1 between the patients and the controls after adjustment for the OxS markers. One possible explanation of why the differences would not achieve significance is the relatively small sample size. But recent studies have reported that small arteries are particularly sensitive to cardiovascular disease-associated alterations as well as to risk prediction, and decrease in compliance of the small arteries with increasing age is more significant than in the large arteries. The significant association between C2, but not C1, and F2-IsoPs in the multivariate model could point to the possibility that in advanced stages of peripheral atherosclerosis OxS-related microvascular alterations become more prominent and contribute to the symptomatology.

Interestingly, only limited data were available about associations between arterial elastic properties and OxS in humans. Previous studies have reported significant correlations of oxLDL with carotid and aortic distensibility, increased F2-IsoPs levels, and reduced endothelial function, as well as diminished arterial compliance in healthy subjects after methionine administration. These findings suggest that oxidative modifications may be a major mechanism capable of justifying the vascular impairment. However, there is wide evidence that atherosclerosis represents a state of heightened OxS, which alters the bioavailability of nitric oxide (NO). It has been shown that endothelial factors (eg, NO) may contribute a functional component to arterial stiffness and endothelial dysfunction is closely related to reduced arterial elasticity. These findings suggest that endothelial dysfunction might be one potential mechanism underlying alterations in the elasticity of atherosclerotic vessels.

Despite the fact that there are several plausible linking mechanisms between OxS and arterial elasticity in atherogenesis, the exact mechanisms by which OxS may affect arterial elasticity are still undergoing investigation. The pathophysiology of atherosclerosis involves many similar inflammatory and OxS-mediated cascades that can lead to vessel remodeling and altered collagen and elastin structure. However, it remains less clear whether the deposition of lipids in the vascular wall and development of atherosclerotic lesions alone contribute to vessel stiffness. Furthermore, atherosclerotic plaque could also secondarily alter endothelial function and thereby worsen stiffening.

Recent investigations suggest that high-grade OxS could modulate the activity of matrix metalloproteinases (eg, MMP-2 and MMP-9), which are associated with destruction of the elastic laminae of arteries and may be involved in the process of arterial stiffening in humans. In addition, increased production of ROS may also influence vessel wall elasticity by enhancing smooth muscle tone or by promoting smooth muscle cell proliferation. However, because endothelial dysfunction, arterial stiffness, and atherosclerosis often coexist, causality remains uncertain. Nevertheless, multifaceted strategies may exert

![Figure 3](image-url)

**FIG. 3.** Scatterplot of baseline diene conjugates of low-density lipoproteins (LDL-BDC) and 8-iso-prostaglandin F2α (F2-IsoPs). A linear association was observed between LDL-BDC and F2-IsoPs for all study subjects (patients, open circles, and controls, closed circles) (N = 66) (R = .32, P = .01).
a beneficial effect on atherosclerosis through improvement of endothelial function as well as arterial elasticity. Although isoprostanes and oxLDL have been considered predictive markers for cardiovascular diseases, very few studies have accomplished measurement of these biomarkers in patients with PAD. We demonstrated elevated F2-IsoPs and LDL-BDC in patients with PAD and associations between F2-IsoPs and arterial elasticity, which might suppose the role of high-grade OxS in the vascular damage in patients with atherosclerosis. The association between F2-IsoPs and LDL-BDC may offer a mechanistic explanation of how increased free radical-catalyzed peroxidation of arachidonic acid as well as enhanced oxidative modifications of LDL are combined to contribute to high-grade oxidative injury in vascular wall.

A weakness of the present study is its cross-sectional observational nature, which limits the evaluation of causal relationships. Also the long-term effects of smoking and medications may have been potential confounders. Yet we were unable to withdraw chronically ill patients from their medications for extended periods of time. And finally, we studied only men, considering that patients with PAD are predominantly male, which makes it difficult to use the current findings for women.

In conclusion, the current study demonstrates for the first time associations between arterial elasticity, measured by PWA, and F2-IsoPs. These observations support the notion that oxidative modifications may play an essential role in alteration of the arterial wall in atherosclerosis. In addition, our findings suggest that assessment of the mechanical properties of the arteries, combined with measurement of OxS-related indices, could provide a potential surrogate complex for characterizing vascular disorders.

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References


