Altered Flow-Mediated Vasodilatation, Low Paraoxonase-1 Activity, and Abnormal High-Density Lipoprotein Subclass Distribution in Takayasu’s Arteritis

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Background: Takayasu’s arteritis (TA) is an idiopathic chronic inflammatory disease that causes occlusion of the elastic large arteries.1-2 During the early stages, the arterial damage is not easily detected, but as the disease progresses, patients may show a variety of symptoms that can be linked to endothelial dysfunction (ED), including hypertension (HT). Nevertheless, ED has not been clearly demonstrated in TA.

ED causes deterioration of endothelial-dependent vasodilation and a series of extensive abnormalities in the integrity and homeostasis of the endothelium. A postulated mechanism involves the participation of oxygen radicals, and chronic activation of the endothelial cells.3 The deterioration in vasodilation has been confirmed by many studies of essential HT4 diabetes types 1 and 25-7 coronary artery disease8 congestive heart failure9 insulin resistance10 and kidney disease.11 Measurement of flow-mediated vasodilatation (FMD) is a non-invasive test that allows the evaluation of endothelial function in the brachial artery. It is a validated method, accepted in the diagnosis and long-term follow-up of patients.12,13

Several clinical and epidemiological studies have demonstrated a negative correlation between high-density lipoprotein (HDL)-cholesterol (C) and the risk of developing coronary heart disease.14-16 This negative correlation has been explained through the role of HDL in reverse cholesterol transport17 as well as by the capacity of these lipoproteins to stimulate the synthesis of nitric oxide by the endothelium18 and assist endothelial function through their associated enzyme, paraoxonase-1 (PON1).19 This enzyme catalyzes the conversion of the proatherogenic low-density lipoprotein (LDL)-lipoperoxides to the corresponding lipohydroxides that are innocuous for the endothelium.19

The HDL include a heterogeneous group of lipoproteins that may be classified into HDL2b, HDL2a, HDL3a, HDL3b, and HDL3c, depending on their decreasing size. These HDL subclasses have different antiatherogenic characteristics, including a different capacity to bind and transport PON1.20,21 Despite its potentially beneficial effects, the relationship of HDL with endothelial function has been barely studied in vivo.

The purpose of this study was to evaluate FMD in TA, the role of HT in ED, and to describe the HDL subclasses and PON1 activity in order to explore the possible contribution these may have in the ED of TA.

Key Words: Endothelial dysfunction; High-density lipoprotein subclasses; Paraoxonase-1; Takayasu’s arteritis; Vasculitis
Methods

Study Population
We included 30 women diagnosed with TA and 30 women volunteers matched for age and body mass index in the study. The TA diagnosis was based on the American College of Rheumatology Criteria for the Classification of Takayasu Arteritis.3 The presence of more than 3 of the following 6 criteria is consistent with a diagnosis of TA: onset age <40 years, claudication of an extremity, decreased brachial artery pulse, >10 mmHg difference in systolic blood pressure (BP) between the arms, a bruit over the subclavian arteries or the aorta, and arteriographic evidence of narrowing or occlusion of the entire aorta, its primary branches, or the large arteries in the proximal upper and lower extremities. The clinical classification of TA was defined as 5 subgroups as proposed by Hata et al.3,22 (1) type I was presumed in patients with involvement of the aortic arch; (2) type IIa was confined to the ascending aorta and aortic arch, and type IIb was confined to the ascending aorta, aortic arch and descending aorta without involvement of the celiac artery; (3) type III was characterized by the involvement of the descending aorta; (4) type IV was presumed in patients with involvement of the abdominal aorta and renal arteries; and (5) type V was presumed in patients with involvement of the entire aorta and its branches. We included 15 patients with HT (normoglycemic without a clinical history of diabetes or other chronic disease) as a comparison group.

Measurement of BP was done using standardized techniques. HT was considered to be present when the diastolic BP was ≥90 mmHg or systolic BP was ≥140 mmHg. The study was approved by the Institutional Ethics Committee and informed consent was given by all the participants. Clinical histories of all participants were taken and all underwent physical examination. A 12-h fasting blood sample into dry tubes without EDTA.

PO1 Activity
PON1 activity was measured using phenylacetate as the substrate.24 Initial rates of hydrolysis were determined spectrophotometrically at 270 nm. The assay mixture included 1 mol/L phenylacetate and 0.9 mmol/L CaCl2 in 20 mmol/L Tris–HCl, pH 8.0, and 100 μL serum (diluted 1:100). The E270 for the reaction was 1.310 mol · L–1 · cm–1. Arylesterase activity was defined as the number of micromoles of phenylacetate hydrolyzed per minute per milliliter of serum.

HDL Subclass Size Distribution
HDLs were separated by ultracentrifugation in a Beckman Optima TLX table centrifuge at 504,000 × g as described previously.22 Briefly, total apo B-containing-lipoproteins (density <1.063 mg/dl) were obtained after 2.16 h, while the total HDL (1.063 <density <1.21 g/ml) took 2.5 h. HDLs were dialyzed against 0.09 mol/L Tris/0.08 mol/L boric acid/3 mmol/L EDTA buffer, pH 8.4. The hydrodynamic diameter of each HDL was estimated by non-denaturing 4–30% gradient polyacrylamide gel electrophoresis using globular proteins as the reference (thyroglobulin, 17 nm; ferritin, 12.2 nm; catalase, 10.4 nm; lactate dehydrogenase, 8.2 nm; albumin, 7.1 nm; high-molecular-weight calibration kit, Amersham Pharmacia Biotech, Buckinghamshire, UK).23 Gels were stained with Coomassie blue 250 for proteins, and the relative proportions of each HDL subclass were estimated by optical densitometry, considering the following size intervals: HDL3c, 7.9–8.45 nm; HDL3b, 8.45–8.98 nm; HDL2a, 9.94–10.58 nm; and HDL2b, 10.58–12.36 nm.26 The relative proportion of each HDL subclass was defined as the percentage of the total HDL area under the curve. The variability coefficient of the method was less than 7%.

High-Resolution Ultrasonography
The left ventricular mass (LVM) was determined from 2-dimensional transthoracic echocardiograms, according to the American Society of Echocardiography guidelines, using the parasternal view at the level of the papillary muscles and the apical 4-chamber view. Ventricular hypertrophy was defined as an increase in LVM of more than 115 g.27

The ultrasound method of evaluating endothelial function has been described previously.28 The diameter of the brachial artery was measured after the patients had rested for at least 30 min. The brachial artery was evaluated in the longitudinal plane 5 cm above the antecubital fossa. Whenever an adequate probe position was obtained, the skin was marked and the arm was held in the same position during the entire study. Reactive hyperemia was induced by inflating a BP cuff to a pressure of 200 mmHg for 5 min. The brachial artery internal diameter was measured 3 times, and averages were taken at rest and in the first minute after deflating the BP cuff. FMD was defined as the percent change in the internal diameter of the brachial artery during reactive hyperemia, which was measured at 1 min, compared with the basal diameter.28

The maximum flow speed in the brachial artery was obtained with pulsed wave Doppler with the sample volume in the center of the artery and a correction angle at 60°, at rest and during the first 15 s after cuff deflation, taking the average of 3 measurements. The maximum speeds considered to be normal were 50–70 cm/s.29 Reactive hyperemia was calculated as the ratio of the maximal velocity divided by the maximal velocity at baseline and was considered to be abnormal if the maximal velocity was ≤2.0-fold of the maximal velocity at baseline.

Measurement of Cytokines
Commercial enzyme-linked immunosorbent assay kits were used for the measurement of serum IL-1, IL-6, and IL-10 levels, according to the manufacturers’ instructions.

Statistical Analysis
The central tendencies, which include mean, median, and percentage, were calculated for quantitative variables. The variable distribution was analyzed through the Kolgomorov-Smirnov test. Multivariate analysis with ANOVA and post-hoc analysis (LSD) were performed for heterogeneous variables. The associations between FMD and its determinants were evaluated with Spearman’s correlation and Mann-Whitney U test. Multiple linear regression analysis was undertaken to determine independent predictors of the vascular function variables. Dichotomous variables and the value of strata were evaluated by the chi-square test. Unless otherwise indicated, data are expressed as mean ± SD. Statistical analyses were performed with SPSS 11 version software (Chicago, IL, USA).

Results
The clinical characteristics of the controls, TA and hypertensive patients are shown in Table 1. All patients were
of the parameters measured by ultrasound was significantly different between the subgroups (Table 3). Concerning the affected vessels, the low FMD subgroup comprised 12 type V, 4 type I, 3 type II and 1 type III TA patients, whereas the high FMD subgroup included 6 type V, 3 type I and 1 type II TA patients. The proportion of the different types of TA patients was not significantly different between groups (P>0.05). Type V was the most frequent TA type (60%), so we further classified the TA patients as type V or non-type V in order to compare the influence of the affected vessels on FMD. Using the FMD cutoff point of 10%\(^\text{e}\) 13 type-V and 8 non-type-V patients were in the low FMD subgroup, whereas 5 type-V and 4 non-type-V patients were in the high FMD subgroup (chi-square >0.05).

We included 15 hypertensive women for comparison of the hemodynamic parameters of our TA patients (Tables 1, 4). Despite the similar BPs (Table 1), the TA patients as a whole (n=30) had a lower FMD (median=7.68%) than the hypertensive patients (19.03%, P=0.016). However, the lowest FMD values were recorded in the TA patients with HT (Table 4). Therefore, the contribution of HT to ED in TA patients could not be discarded; however, its role was probably masked by the antihypertensive and antiinflammatory treatments. The drugs taken by the TA patients at the time of the study were angiotensin-converter enzyme inhibitors (ACEIs) (n=12) and prednisone (n=12). Ten TA patients were not taking any medication. The TA patients were receiving other antihypertensive drugs (not in combination with ACEI) at the time of the study, such as \(\beta\)-blockers (n=2), calcium-channel blockers (n=3), and diuretics (n=1). We compared the FMD of subjects who were taking ACEIs, including 14 patients from the HT group (TA and HT, n=20), with those who were not receiving this type of drug (n=55). The FMD was similar between the groups (median=15.00 and 11.84%, respectively, P=0.431), thus indicating a lack of association between ACEIs and FMD. In contrast, patients receiving prednisone (n=12) had a lower FMD (median=7.76%) than the subjects who were not under prednisone treatment (controls and hypertensive patients, n=63, median=15.51, P=0.029).

Inflammatory markers may also be indicators of ED, so we determined that the levels of IL-1, IL-6 and IL-10. IL-1 and IL-10 were not different in the TA group when compared with the controls and the hypertensive group (data not shown). In contrast, the IL-6 concentration was significantly higher in the TA group (median=3.76 pg/ml, 0–19.77 pg/ml).

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**Table 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th>Clinical variable</th>
<th>Control (n=30)</th>
<th>TA patients (n=30)</th>
<th>Hypertensive patients (n=15)</th>
<th>P value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>35.6±12.5</td>
<td>36.5±11.8</td>
<td>40.2±8.5</td>
<td>NS</td>
</tr>
<tr>
<td>Females</td>
<td>30</td>
<td>30</td>
<td>15</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>642±11.6</td>
<td>62±11.4</td>
<td>63.5±12.4</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155±27</td>
<td>157±25</td>
<td>153±24</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>26.1±24.0</td>
<td>25.5±4.1</td>
<td>26.8±5.1</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>114±12.13</td>
<td>139±22.9</td>
<td>137.0±18.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>60.0±11.0</td>
<td>84.8±15.8</td>
<td>90.7±12.7</td>
<td>0.000</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.87±1.84</td>
<td>5.03±1.87</td>
<td>4.79±0.72</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>3.39±1.47</td>
<td>3.49±1.53</td>
<td>2.57±0.44</td>
<td>0.094</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.06±0.44</td>
<td>0.88±0.28</td>
<td>1.21±0.19</td>
<td>0.000</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.58±0.95</td>
<td>1.60±0.84</td>
<td>1.92±0.85</td>
<td>0.000</td>
</tr>
<tr>
<td>Intima-media thickness (mm)</td>
<td>4.2±1.6</td>
<td>6.7±1.5</td>
<td>5.6±1.8</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values are mean±standard deviation (SD).
\(^a\)ANOVA test; \(^b\)logarithmically transformed for analysis; \(^c\)LSD post hoc test P<0.005 vs control; \(^d\)LSD post hoc test P<0.05 vs hypertensive patients group; \(^e\)LSD post hoc test P<0.05 vs control.

TA, Takayasu’s arteritis; BMI, body mass index; BP, blood pressure; LDL, low-density lipoproteins; HDL, high-density lipoproteins.

**Table 2. Antihypertensive Treatment and Clinical Classification of the TA Patients\(^a\)**

<table>
<thead>
<tr>
<th>Antihypertensive treatment</th>
<th>Clinical classification of TA</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Type I</td>
<td>3 (10)</td>
</tr>
<tr>
<td></td>
<td>Type II</td>
<td>3 (10)</td>
</tr>
<tr>
<td></td>
<td>Type III</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td></td>
<td>Type V</td>
<td>11 (36.6)</td>
</tr>
<tr>
<td>No</td>
<td>Type I</td>
<td>4 (13.3)</td>
</tr>
<tr>
<td></td>
<td>Type II</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td></td>
<td>Type III</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Type V</td>
<td>7 (23.3)</td>
</tr>
</tbody>
</table>

\(^a\)According to Hata et al.\(^2\)

Abbreviation see in Table 1.

female, and there were no significant differences in age, weight, height, total cholesterol or triglycerides. Significant differences between the TA patient and control groups were found for systolic and diastolic BPs (Table 1), so for this reason, we included 15 women with HT as a comparison group and their characteristics are also shown in Table 1.

The classification of TA patients by hypertensive therapy is shown in Table 2. Type V was the most frequent (60%) and the distribution of the different types of the disease was similar in patients with and without antihypertensive treatment (P>0.05).

Regarding the results of vascular ultrasound of the brachial artery, reactive hyperemia was not significantly different between TA patients and the control group (2.3±0.9 and 2.1±1.0, respectively, P>0.05). In contrast, the maximal speed of the brachial artery was lower in TA patients than in the control subjects both at baseline (31.5±15.0 and 58.7±21.4 cm/s, respectively, P<0.001) and after reactive hyperemia (64.9±41.3 vs 129.0±55.7 cm/s, P<0.001). These differences appeared simultaneously with low basal vessel sizes in the TA patients (27.7±6.8 mm) as compared with controls (33.1±4.5 mm, P<0.005). Mean FMD was lower in patients with TA (median=7.68%) as compared with controls (median=15.12%, P<0.05), but the FMD range was abnormally large in the former group (from −20.33 to 53.8%) than in the latter (−2.06 to 47.86%). Consequently, we divided the TA patients into 2 subgroups using as a FMD cutoff point of 10% (Table 3)\(^2\) Despite the clear dichotomy concerning the FMD within the TA group, any of the parameters measured by ultrasound was significantly different between the subgroups (Table 3). Concerning the affected vessels, the low FMD subgroup comprised 12 type V, 4 type I, 3 type II and 1 type III TA patients, whereas the high FMD subgroup included 6 type V, 3 type I and 1 type II TA patients. The proportion of the different types of TA patients was not significantly different between groups (P>0.05). Type V was the most frequent TA type (60%), so we further classified the TA patients as type V or non-type V in order to compare the influence of the affected vessels on FMD. Using the FMD cutoff point of 10%\(^\text{e}\) 13 type-V and 8 non-type-V patients were in the low FMD subgroup, whereas 5 type-V and 4 non-type-V patients were in the high FMD subgroup (chi-square >0.05).

We included 15 hypertensive women for comparison of the hemodynamic parameters of our TA patients (Tables 1, 4). Despite the similar BPs (Table 1), the TA patients as a whole (n=30) had a lower FMD (median=7.68%) than the hypertensive patients (19.03%, P=0.016). However, the lowest FMD values were recorded in the TA patients with HT (Table 4). Therefore, the contribution of HT to ED in TA patients could not be discarded; however, its role was probably masked by the antihypertensive and antiinflammatory treatments. The drugs taken by the TA patients at the time of the study were angiotensin-converter enzyme inhibitors (ACEIs) (n=12) and prednisone (n=12). Ten TA patients were not taking any medication. The TA patients were receiving other antihypertensive drugs (not in combination with ACEI) at the time of the study, such as \(\beta\)-blockers (n=2), calcium-channel blockers (n=3), and diuretics (n=1). We compared the FMD of subjects who were taking ACEIs, including 14 patients from the HT group (TA and HT, n=20), with those who were not receiving this type of drug (n=55). The FMD was similar between the groups (median=15.00 and 11.84%, respectively, P=0.431), thus indicating a lack of association between ACEIs and FMD. In contrast, patients receiving prednisone (n=12) had a lower FMD (median=7.76%) than the subjects who were not under prednisone treatment (controls and hypertensive patients, n=63, median=15.51, P=0.029).

Inflammatory markers may also be indicators of ED, so we determined that the levels of IL-1, IL-6 and IL-10. IL-1 and IL-10 were not different in the TA group when compared with the controls and the hypertensive group (data not shown). In contrast, the IL-6 concentration was significantly higher in the TA group (median=3.76 pg/ml, 0–19.77 pg/ml).
Table 3. Comparison of TA Patients With Low and High FMD

<table>
<thead>
<tr>
<th>Clinical variable</th>
<th>Low FMD (n=20)</th>
<th>High FMD (n=10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of TA (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>135±98</td>
<td>124±130</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.8</td>
<td>26.5±4</td>
<td>NS</td>
</tr>
<tr>
<td>Basal vessel size (mm)</td>
<td>25.8±4</td>
<td>23.2±4.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Maximal velocity at baseline (cm/s)</td>
<td>31.1±5.3</td>
<td>32.3±14.8</td>
<td>NS</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>3.29±[–20.33–14.02]</td>
<td>28.5±[17.10–53.8]</td>
<td>0.000</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>152.9±50.8b</td>
<td>168.6±71.0a</td>
<td>NS</td>
</tr>
</tbody>
</table>

Low-FMD, <10%; high-FMD, >10%.
Values are mean±SD or * as median.
*P<0.005 vs controls; **P<0.001 vs control; †P<0.05 vs control.
FMD, flow-mediated vasodilatation; LVM, left ventricular mass. Other abbreviations see in Table 1.

Table 4. Brachial Artery Ultrasound Parameters in Response to Hyperemia

<table>
<thead>
<tr>
<th>Ultrasound parameter</th>
<th>Controls (n=30)</th>
<th>Normotensive TA patients (n=10)</th>
<th>Hypertensive TA patients (n=20)</th>
<th>Hypertensive patients (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal vessel size (mm)</td>
<td>28.8±3.9</td>
<td>25.9±2.6d</td>
<td>25.2±5.2±4</td>
<td>33.2±3.1±s</td>
<td>0.000</td>
</tr>
<tr>
<td>Maximal velocity at baseline (cm/s)</td>
<td>58.7±21.4</td>
<td>29.4±13.1±d</td>
<td>32.6±16.1±d</td>
<td>92.3±19.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Maximal velocity at 1 min (cm/s)</td>
<td>129.0±55.7</td>
<td>64.1±31.6</td>
<td>65.3±46.1</td>
<td>138.2±38.4</td>
<td>0.000</td>
</tr>
<tr>
<td>Reactive hyperemia</td>
<td>2.3±0.9</td>
<td>1.8±0.69</td>
<td>1.5±0.3</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>FMD (%)</td>
<td>15.12 [–2.0–47.8]</td>
<td>6.66 [–8.0–53.8]</td>
<td>9.16 [–20.33–52.9]</td>
<td>19.03 [9.5–30.5]</td>
<td>0.000</td>
</tr>
<tr>
<td>LVM (g)</td>
<td>94.4±21.3</td>
<td>168.2±62.4±c</td>
<td>153.1±56.5±c</td>
<td>122.0±27.5b</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*LSD post hoc test P<0.005 vs control group; †LSD post hoc test P<0.05 vs control group; ‡LSD post hoc test P<0.05 vs HT group; §LSD post hoc test P<0.005 vs HT group; ¶LSD post hoc test P<0.005 vs TA hypertensive group; ††Mann-Whitney U test P<0.05 vs hypertensive group.
Abbreviations see in Tables 1,3.

Table 5. Subclasses of HDL and PON1 Activity

| Ultrasound parameter | Controls (n=30) | TA patients (n=30) | Hypertensive patients (n=15) | P value*
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL2b (%)</td>
<td>15.8±12.2</td>
<td>22.4±11.4b</td>
<td>18.5±4.1</td>
<td>0.067</td>
</tr>
<tr>
<td>HDL2a (%)</td>
<td>13.3±6.1</td>
<td>13.9±3.5</td>
<td>16.3±1.2b</td>
<td>0.079</td>
</tr>
<tr>
<td>HDL3a (%)</td>
<td>33.8±7.1</td>
<td>34.1±8.5</td>
<td>32.1±2.2</td>
<td>0.660</td>
</tr>
<tr>
<td>HDL3b (%)</td>
<td>22.6±5.8</td>
<td>18.6±5.9</td>
<td>18.3±1.5</td>
<td>0.006</td>
</tr>
<tr>
<td>HDL3c (%)</td>
<td>14.9±6.8</td>
<td>11.6±6.9b</td>
<td>14.7±2.1</td>
<td>0.093</td>
</tr>
<tr>
<td>PON1 (μmol·min⁻¹·ml⁻¹)</td>
<td>200±184.0</td>
<td>134.7±64.8</td>
<td>120.5±39.7</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*ANOVA test; †LSD post hoc test P<0.05 vs Control group; ‡LSD post hoc test P<0.05 vs Control group.
HDL fractions are expressed as % of total HDL protein.
PON1, paraoxonase-1. Other abbreviations see in Table 1.

than in the controls (median=0.1 pg/ml, 0–1.52 pg/ml, P= 0.003).
In order to obtain more insight to the possible contribution of systolic and diastolic BP, lipid profile, IL-6 concentration and medications to FMD, we performed a stepwise regression model that included these parameters as independent variables and FMD as the dependent variable (n=75). In this model, systolic BP and LDL-C were the 2 predictors of FMD (β=–0.454, P=0.002, and β=–0.394, P=0.008 for SBP and LDL-C, respectively).
Regarding the lipid profile, the LDL-C concentration was higher in the TA group than in the hypertensive patients (Table 1); HDL-C was low in TA patients when compared with the control and HT groups (Table 1). These lower plasma levels of HDL-C in the TA patients were concurrent with relative lower proportions of small HDL3b and higher proportions of large HDL2b subclasses as compared with the control group (Table 5). The relative proportion of small HDL3b had a positive correlation with PON1 activity in the 75 subjects included in the study (r=0.290, P=0.013). These findings concur with a significantly diminished enzyme activity in TA patients (Table 5). The HT group also has low relative proportions of HDL3b and low PON1. In order to get more insight to the possible association between HDL subclasses and PON1 activity, we performed a stepwise multiple regression analysis that included the different HDL subclasses and lipid profile; HDL3b and LDL-C were the only variables that explained the plasma PON1 activity (β=0.501, P=0.001 and β=0.364, P=0.017, for HDL3b and LDL-C, respectively).
HDL3b positively correlated with maximal basal velocity (r=0.264, P<0.05). FMD inversely correlated with HDL2a and HDL3a (r=–0.266, P=0.05, and r=–0.312, P=0.015, respectively) and positively with HDL3c (r=0.303, P<0.05). The negative correlation between FMD and HDL3a remained when the TA group was analyzed independently (r=–0.393, P<0.05). Considering that TA patients had lower basal velocities that may affect the endothelial response, we per-
formed our correlation analysis while correcting it for basal velocity. Under this condition, correlations between FMD and HDL subclasses remained significant (data not shown).

Diastolic and systolic BPs correlated negatively with blood velocity at base line and positively with the intima-media thickness (r = -0.300, P < 0.05, and r = -0.348, P < 0.01; r = 0.473, P < 0.000 and r = 0.293, P < 0.05, for velocity and IMT, respectively). Diastolic and systolic BPs did not correlate with FMD (data not shown) but we observed a positive correlation between diastolic BP and HDL3a (r = -0.306, P < 0.05) and a negative correlation with PON1 activity (r = -0.262, P < 0.05).

The duration of TA is a potential confusing factor for ED, so we analyzed FMD by grouping the TA patients according to time. Our results showed that the duration of TA did not affect the severity of ED; when patients were classified according to the length of the disease, 11 of 17 (65%) who had had TA for less than 10 years showed ED. In comparison, ED was observed in 10 of 13 (76%) patients who had had TA for more than 10 years, so the difference between groups was not significant.

Because low FMD in TA patients may be the result of low basal velocities, we performed a stepwise multiple regression analysis including the HDL subclasses, PON1 activity and BP as independent variables, and the basal velocity as the dependent variable. In this model, only diastolic BP was a predictor of the basal velocity (\(\beta = -0.348, P = 0.006\)). When the TA group was analyzed independently, pharmacological treatment was also included as a dependent variable. Surprisingly, only prednisone predicted the basal velocity in this group (\(\beta = 0.434, P = 0.017\)). Indeed, when the TA patients were grouped by prednisone intake, those that received the drug (n = 12) had a significantly higher basal blood velocity (39.4 ± 6.4 cm/min) as compared with those who were not receiving this medication (26.3 ± 11.8, P < 0.05).

The LVM was significantly higher in TA patients than in controls (Table 3). In the whole group (n = 75), LVM had a positive correlation with systolic BP (r = 0.229, P < 0.05) and diastolic BP (r = 0.246, P < 0.05), but also with large HDL2b (r = 0.360, P < 0.005), and negative correlations with small particles (HDL3b and HDL3c: r = -0.356, and r = -0.423, respectively, P < 0.001 for both). The correlations between LVM and HDL particles remained similar when TA patients were analyzed independently (r = 0.429, P < 0.05, and r = -0.518, P < 0.005, for HDL2b, and HDL3c, respectively). Accordingly, in a stepwise multiple regression analysis using LVM as dependent variable and HDL subclasses, BPs and PON1 activity as independent variables, only HDL3b and HDL2a predicted LVM in the model (Table 6).

### Table 6. Summary of Stepwise Multiple Regression Analysis for LVM

<table>
<thead>
<tr>
<th></th>
<th>(\beta)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL3b</td>
<td>-0.628</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL2a</td>
<td>-0.301</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

For this model, multiple \(r = 0.504, r^2 = 0.254, P = 0.000\).

Abbreviations see in Tables 1, 3.

**Discussion**

We have demonstrated impaired FMD and low maximal basal blood flow velocities simultaneously with low serum PON1 activity and reduced proportion of small HDL3b subclass in patients with TA.

The measurement FMD in the brachial artery using ultrasound is a noninvasive and accurate indicator of NO production. Therefore, our results suggest ED in TA patients and the low basal velocities could determine the abnormal endothelial response. In order to diminish the possible influence of a low basal velocity on FMD, we carried out careful ultrasound measurements in the opposite arm to that with the affected vessels. Furthermore, we performed a partial correlation analysis by correcting for basal maximal velocity.

ED is a common feature in HT, so because TA is frequently associated with HT we suggest that this condition could induce ED. The TA patients indeed showed significantly higher diastolic and systolic BPs than controls. Therefore, we included a group of women (HT group) and divided the TA group into hypertensive and normotensive patients for comparison. Interestingly, TA patients recorded lower values of FMD, basal vessel size, basal velocity and velocity at 1 min, than either the controls or HT group. These differences suggest that high BP has little, if any, impact on ED in TA patients. Nevertheless, when we performed a multiple linear regression in order to explain statistically the low FMD in the TA patients, systolic BP happened to be one of the parameters involved in this abnormality. This observation agrees with previous reports that related the high BP and ED.

The low FMD in TA patients could also have other etiologies, such as the affected vessel, drug intake, and inflammation. The IL-6 concentration was increased significantly in TA patients, but the statistical analysis failed to demonstrate a direct contribution of this interleukin to the low FMD. Regarding the drug intake, most TA subjects were under antihypertensive and/or anti-inflammatory treatment. ACEIs were not related to an enhancement of FMD. These results coincide with a previous report that demonstrated a lack of normalization of FMD with enalapril in hypertensive patients. Furthermore, the type of vessel affected can be discarded as an important contributor to low FMD because the distribution of the different types of TA was similar in the low and high FMD subgroups.

Regarding antiinflammatory treatments used by TA patients, prednisone was associated with a decreased FMD. The effect of corticosteroids on FMD is still controversial; previous reports have demonstrated that they (prednisolone and prednisone) do not affect FMD in rheumatoid arthritis or in systemic lupus erythematosus. In contrast, short-term treatment with corticosteroids in Behçet’s disease results in impaired endothelial function. Therefore, it is possible that the deleterious effect of corticosteroids on FMD is variable and is determined by the pathological process.

The low maximal velocities observed in TA patients is another new finding from our study that should be further evaluated as a different clinical marker of TA. Low maximal velocities may be the result of stenosis of the large arteries, particularly the ascending aorta and the aortic arch, decreasing blood flow to the arms. Thus, it could be argued that low basal velocities have conditioned the decreased FMD that was observed in the TA patients. Nevertheless, we clearly demonstrated that there were 2 subgroups of patients based on their FMD and subjects with high or low FMD had a similar decrease in basal and 1-min velocities. In the
same context, multiple regression analysis showed that in the whole group the maximal flow velocity at 1 min was predicted through the diastolic BP. Surprisingly, those patients who received prednisone showed better basal velocities. This observation should be noted and further evaluated in the treatment of patients with TA.

Studies in vitro have shown that HDLs regulate endothelial function, probably by stimulating NO synthesis. In this context, TA patients had lower levels of small HDL3b and a high proportion of large HDL2b, suggesting that the HDL subclasses may be also important in endothelial function. However, a weak point of this study is the design, which did not allow to establish specifically the contribution of the HDL subclasses to low FMD. Further in vivo studies are needed to determine whether or not the HDL subclasses have a different contribution to endothelial function.

In addition to the possible direct role of HDLs on endothelial function, these lipoproteins may indirectly protect the endothelium from oxidative stress by the intermedation of PON1, which eliminates LDL-lipoperoxides and as a consequence interrupts the spreading of free radicals, which inactivate NO. Our data demonstrated significant low plasma levels of PON1 activity in TA patients and low PON1 activity has also been observed in other physiological situations characterized by ED, such as HT, chronic renal failure, and peripheral artery disease, as well as diabetes mellitus. Considering all this evidence, we expected that PON1 would correlate with FMD, but it did nor did it predict the FMD in the multiple regression analysis. Low PON1 activity in TA patients is probably associated with HT. Because the group of hypertensive patients had comparably low levels of enzyme activity, these results coincide with those from previous reports that have demonstrated low PON1 activity in hypertensive patients. Therefore, low PON1 activities may be related to increased BP and is another feature of TA.

The association of PON1 to HDL is strongly dependent on the lipoprotein structure, especially the surface tension. Because surface tension is dependent on the lipoprotein’s diameter, the amount of enzyme associated with HDL is related to size. Therefore, low PON1 activity may be related to an abnormal size distribution of HDLs, because HDL3b levels were significantly lower in TA and HT patients in comparison with controls. Furthermore, PON1 activity correlated with the relative proportion of HDL3b. Considering that PON1 activity determined using phenylacetate as the substrate is an estimation of the mass of the active enzyme in plasma, this correlation suggests that in the study population the HDL3b subclass was the most important carrier of PON1.

Interestingly, LVM correlated positively with large HDL2b and negatively with small HDL3b and HDL3c, and some HDL subclasses predicted LVM in the stepwise regression analysis. Previous studies have identified a relationship between LVM and HDL-C in both hypertensive patients and the general population. Our results further suggest that not only the HDL-C plasma concentration plays a role in left ventricular (LV) size, but also the structure of these lipoproteins. So far, there is not a clear physiological link between HDL concentration, HDL subclass and LVM; it has been suggested that insulin resistance is the key factor that induces both LV hypertrophy and low HDL-C. Moreover, we have formerly demonstrated that insulin resistance induces an abnormal HDL size distribution. As a consequence, the necessity for a future study designed to analyze the insulin sensitivity of TA patients seems to be sufficiently supported by these results.

To summarize, this study demonstrates that TA patients have ED simultaneously with low plasma PON1 activity and a reduced proportion of HDL3b. These results, for the first time, describe additional features of the cluster of abnormalities commonly observed in TA patients. Whether these abnormalities contribute to the development and enhancement of the clinical symptoms in TA patients continues to be elucidated.

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References

18. Mineo C, Deguchi H, Griffin JH, Shaul PW. Endothelial and anti- 
thrombotic actions of HDL. *Circ Res* 2006; **98**: 1352–1364.

19. Aviram M, Rosenblat M, Bigaier CL, Newton RS, Primo-Parma 
SL, La Du BN. Paraoxonase inhibits high-density lipoprotein oxida-
tion and preserves its functions: A possible peroxiderve role for 

20. Deakin S, Leviev I, Gomaraschi M, Calabresi L, Franceschini G, 
James RW. Enzymatically active paraoxonase-1 is located at the 
external membrane of producing cells and released by a high affinity, 
saturable, desorption mechanism. *J Biol Chem* 2002; **277**: 4301–
4308.

exert potent protection of atherogenic LDL against oxidative stress. 

22. Arend WP, Michel BA, Bloch DA, Hunder GG, Calabrese LH, 
Edworthy SM, et al. The American College of Rheumatology 1990 
criteria for the classification of Takayasu arteritis. *Arthritis Rheum* 
1990; **33**: 1129–1134.

23. Hata A, Noda M, Moriwaki R, Numano F. Angiographic findings of 
S155–S163.

24. Gan KL, Smolen A, Ecker HW, La Du BN. Protein purification of 
human serum paraoxonase/arylesterase: Evidence for one esterase 
catalyzing both activities. *Drug Metab Dispos* 1991; **19**: 100–106.

25. Huesca-Gómez C, Franco M, Luc G, Montano LF, Masso F, Posadas-
Romero C, et al. Chronic hypothyroidism induces abnormal structure 
of high-density lipoproteins and impaired kinetics of apolipoprotein 

26. Huesca-Gómez C, Carreon-Torres E, Nepomuceno-Mejía T, Sanchez-
cholesterol ester transfer protein and lecithin: Cholesterol acyl 

27. Krishnan R, Becker RJ, Beiglmy LM, Lopez-Candales A. Impact of 
body mass index on markers of left ventricular thickness and mass 
calculation: Results of a pilot analysis. *Echocardiography* 2005; **22**: 

28. Correnti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau 
F, Creager MA, et al. Guidelines for the ultrasound assessment of 
endothelial-dependent flow-mediated vasodilation of the brachial 

29. Roman MJ, Nasir TZ, Gardin JM, Gerhard-Herman M, Jaff M, 
Möhler E. Clinical applications of noninvasive vascular ultrasound in 
cardiovascular risk stratification: A report from the American Society of 
Echocardiography and the Society of Vascular Medicine and 

30. Higashi Y, Sasaki S, Kurisu S, Yoshimizu A, Sasaki N, Matsuura H, 
et al. Regular aerobic exercise augments endothelium-dependent 
vascular relaxation in normotensive as well as hypertensive subjects: 
Role of endothelium-derived nitric oxide. *Circulation* 1999; **100**: 
1194–1202.

Fixed combination of perindopril and indapamide at low dose improves 
endothelial function in essential hypertensive patients after acute 

32. Hafström I, Rohani M, Deneberg S, Wörmert M, Joggestrand T, 
Frostegård J. Effects of low-dose prednisolone on endothelial func-
tion, atherosclerosis, and traditional risk factors for atherosclerosis in 
patients with rheumatoid arthritis: A randomized study. *J Rheumatol* 
2007; **34**: 1810–1816.

33. Park MC, Lee SW, Park YB, Lee SK. Serum cytokine profiles and 
their correlations with disease activity in Takayasu’s arteritis. *Rheumatology* 
2006; **45**: 545–548.

arterial hypertension with enalapril does not result in normalization of 
endothelial dysfunction of the conduit arteries. *Angiology* 2006; 
**57**: 187–192.

35. Lima DS, Sato EI, Lima VC, Miranda F Jr, Hatta FH. Brachial endo-
thelial function is impaired in patients with systemic lupus erythemato-

36. Protogerou AD, Stikakis PP, Stamatakopoulos KS, Papamichail C, 
Aznaouridis K, Karatzis E, et al. Interrelated modulation of endothe-
lium function in Behcet’s disease by clinical activity and corticosteroid 

al. Paraoxonase-1 activity modulates endothelium function in patients 
with peripheral arterial disease. *Atherosclerosis* 2005; **183**: 349–354.

38. Keynes RG, Griffiths CH, Hall C, Garthwaite J. Nitric oxide consump-
tion through lipid peroxidation in brain cell suspensions and homog-

Oxidative stress in white coat hypertension, role of paraoxonase. *J Hy-
pertens* 2004; **18**: 523–528.

40. Dantoine TF, Debord J, Charmes JP, Merle L, Marquet P, Lachatre 

41. Abbott CA, Mackness MI, Kumar S, Boulton AJ, Durrington PN. 
Serum paraoxonase activity, concentration, and phenotype distribution 
in diabetes mellitus and its relationship to serum lipids and lipopro-

Pasqualini L, et al. High-density lipoprotein cholesterol and left ven-
tricular hypertrophy in essential hypertension. *J Hypertens* 2001; **19**: 
2265–2270.

43. Anan F, Yonemochi H, Masaki T, Takahashi N, Fukunaga N, Teshima 
Y, et al. High-density lipoprotein cholesterol and insulin resistance 
are independent and additive markers of left ventricular hypertrophy 

44. Pérez-Méndez O, Torres-Tamayo M, Posadas-Romero C, Vidaure 
C, et al. Chronic hypothyroidism induces abnormal structure 
of human serum paraoxonase/arylesterase: Evidence for one esterase 
catalyzing both activities. *Drug Metab Dispos* 1991; **19**: 100–106.