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# Metabolic syndrome and preclinical atherosclerosis: focus on femoral arteries

Gaetano Vaudo*, Simona Marchesi, Donatella Siepi, Matteo Brozzetti, Massimo Raffaele Mannarino, Matteo Pirro, Giuseppe Schillaci, Giovanni Ciuffetti, Graziana Lupattelli, Elmo Mannarino

*Internal Medicine, Angiology and Atherosclerosis, University of Perugia School of Medicine, S. Maria della Misericordia Hospital, Piazzale Menghini, 1, 06100, Italy*

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**Abstract**

Several evidences revealed the relationship between the earliest stages of atherosclerosis and the components of metabolic syndrome. The aim of this study was to disclose preclinical atherosclerotic lesions in a cross-sectional observational study involving 147 patients with metabolic syndrome by the assessment of brachial flow–mediated vasodilation (FMV) and intima-media thickening at both carotid and femoral sites. The purpose was to investigate the association of this metabolic disorder with prevalent atherosclerotic damage in different vascular sites. A control group of 87 healthy subjects was also investigated. Patients had lower values of FMV and a higher mean intima-media thickness (IMT) at both the carotid and femoral sites with respect to controls. Flow-mediated vasodilation had a positive correlation with high-density lipoprotein (HDL) cholesterol and a negative one with low-density lipoprotein (LDL) cholesterol, glycemia, and insulinemia. Carotid mean IMT was directly related to LDL cholesterol and age, and inversely with HDL cholesterol; femoral mean IMT had a direct association with LDL cholesterol, triglycerides, glycemia, and insulinemia and an inverse correlation with HDL cholesterol and LDL size. LDL cholesterol, HDL cholesterol, insulin, and brachial artery diameter were predictive of brachial FMV ($\beta$ = −0.17, 0.21, −0.27, and −0.29, respectively; $P < .05$), whereas age, LDL cholesterol, and HDL cholesterol were independent predictors of mean carotid IMT ($\beta$ = 0.19, 0.37, and −0.27, respectively; $P < .05$); on the other hand, LDL cholesterol, triglycerides, and insulin were independent predictors of mean femoral IMT ($\beta$ = 0.32, 0.26, and 0.25, respectively; $P < .05$). In conclusion, the present study documented an altered endothelial function and intima-media thickening in patients with metabolic syndrome without overt cardiovascular disease. Moreover, it focused on the strong influence of metabolic syndrome on preclinical atherosclerotic lesions at the femoral site.


## 1. Introduction

Metabolic syndrome, as defined by the presence of at least 3 of the following factors—high triglyceride, low high-density lipoprotein (HDL), and high fasting glycemia levels, hypertension, and abdominal obesity—represents a cardiovascular risk condition and is associated with increased coronary atherosclerosis [1-5].

Several evidences have revealed the relationship existing between the earliest stages of atherosclerosis, such as the impairment of endothelial function and the intima-media thickening of large arteries, and the components of metabolic syndrome [6-8]. These 2 early features of atherosclerosis are sustained by different pathophysiological mechanisms. In fact, endothelial impairment expresses the endothelial decrease in nitric oxide bioavailability with a consequent loss of arterial capacity to dilate [9]; on the other hand, arterial thickening indicates a morphological lesion determined by lipid infiltration into the arterial wall [10,11].

It is well established that metabolic syndrome has a predictive power on the development of carotid atherosclerosis [12-14], but less is known about femoral involvement. However, it has been speculated that atherosclerosis starts earlier in the femoral artery compared with the carotid artery [15]. Several observations suggest that patients with peripheral atherosclerotic involvement are characterized by a lipid pattern similar to metabolic syndrome, specifically, characterized by low HDL and high triglycerides [16,17].

* Corresponding author. Tel.: +39 0755784015; fax: +39 0755784022.
*E-mail address:* gvaudo@unipg.it (G. Vaudo).

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On the basis of these considerations, the aim of the study was to investigate the association between metabolic syndrome and preclinical atherosclerosis by the assessment of endothelial function expressed as brachial flow–mediated vasodilation (FMV) and intima-media thickening at both carotid and femoral sites, with the intent to investigate whether this metabolic disorder may favor a different degree of atherosclerotic involvement in the vascular tree.

## 2. Materials and methods

This cross-sectional observational study was carried out by enrolling 147 outpatients with metabolic syndrome who were referred to our lipid clinic. Inclusion criteria were the presence of at least 3 of the following parameters: high triglyceride, low high-density lipoprotein (HDL), and high fasting glycemia levels, hypertension, and abdominal obesity [1].

Exclusion criteria were smoking habit (current smoking status or in the past 10 years), known endocrine disease such as hypothyroidism, Cushing disease, history of cardiovascular diseases (myocardial infarction, angina, ischemic stroke, heart failure, intermittent claudication), medical treatment with steroids, lipid-lowering drugs, antioxidants, and hormone replacement therapy, recent infectious diseases, connective tissue diseases, neoplasms, and renal or liver insufficiency.

A control group of 87 subjects matched for age and sex was recruited among the staff employees of our university; they were normolipemic, had no diabetes mellitus, hypertension, smoking habits, and previous cardiovascular events, and were not using drugs acting on endothelial function.

All subjects underwent clinical examination, measurement of brachial artery FMV, carotid and femoral intima-media thickness (IMT), and assessment of serum lipids.

The study protocol was approved by the ethics committee of our institution; all subjects gave their written consent.

After an overnight fasting, blood was drawn and the following parameters were determined: total cholesterol, triglycerides (enzymatic colorimetric method), HDL cholesterol (enzymatic colorimetric method after precipitation with polyethylene glycol), low-density lipoprotein (LDL) cholesterol (Friedewald formula) [18], insulin, glycemia, and LDL size.

Gradient gel electrophoresis on 2% to 16% polyacrylamide was used to classify LDL particle size. After a 30-minute prerun at 50 V, plasma samples, standard proteins (thyroglobulin, ferritin, catalase, dehydrogenase lactate, and albumin) and latex beads were applied. Electrophoresis was carried out at 200 V for 24 hours in a tris(hydroxymethyl) aminomethane (0.014 mol/L)-glycine (0.109 mol/L) buffer, pH 8.3. Gel staining was performed with methanol, acetic acid, and Coomassie blue. A densitometric scan at 590 nm followed. A quadratic equation (Stokes polynomial regression) was used to convert migration distance into particle diameter (expressed in nanometers) [19].

### 2.1. Assessment of brachial endothelial function

Flow-mediated vasodilation was assessed in the brachial artery by ultrasonography [20]. The measurements were performed on the nondominant arm while the patient was in the supine position, after 10 to 20 minutes rest in a quiet, dark room with a temperature of 22°C. The brachial artery was scanned longitudinally just above the antecubital crease with a linear multifrequency 5- to 12-MHz transducer (HDI 3500, Advanced Technology Laboratories, USA). The diameter of the brachial artery was measured at the R wave of the electrocardiogram, on the interface between media and adventitia of the anterior and posterior wall. Gain settings were optimized to identify the lumen and the vessel wall interfaces and were not modified during the examination. Hyperemia was induced by inflation of a pneumatic cuff (12.5 cm wide) at 230 to 250 mm Hg for 4 minutes on the most proximal portion of the forearm. Arterial diameter measurement was repeated 45 to 60 seconds after sudden deflation of the cuff. Tracings were recorded on videotape and read by one investigator who was unaware of the subject's clinical data and temporal sequence. The average of 3 measurements of basal and posthyperemia diameter was used for the analysis. Flow-mediated vasodilation was expressed as the relative increase in brachial artery diameter during hyperemia, and defined as $100 \times$ [(posthyperemia diameter − basal diameter)/basal diameter]. Blood flow was measured as arterial cross-sectional area ($\pi r^2$) times mean Doppler velocity corrected for angle. The intraobserver between-occasion reproducibility of FMV in our laboratory was assessed in 10 subjects examined 2 days apart. The mean ± SD difference between the 2 examinations was 1.0% ± 1.5%.

### 2.2. Carotid and femoral IMT

Carotid and femoral arteries were examined with high-resolution B-mode ultrasonography [21]. The examination was performed with a commercially available ultrasound device (HDI 3500, Advanced Technology Laboratories, USA) equipped with a linear multifrequency 5- to 12-MHz transducer. Subjects were examined while in the supine position, and all measurements were obtained at end diastole by electrocardiographic triggering. The ultrasound images were stored on an S-VHS videotape and analyzed with an image processing workstation (Kontron KS-200, Munich, Germany). On a longitudinal 2-dimensional ultrasound image of the carotid and femoral artery, the near and far arterial walls are displayed as 2 bright white lines separated by a hypoechogenic space. The distance between the leading edge of the first bright line on the far wall (lumen-intima interface) and the leading edge of the second bright line (media-adventitia interface) indicates the IMT of the far wall. For the near wall, IMT was calculated as the distance between the trailing edge of the first bright line and the trailing edge of the second bright line. A 1.5-cm segment of the common carotid artery (immediately caudal to the

bifurcation), the bifurcation of the common carotid artery, and the proximal 1.5-cm segment of the internal carotid artery were considered.

Similarly, we examined the distal 1.5-cm segment of the common femoral artery and the proximal 1.5-cm segment of the superficial femoral artery. Tracings were read by 2 observers who were unaware of the patients' clinical data. Each subject was characterized by mean carotid IMT (defined as the average of 36 IMT readings: common, bifurcation and internal carotid arteries, right and left side, far and near wall, 3 sampling points per segment) and mean femoral IMT (defined as the average of 24 IMT readings: common and superficial femoral arteries, right and left side, far and near wall, 3 sampling points per segment). We also calculated the mean IMT of each segment, including common carotid, bifurcation, internal carotid, common femoral, and superficial femoral arteries. The intraobserver coefficient of variation was 3.9% in the carotid site (mean ± SD of the difference, 0.018 ± 0.031 mm) and 5.2% in the femoral site (mean ± SD of the difference, 0.006 ± 0.028 mm). Corresponding interobserver values were 5.6% in the carotid site (0.028 ± 0.032 mm) and 7.7% in the femoral site (0.029 ± 0.034 mm).

### 2.3. Statistical analysis

Data are presented as mean ± SD. Student $t$ test was performed to compare parametric variables between groups, $\chi^2$ for qualitative variables, and Mann-Whitney $U$ test for parameters with a nonnormal distribution. Vascular parameters were analyzed by dividing the study population into 2 groups: patients with 3 metabolic syndrome parameters (group A) and those with more than 3 metabolic syndrome factors (group B). Pearson correlation coefficients tested univariate association between study variables; Spearman correlation was used for nonparametric variables. Multiple regression analysis was performed with carotid mean IMT, mean femoral IMT, and brachial FMV as dependent variables. The independent variables in the model were age, triglyceride, HDL cholesterol, and LDL cholesterol levels, LDL size, waist circumference, and insulin. The FMV model also included brachial artery diameter. $P < .05$ was considered statistically significant. The sample size was calculated on the basis of our previous experiences and conservatively assuming 15% of FMV, 6% SD, and a 0.8-mm IMT, with 0.1-mm SD to detect a difference with an $\alpha$ value of .05. Data were analyzed by SPSS statistical software package, release 13.0 (SPSS, Chicago, IL).

Table 1
Demographic, clinical, and lipid parameters of the population study

| | Patients (n = 147) | Controls (n = 87) | P |
|---|---|---|---|
| Age (y) | 52 ± 12 | 54 ± 6 | .25 |
| Male/Female | 101/46 | 50/37 | .06 |
| Body mass index (kg/m$^2$) | 27 ± 1 | 26 ± 1 | <.05 |
| Systolic blood pressure (mm Hg) | 147 ± 13 | 141 ± 15 | <.05 |
| Diastolic blood pressure (mm Hg) | 82 ± 9 | 81 ± 7 | .66 |
| Waist circumference (cm) | 101 ± 6 | 81 ± 3 | <.05 |
| Total cholesterol (mmol/L) | 7.1 ± 0.7 | 5.2 ± 0.6 | <.05 |
| LDL cholesterol (mmol/L) | 4.9 ± 0.7 | 3.2 ± 0.4 | <.05 |
| HDL cholesterol (mmol/L) | 1.3 ± 0.2 | 1.4 ± 0.3 | <.05 |
| Triglycerides (mmol/L) | 2.1 ± 0.7 | 1.6 ± 0.6 | <.05 |
| Log triglycerides | 2.2 ± 0.2 | 2.1 ± 0.1 | <.05 |
| LDL size (nm) | 25.2 ± 0.7 | 26.1 ± 0.6 | <.05 |
| Glycemia (mmol/L) | 5.9 ± 0.5 | 5.1 ± 0.4 | <.05 |
| Insulin (IU/L) | 13.5 ± 3.1 | 10.4 ± 1.7 | <.05 |

Data are presented as mean ± SD.

Table 2
Demographic, clinical, and lipid parameters of the population study divided on the basis of sex

| | Males (n = 101) | Females (n = 46) | P |
|---|---|---|---|
| Age (y) | 51 ± 11 | 56 ± 12 | <.05 |
| Body mass index (kg/m$^2$) | 27 ± 1 | 27 ± 1 | .44 |
| Systolic blood pressure (mm Hg) | 143 ± 15 | 138 ± 15 | .10 |
| Diastolic blood pressure (mm Hg) | 83 ± 8 | 79 ± 10 | <.05 |
| Waist circumference (cm) | 101 ± 5 | 100 ± 7 | .51 |
| Total cholesterol (mmol/L) | 7.2 ± 0.6 | 6.7 ± 0.8 | <.05 |
| LDL cholesterol (mmol/L) | 4.9 ± 0.6 | 4.7 ± 0.6 | .07 |
| HDL cholesterol (mmol/L) | 1.2 ± 0.2 | 1.3 ± 0.2 | .44 |
| Triglycerides (mmol/L) | 2.2 ± 0.8 | 1.8 ± 0.7 | <.05 |
| Log triglycerides | 2.3 ± 0.1 | 2.2 ± 0.2 | <.05 |
| LDL size (nm) | 25.1 ± 0.7 | 25.3 ± 0.7 | .11 |
| Glycemia (mmol/L) | 5.9 ± 0.6 | 5.9 ± 0.4 | .84 |
| Insulin (IU/L) | 13.5 ± 3.3 | 13.6 ± 2.6 | .83 |

Data are presented as mean ± SD.

## 3. Results

Demographic, clinical, and lipid parameters of the 147 study patients and 87 controls are reported in Table 1. As expected, patients had higher values of body mass index, systolic blood pressure, waist circumference, total cholesterol, LDL cholesterol, triglycerides, glycemia, and insulin than controls; HDL cholesterol was lower in patients with metabolic syndrome.

Moreover, when patients were divided on the basis of sex, triglycerides were higher in men than in women (see Table 2).

Table 3
Ultrasound characteristics of the population study

| | Patients (n = 147) | Controls (n = 87) | P |
|---|---|---|---|
| Mean IMT (mm) | | | |
| Common carotid | 1.10 ± 0.05 | 0.89 ± 0.09 | <.05 |
| Bifurcation | 1.32 ± 0.05 | 1.17 ± 0.19 | <.05 |
| Internal carotid | 1.33 ± 0.14 | 1.04 ± 0.11 | <.05 |
| Carotid | 1.35 ± 0.09 | 1.10 ± 0.10 | <.05 |
| Common femoral | 1.44 ± 0.09 | 1.08 ± 0.09 | <.05 |
| Superficial femoral | 1.36 ± 0.11 | 1.41 ± 0.12 | <.05 |
| Femoral | 1.41 ± 0.12 | 1.08 ± 0.07 | <.05 |
| Brachial FMV (%) | 4.5 ± 2.3 | 7.2 ± 2.6 | <.05 |
| Brachial artery diameter (mm) | 4.0 ± 0.8 | 4.1 ± 0.6 | <.05 |

Data are presented as mean ± SD.

Table 4
Vascular parameters dividing patients with metabolic syndrome with 3 metabolic syndrome factors (group A) and with more than 3 metabolic syndrome factors (group B)

| | Group A (n = 103) | Group B (n = 44) | P |
|---|---|---|---|
| Mean IMT (mm) | | | |
| Common carotid | 1.10 ± 0.06 | 1.10 ± 0.04 | .54 |
| Bifurcation | 1.32 ± 0.05 | 1.31 ± 0.04 | .23 |
| Internal carotid | 1.32 ± 0.15 | 1.36 ± 0.10 | <.05 |
| Carotid | 1.35 ± 0.10 | 1.34 ± 0.05 | .66 |
| Common femoral | 1.42 ± 0.08 | 1.47 ± 0.10 | <.05 |
| Superficial femoral | 1.34 ± 0.10 | 1.39 ± 0.13 | <.05 |
| Femoral | 1.36 ± 0.13 | 1.48 ± 0.11 | <.05 |
| Brachial FMV (%) | 4.7 ± 2.2 | 4.3 ± 2.2 | .29 |
| Brachial artery diameter (mm) | 4.0 ± 0.7 | 4.1 ± 0.8 | 0.13 |

Data are presented as mean ± SD.

Ultrasound measurements are reported in Table 3. Patients had lower values of FMV and a higher mean IMT at both the carotid and femoral sites than did controls.

Vascular parameters were analyzed by dividing patients with metabolic syndrome on the basis of the number of metabolic syndrome factors (group A with 3 factors and group B with >3 factors); group B patients showed a higher IMT at the internal carotid and in the femoral site (see Table 4).

## 4. Correlates of ultrasound measurements

At univariate analysis as shown in Table 5, FMV had a positive correlation with HDL cholesterol and a negative correlation with LDL cholesterol, glycemia, and insulinemia. Carotid mean IMT was directly related to LDL cholesterol and age, and inversely with HDL cholesterol. Femoral mean IMT had a direct association with LDL cholesterol, triglycerides, glycemia, and insulinemia, and an inverse correlation with HDL cholesterol and LDL size (Fig. 1). Moreover, there was a direct correlation between carotid mean IMT and femoral mean IMT ($r = 0.27$, $P < .05$), brachial FMV was not associated with carotid and femoral mean IMT. Multivariate regression analysis showed that LDL cholesterol, HDL cholesterol, insulin, and brachial artery diameter were predictive of brachial FMV ($\beta = -0.17$, 0.21, −0.27, and −0.29, respectively; all $P < .05$; see Table 6), whereas age, LDL, and HDL cholesterol were the independent predictors of mean carotid IMT ($\beta = 0.19$, 0.37, and −0.27, respectively; all $P < .05$; see Table 6); on the other hand, LDL cholesterol, triglycerides and insulin were the independent predictors of mean femoral IMT ($\beta = 0.32$, 0.26, and 0.25, respectively; all $P < .05$; see Table 6).

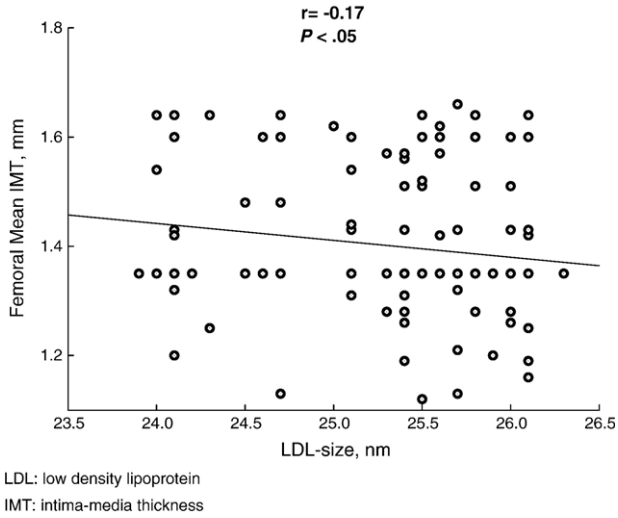


Fig.1. Univariate correlation between femoral mean IMT and LDL size in patients affected by metabolic syndrome.

## 5. Discussion

The present study investigated the preclinical atherosclerotic involvement in a cross section of patients affected by metabolic syndrome without previous cardiovascular events, evaluating in detail the brachial FMV and intima-media thickening at different sites: the carotid and femoral sites.

Brachial FMV represents an early sign of atherosclerotic involvement indicating the endothelial detriment in nitric oxide release. In metabolic syndrome, there is a synergistic action of different factors, each one able to impair endothelial reactivity. In our patient population, the endothelial function was mainly modulated by LDL cholesterol,

Table 5
Univariate correlations between carotid mean IMT, femoral mean IMT, brachial FMV, and lipid and clinical parameters

| | Brachial FMV | | Carotid mean IMT | | Femoral mean IMT | |
|---|---|---|---|---|---|---|
| | r | P | r | P | r | P |
| Age | −0.19 | <.05 | 0.25 | <.05 | 0.01 | .87 |
| LDL cholesterol | −0.23 | <.05 | 0.38 | <.05 | 0.29 | <.05 |
| HDL cholesterol | 0.17 | <.05 | −0.16 | <.05 | −0.20 | <.05 |
| Triglycerides | −0.01 | .77 | 0.01 | .86 | 0.24 | <.05 |
| LDL size | 0.04 | .52 | 0.02 | .74 | −0.17 | <.05 |
| Glycemia | −0.27 | <.05 | −0.07 | .26 | 0.18 | <.05 |
| Insulin | −0.29 | <.05 | −0.05 | .50 | 0.23 | <.05 |
| Waist circumference | −0.07 | .22 | 0.07 | .26 | 0.06 | .35 |

Table 6
Predictors of brachial FMV and mean carotid and femoral IMT

| | Brachial FMV | | Carotid mean IMT | | Femoral mean IMT | |
|---|---|---|---|---|---|---|
| | β | P | β | P | β | P |
| Age | −0.09 | .95 | 0.19 | <.05 | 0.08 | .35 |
| Waist | 0.002 | .98 | 0.09 | .15 | 0.06 | .28 |
| LDL cholesterol | −0.17 | <.05 | 0.37 | <.05 | 0.32 | <.05 |
| LDL size | 0.01 | .87 | 0.01 | .88 | 0.02 | .67 |
| HDL cholesterol | 0.21 | <.05 | −0.27 | <.05 | −0.01 | .88 |
| Triglycerides | −0.05 | .78 | −0.02 | .96 | 0.26 | <.05 |
| Insulin | −0.27 | <.05 | 0.10 | .96 | 0.25 | <.05 |
| Brachial diameter | −0.29 | <.05 | – | – | – | – |

P of the models, <.001.

which exerts a recognized endothelial damage by several mechanisms, such as the inhibition of nitric oxide synthase [22] and the breakdown of endothelial nitric oxide. High-density lipoprotein cholesterol, as suggested in previous reports, has a protective action on endothelial function. In fact, HDL cholesterol antagonizes the oxidative state through antioxidant enzymes and proteins, including platelet-activating factor acetylhydrolase [23], paraoxonase [24], and apoprotein J [25], all able to reduce the LDL oxidative potential. Moreover, HDL has anti-inflammatory properties such as the inhibition of adhesion molecule expression and the inhibition of sphingosine kinase, an enzyme involved in the modulation of endothelial adhesion molecule by tumor necrosis factor α [26,27].

We also observed that brachial FMV is modulated by insulin: increased insulin levels underlie an insulin resistance state, a "core" defect in subjects with metabolic syndrome. The negative influence of insulin resistance on endothelial function is mediated by a number of mechanisms all conditioning reduced nitric oxide levels: inhibition of phosphoinositol-3 kinase pathway [28], augmentation in free fatty acids [29], increased levels of proinflammatory adipokines [30], production of reactive oxygen species [31], reduced HDL with consequent lower antioxidant and anti-inflammatory effects, and increased blood pressure levels [32].

Patients affected by metabolic syndrome had a higher carotid and femoral IMT, a finding that is in concordance with previous observations [12-14]. Carotid IMT seems to be modulated mainly by LDL and HDL cholesterol; however, at femoral sites, IMT was associated with LDL size and HDL cholesterol and independently predicted by LDL cholesterol, triglycerides, and insulin. The relevance of LDL on intima-media thickening, along with the protective action exerted by HDL on the arterial wall, is well established [33-35]. As in the case of endothelial dysfunction, the high susceptibility to oxidation of LDL could be the key mechanism explaining the capacity of this lipid subfraction to penetrate into the intima-media space. In fact, oxidized LDLs are able to access and to deposit inside the vascular wall by means of their high affinity for proteoglycans of intima; nevertheless LDL-proteoglycan complexes show increased susceptibility to oxidation [36,37]. It is known that HDL cholesterol reduces the oxidative charge of LDL, consequently protecting vascular surface [38].

The main finding of the present study is the evidence of a selective impact of metabolic syndrome components on the intima-media thickening at femoral site.

The relevance of lipid metabolism on atherosclerosis at a peripheral site is a controversial topic. Previous observations have suggested that patients affected by peripheral arterial disease are characterized by low HDL cholesterol, normal levels of LDL cholesterol, and an altered clearance of postprandial triglycerides describing a metabolic cluster, similar to that acting in metabolic syndrome, indicating a potential different impact of lipid factors on vascular sites [16,17,39]. More recently, in a large-cross sectional study, it has been documented that metabolic syndrome is more prevalent in patients affected by peripheral arterial disease than in patients with coronary and cerebrovascular atherosclerosis [4]. In metabolic syndrome, there is a concurrence of several mechanisms promoting the atherosclerotic process such as decreased fibrinolysis, oxidative stress, small dense LDL cholesterol, and increased inflammation [39,40]. In addition, Sigurdardottir et al [41] documented that oxidized LDLs are associated with echolucent plaque in femoral arteries of patients with metabolic syndrome. In our case, we documented an association between intima media thickening and small, dense LDL only at the femoral site, suggesting that this vascular site is more susceptible to the oxidative charge of this lipid fraction.

The limitations of the study were that it was cross-sectional and limited to middle-aged white patients.

In conclusion, the present study demonstrated the presence of impaired altered endothelial function and intima-media thickening in patients with metabolic syndrome without overt cardiovascular disease. Moreover, it focused on the main influence of metabolic syndrome on preclinical atherosclerotic lesions at the femoral level. Future studies are needed to confirm these findings and to investigate the underlying mechanism of this difference.

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