

## Carotid Intimal-Medial Thickening and Endothelial Function in Coronary Artery Disease

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### OBJECTIVE

To investigate the correlation between the endothelial function and the carotid intimal-medial thickening (IMT) in a population of patients with coronary artery disease, as well as that between the endothelial function and carotid IMT with the severity of the coronary lesions.

### METHODS

Forty-three patients aged  $60.5 \pm 9.2$  years, (67.4% males) with coronary artery disease at the coronariography were studied. Endothelial function was assessed using the brachial artery reactivity test (BART), which measured the percentage of flow-mediated dilatation (%FMD). The carotid IMT was evaluated through vascular ultrasound.

### RESULTS

The mean %FMD was  $4.7 \pm 3.6$  and the mean carotid IMT was  $1.08 \pm 0.23$  mm. The carotid MIT and %FMD measurements showed a statistically significant correlation, with Spearman's coefficient of 0.315,  $p = 0.042$ , demonstrating that lower %FMD values corresponded to an increased carotid IMT ( $r = -0.315$ ,  $p = 0.042$ ). There was no correlation between %FMD or IMT and the severity of coronary lesions.

### CONCLUSION

The presence of a correlation between carotid IMT and %FMD demonstrates a concomitance of anatomical and functional vascular alterations in coronary artery disease, regardless of the severity of the atherosclerotic lesions.

### KEY WORDS

Endothelial function, carotid, coronary disease, atherosclerosis.

Endothelial dysfunction has an important role in plaque formation and the clinical course of atherosclerosis. The intimal-medial thickening (IMT) of the carotid is an early vascular alteration that precedes plaque formation. However, the correlation between these parameters in coronary artery disease is yet to be defined.

Coronary artery disease persists as the main cause of death worldwide. It is currently known that the endothelium has a vital participation in the pathogenesis of atherosclerosis, triggering an inflammatory response that is responsible for the atherosclerotic plaque formation and instability, with a direct influence on the clinical course of atherosclerosis and other cardiovascular diseases, such as arterial hypertension and cardiac failure.

Until the beginning of the 90s, endothelial function was evaluated through invasive methods, with the intracoronary injection of acetylcholine<sup>1</sup>. In 1992, Celermajer et al<sup>2</sup> standardized the brachial artery reactivity test (BART), in which the endothelial function is assessed through a simple vascular ultrasonographic examination.

The vascular intimal-medial thickening (IMT) corresponds to the initial stage of atherosclerosis, preceding plaque formation. The ultrasonography of the carotid artery allows IMT to be easily measured, and the presence and severity of the carotid intimal-medial thickening have been correlated to coronary atherosclerosis<sup>3,4</sup>.

Although the presence of endothelial dysfunction and carotid intimal-medial thickening have a prognostic value that has been well demonstrated as independent predictors of future cardiovascular events (acute myocardial infarction, stroke and death), the value of these methods as indicators of possible coronary artery disease is yet to be defined and remains unclear.

The main objective of this study was to investigate a correlation between the brachial artery reactivity test (BART) and carotid intimal-medial thickening (IMT) in a population of patients with coronary artery disease. As a secondary objective, we investigated a correlation between these two tests and the severity of the atherosclerotic lesions detected by coronary angiography.

## METHODS

The sample consisted of patients referred to the Laboratory of Hemodynamics of the Felício Rocho and Socor Hospitals, in Belo Horizonte, Minas Gerais, Brazil, between March 2002 and March 2003. The coronary angiography was requested by the assistant physician, in order to investigate thoracic pain or ischemia detected through a functional test (ergometric stress test, myocardial scintigraphy or stress echocardiogram (STRESS ECHO)).

The patients were selected consecutively immediately after undergoing the elective coronary angiography. All

patients who presented evidence of any degree of luminal obstruction were included.

The exclusion criteria were: normal coronary angiography (smooth coronary arteries); diagnosis of acute coronary syndrome (unstable angina or acute myocardial infarction) within seven days prior to the examination; clinical or laboratory evidence of any chronic disease such as hepatopathies, valvopathies, cardiac failure, thyroid disease, chronic renal failure with serum creatinine  $\geq 2.0$  mg%, conjunctive tissue diseases and neoplasias.

After signing the written informed consent, the patients were referred to the Bias Fortes Outpatient Clinic, from Hospital das Clínicas of the Federal University of Minas Gerais, in order to fill out the research protocol, along with the assessment of the clinical picture and risk factors for coronary disease.

Smoking was defined as the habit of smoking during the previous three months. Dyslipidemia was defined as serum LDL cholesterol levels  $> 160$  mg/dL and hereditary factor for coronary artery disease, such as the presence of at least one first-degree relative with a diagnosis of the disease or sudden death, at an age younger than 55 yrs in the male sex and 65 yrs in the female sex. Systemic arterial hypertension was considered according to the information provided by the patient or use of anti-hypertensive medication. The presence of diabetes mellitus was defined according to the information provided by the patient, use of oral hypoglycemic drugs and/or insulin or through fasting glycemia levels (minimum of two measurements above 126 mg/dL).

All patients were referred to the vascular laboratory of Ecograf, a Diagnostic Medicine Group in Belo Horizonte, Minas Gerais, Brazil, in order to undergo the endothelial function test and carotid ultrasonography.

*Specific procedures – coronary angiography and left ventricle angiography* - The coronary angiography was analyzed subjectively by two independent interventionist cardiologists. In case of discordance, a third specialist was called in for another evaluation. Lesions with a compromised luminal diameter  $< 50\%$  were called parietal irregularities. The results were analyzed in patients with lesions  $< 50\%$ , lesions between 50% and 70% and lesions  $\geq 70\%$ .

*Endothelial function test through the flow-mediated dilatation of the brachial artery* - The brachial artery reactivity test (BART) was performed according to the original description by Celermajer et al<sup>2</sup>; drugs with effect on the endothelial function were discontinued prior to the test. Calcium channel blockers, nitrates and angiotensin-converting enzyme inhibitors were withdrawn 24 hours before the test. Beta blockers were withdrawn 48 hrs before the test. All tests were carried out in the morning and after a minimum fasting period of 8 hours.

The patient remained in a resting position, in dorsal decubitus, for 10 minutes prior to the test. The diameter of the brachial artery was measured through a vascular ultrasound, using a linear transducer with a 7.0 MHz frequency in an ASPEN equipment (Acuson, Mountain View, California – USA). The images were obtained during resting, during reactive hyperemia, again during resting and after the administration of sublingual nitroglycerin.

With the transducer positioned 2 to 15 cm above the cubital fossa, the measurement of the diameter of the brachial artery was performed (in mm) in basal conditions. Next, the insufflation of a pneumatic cuff positioned 5 cm below the cubital fossa at 250 mmHg for 5 minutes was carried out, in order to produce distal ischemia. Sixty seconds after cuff desufflation, the recording of images in a state of reactive hyperemia was performed. After 15 minutes of wait, 400 µcg of sublingual nitroglycerin was administered and 4 minutes later, the last recording of arterial diameter was performed. The variation of arterial diameter during resting and after reactive hyperemia was expressed as the percentage of flow-mediated dilatation (%FMD). The means of four measurements in each situation were obtained: basal, after reactive hyperemia, and after nitroglycerin. All images were obtained by the same examiner, who was blinded for the clinical picture and the coronary angiography results.

**Measurement of the carotid intimal-medial thickening (IMT)** - The measurement of the carotid intimal-medial thickening was performed according to the technique described in previous studies<sup>5,6</sup>, during the initial 10 minutes of the resting period, before the brachial artery reactivity test was performed, by the same examiner. The same vascular transducer described above was used, by positioning it longitudinally on the patient's carotid artery, bilaterally. The highest of the values obtained from both carotids, expressed in millimeters (mm) was considered for analysis.

**Statistical analysis** - The quantitative variables were described by means and standard deviations. Qualitative variables were described by frequency and percentage. The correlation between the %FMD, carotid intimal-medial thickening and the severity of atherosclerotic lesions by coronary angiography was obtained through the Spearman's rank correlation coefficient (rs), as the distribution of the variables was not normal. The results were considered significant when  $p < 0.05$ .

The present study represents a sub-study of a broader project, and the sample calculation for such project was carried out considering an alpha error of 0.05 and a beta error of 0.20. Considering that the sample size was determined *a priori*, the power of this study was estimated at being 74.8% for an alpha error of 0.05, according to the PS Power and Sample Size Calculation Software (Dupont and Plummer, 1997, available at <http://www.mc.vanderbilt.edu/prevmed/ps.htm>).

**Ethical aspects** - All patients signed a written informed consent prior to the study. The study was carried out according to the norms in the 196/96 resolutions on research involving human beings published by the Health Ministry and National Council of Health, and approved by the Ethics Committee on Research of Hospital das Clinicas of the University of São Paulo School of Medicine.

**Objectives** - The main objective of this study was to investigate a correlation between BART and carotid intimal-medial thickening in a population of patients with an ample spectrum of coronary artery disease, demonstrated objectively through a cineangiography.

A secondary objective was to evaluate a correlation between the endothelial function and the carotid intimal-medial thickening with the severity of the coronary artery disease expressed by the degree of luminal obstruction (in %), determined through an atherosclerotic plaque.

## RESULTS

Forty-two patients were assessed with 66.7% of them being males. Mean age was  $60.7 \pm 9.2$  yrs, arterial hypertension was present in 35 (83.3%) of the patients and dyslipidemia in 14 (33.3%) of them (Table I).

**Clinical picture** - Atypical thoracic pain was present in 19 (45.2%) of the patients, being the most frequent clinical picture, followed by stable angina in 12 patients (28.6%). Six patients (14.3%) were asymptomatic (Table I).

**Coronary angiography** - Regarding the extension of coronary disease, 12 (28.6%) of the patients presented just parietal coronary alterations, 5 (11.9%) had lesions between 50% and 70% and 25 (59.5%) had lesions  $\geq 70\%$ . Of the patients with coronary artery disease and at least one lesion  $\geq 50\%$ , 10 (23.8%) were uniarterial lesions, 8 (19.0%) were biarterial and 10 (23.8%) were triarterial lesions. Sixteen patients (38.1%) presented

**Table 1 - Risk factors and clinical picture**

Characteristics	n = 42	Porcentual
Hypertension	35	83.3
Diabetes	10	23.8
Dyslipidemia	14	33.3
Family history	9	21.4
Smoking	4	9.5
Previous event	13	31.0
Asymptomatic	6	14.3
Atypical pain	19	45.2
Dyspnea	7	16.7
Previous AMI	3	7.1
Previous unstable angina	2	4.8
Stable angina	12	28.6
Altered ET/SC	3	7.1
Others	4	9.5

AMI = acute myocardial infarction; ET / SC = ergometric test/ myocardial scintillography.

some segmental deficit assessed by ventriculography. The concordance among the interventionist cardiologists who performed the coronary angiographies was evaluated through Kappa method, with an  $81.1 \pm 8.5\%$  rate.

**Endothelial function** - The mean of the percentage of vasodilation after the administration of sublingual nitroglycerin was  $9.6 \pm 4.2$ . Gender and age did not present a correlation with %FMD ( $r = -0.280$ ,  $p = 0.07$  and  $r = -0.088$ ,  $p = 0.57$ , respectively).

The degree of coronary obstruction did not correlate with %FMD either ( $r = -0.008$ ,  $p = 0.96$ ). The mean of % FMD in the studied population was  $4.7 \pm 3.6$ . Figures 1 and 2 show BART images of one of the patients from the study group. There was a %FMD of 12, which represents normal endothelial function.

**Carotid intimal-medial thickening** - The mean of

the carotid intimal-medial thickening was  $1.08 \pm 0.23$  mm (Fig. 2). The measurement of the carotid intimal-medial thickening did not show a correlation with gender and age ( $r = -0.099$ ,  $p = 0.53$  and  $r = 0.278$ ,  $p = 0.074$ , respectively). The severity of coronary artery disease (degree of obstruction) did not correlate with the carotid intimal-medial thickening either ( $r = -0.063$ ,  $p = 0.69$ ).

**Correlation between %FMD and carotid intimal-medial thickening** - The measurements of the carotid intimal-medial thickening and %FMD presented a statistically significant correlation, with Spearman's coefficient of  $-0.315$ ,  $p=0.042$  (Fig.3). When we evaluate this correlation from another point of view, considering the carotid intimal-medial thickening as a binary variable and present if  $> 1.0$  mm, we observe

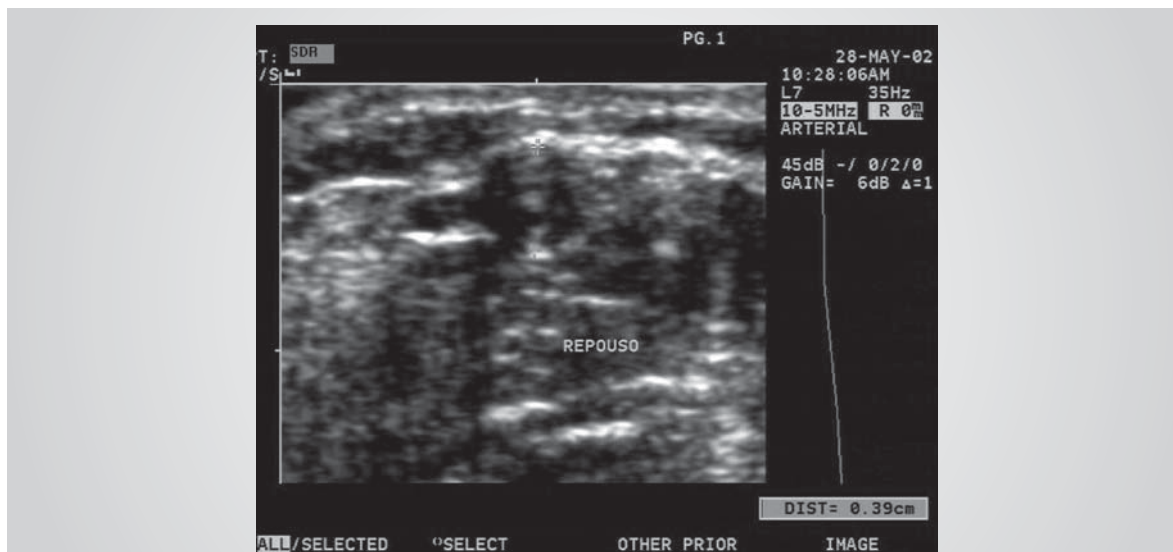


Fig.1 – Transversal section of the brachial artery in a patient from the study sample, showing a basal diameter of 3.9 mm.

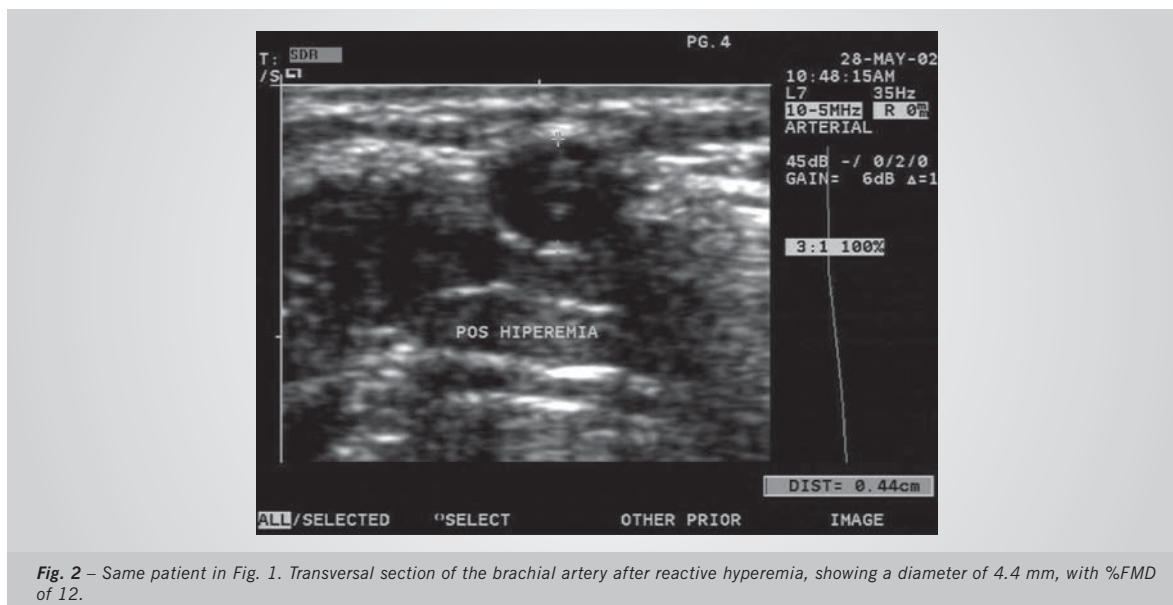


Fig. 2 – Same patient in Fig. 1. Transversal section of the brachial artery after reactive hyperemia, showing a diameter of 4.4 mm, with %FMD of 12.

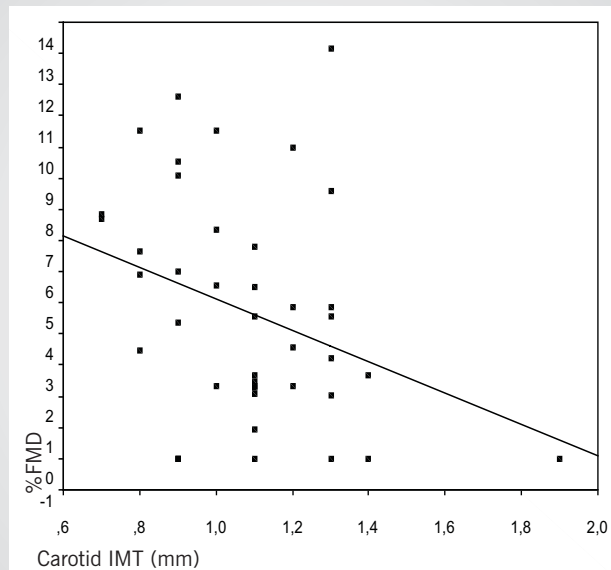


Fig. 3 – Dispersion of the measurement of the carotid intimal-medial thickening and %FMD.

that age and gender still do not show a correlation with intimal-medial thickening, but in a concordant way with the previous analysis, %FMD was  $6.0 \pm 3.7$  in the 18 patients without intimal-medial thickening, a statistically significant difference with a p value = 0.04 (Table II).

**Table 2 – Age, gender and FMD percentage in patients without and with carotid IMT**

Group	Without carotid IMT (n=18)	With carotid IMT (n=24)	p
Age (yrs)	58.7 ± 10.8	62.2 ± 7.7	0.22
Male sex (%)	12 (66.7)	16 (66.7)	1.00
% DMF	6.0 ± 3.7	3.7 ± 3.3	0.04

*IMT - intimal-medial thickening > 1 mm; %FMD - percentage of flow-mediated dilation.*

## DISCUSSION

The study of coronary artery disease in its more initial, sub-clinical phases has been the object of increasing interest. The importance of endothelial dysfunction as an independent predictor of cardiovascular events has been well demonstrated in previous studies<sup>7-9</sup>. Similarly, the presence of carotid IMT is related to the occurrence of acute myocardial infarction and ischemic stroke, with a relative risk between 2 and 6<sup>10</sup>.

The importance of these tests in the early diagnosis of atherosclerotic disease, however, remains indefinite. Schroeder and cols.<sup>11</sup> studied the value of the brachial artery reactivity test in the screening of coronary artery disease in 122 patients who were referred to coronary angiography and established a comparison with other diagnostic methods, such as the presence of thoracic angina, the ergometric treadmill test and myocardial scintillography.

These authors defined a cutoff through the ROC curve, finding the value of  $\%FMD \leq 4.5$  as being the highest sensitivity and specificity value to predict coronary disease. The test sensitivity and specificity were 71.3% and 81.0%, respectively. The highest sensitivity was that of the myocardial scintillography (100%) followed by the presence of angina pain (95.1%) and the ergometric test (82.4%). Regarding the test specificity, the values for the ergometric test were 57.1% and for the presence of angina, 23.8%. The data above show a good diagnostic value for the brachial artery reactivity test in terms of sensitivity and specificity and the authors suggested that the test can have, in the future, a use in the screening of coronary artery disease, but one must consider the possibility of a bias caused by patient selection in this study.

Although BART has been employed in several studies, there is no consensus regarding a reference value for the %FMD that would define endothelial dysfunction. In most studies, the test is described in different groups of patients, with values that are compared to one another, without the objective to define the presence or absence of endothelial dysfunction from a certain %FMD on. In 2001, a guideline on the use of BART was published containing important directions regarding the adequate methodology, but without establishing a value of %FMD that would define endothelial function<sup>12</sup>.

In our sample of patients with a ample spectrum of coronary artery disease, the mean of %FMD of  $4.7 \pm 3.6$  was decreased, which was expected considering the role of endothelium as an initial event of atherogenesis. This value is a little below those found by other authors while studying the endothelial function in patients with coronary disease demonstrated by coronary angiography. Chan

and cols.<sup>13</sup> found a mean of %FMD of  $5.9 \pm 3.8$ . It is important to emphasize that the value found in our study,  $4.7 \pm 3.6$ , is much lower than the mean values found in healthy individuals without risk factors for atherosclerotic disease, between  $8.2 \pm 3.1$  and  $21.1 \pm 2.0$ .<sup>2,14,15</sup>

In a study conducted by our group and published in 2003, we found a %FMD of  $8.9 \pm 5.7$ , studying 26 individuals with normal coronaries<sup>16</sup>.

There was no correlation between %FMD and the severity of coronary disease regarding the degree of obstruction of the atherosclerotic lesions, as also demonstrated by Enderle and cols.<sup>17</sup>, but in contrast with the results by Neunteufl and cols.<sup>15</sup>. Demographic differences, such as the percentage of symptomatic, diabetic, hypertensive, dyslipidemic, patients or smokers, might have determined these discrepant results. The criteria employed to define the severity of coronary disease differ among the studies. Neunteufl and cols stratified the patients in a larger number of subgroups according to the degree of obstruction of the atherosclerotic lesions: < 30, 30-50, 50-70, 70-90, 90-99 and 100%, whereas the stratification employed by us consisted of only 3 subgroups: < 50%, between 50% and 70% and > 70%.

Especially important is the need to consider that, in all studies, the severity of the coronary artery disease was established considering only the degree of luminal obstruction, in a subjective way. Although the interobserver concordance was satisfactory in our study, with a Kappa value > 80%, the computerized quantitative coronary angiography (QCA) would have allowed a more appropriate evaluation and a comparison among the studies, eliminating the interventionist cardiologist's subjectivity and the interobserver variation. Other angiographic characteristics, such as the number of significant lesions by vessel, which would represent a more diffuse disease, could be employed, but there is no consensus or normative data regarding such use. The coronary angiography was used in our study and by the other authors as a reference pattern to define coronary artery disease, a concept that has been discussed after the introduction of intracoronary ultrasound.

Carotid intimal-medial thickening mean in this studied sample was  $1.08 \pm 0.23$  mm. This elevated value is expected and is in accordance with the coronary involvement previously established on the bedside. In contrast with Enderle and cols.<sup>17</sup>, there was no correlation between the arterial intimal-medial thickening and the degree of coronary obstruction, but the same observations regarding the definition of disease severity discussed above must be considered.

The correlation between %FMD and carotid intimal-medial thickening has been evaluated in few studies, and always with a small sample of patients. The negative

correlation coefficient between %FMD and carotid intimal-medial thickening shows that, in this group of patients, lower values of %FMD were correlated with a higher degree of intimal-medial thickening ( $r = -0.315$ ,  $p = 0.042$ ). These data are in accordance with those demonstrated by Enderle and cols.<sup>17</sup> in a group of patients highly suspected of having coronary disease ( $r = -0.317$ ,  $p = 0.0004$ ). Campuzano and cols.<sup>18</sup>, studying a group of 39 patients with risk factors for atherosclerosis and 13 without those factors, found a difference in %FMD between the groups ( $2.77 \pm 2.57$  versus  $11.98 \pm 4.61\%$ ,  $p$  value < 0.01), as well as a difference in IMT values ( $0.85 \pm 0.24$  versus  $0.57 \pm 0.14$  mm,  $p$  value = 0.001), in addition to an inverse correlation between these variables ( $r = -0.357$ ;  $p < 0.01$ ). Juonala et al<sup>19</sup>, studying adults between 24 and 39 yrs with no evidence of CAD, similarly found a negative and significant correlation between %FMD and IMT in a multivariate analysis ( $r = -0.34$ ,  $p = 0.001$ ); additionally, the number of risk factors correlated with IMT in patients with lower %FMD. In the remaining studies, the evaluation of the correlation of the two methods was carried out in hypertensive patients and not in those with coronaropathies. Ghiadoni et al.<sup>5</sup> evaluated the endothelial function and carotid IMT in 44 hypertensive patients and 30 normotensive individuals and found an inverse correlation between the two tests in the hypertensive group, only ( $r = -0.68$ ,  $p = 0.0003$ ). Different results were reported by Barenbrock and et al<sup>6</sup>, who did not find a significant correlation between endothelial function and arterial IMT, when studying previously untreated hypertensive patients with no evidence of coronary disease ( $r = -0.17$ ,  $p = 0.32$ ). Table III shows a review of the outcomes of the main studies that evaluated these questions discussed here. Further studies are necessary to determine whether there is a correlation between these vascular alterations (endothelial dysfunction and IMT) and the severity of coronary artery disease.

## CONCLUSION

In this study, the presence of a correlation between the percentage of flow-mediated dilation and carotid intimal-medial thickening suggests the concomitance of functional and anatomical alterations in coronary artery disease. We did not find a correlation between these vascular alterations (endothelial dysfunction and intimal-medial thickening) and the severity of atherosclerotic lesions detected at the coronary angiography. The clinical importance of these methods remains to be determined. A better test standardization, studies with larger sample sizes and different criteria of selection, might, in the future, allow situating the initial vascular damage evaluation in the context of prevention, diagnosis and stratification of atherosclerotic disease risk.



**Table 3 - Comparison among findings from different studies regarding endothelial function and intimal-medial thickening**

Author	Chan et al <sup>13</sup>	Gokce et al <sup>9</sup>	Enderle et al <sup>17</sup>	Neunteufl et al <sup>15</sup>	Ghiadoni et al <sup>5</sup>	Juonala et al <sup>19</sup>	Campuzano et al <sup>18</sup>	Barenbrock et al <sup>6</sup>	Chequer et al <sup>19</sup>
Sample size	152	187	101	44	44	2.109	52	20	42
Selection	CAD	Pre-op.	CAD	CAD	SAH	Young adults	With and without risk factors	SAH	CAD
%FMD	5.9 ± 3.8	6.7 ± 4.7	3.7 ± 4.1	5.7 ± 4.8	-----		2.77 ± 2.57	3.4 ± 3.6	4.7 ± 3.6
							11.98 ± 4.61	6.4 ± 1.,	
IMT	0.86 ± 0.13	-----	0.59 ± 0.35	-----	1.2 ± 0.3		0.85 ± 0.24	0.66 ± 0.02	1.08 ± 0.23
							0.57 ± 0.14	0.84 ± 0.03	
%DMF/IMT	r = -0.195	-----	r = -0.317	-----	r = -0.58	r = -0.34	-0.357	r = -0.17	r = -0.315
P	p = (NS)		p = 0.0004		P = 0.0003	P = 0.001	p < 0.01	p = 0.32	p = 0.042
%DMF/CAD	-----	-----	NS	r = -0.51	-----			-----	r = -0.008
P				P < 0.0001					p = 0.96
IMT/CAD	-----	-----	r = 0.324	-----	-----			-----	r = -0.063
P			p = 0.0003						p = 0.69

*IMT - intimal-medial thickening > 1mm; %FMD - percentage of flow-mediated dilation; CAD - coronary artery disease; SAH - systemic arterial hypertension; pre-op - pre-operative.*

**Potencial Conflict of Interest**

No potential conflict of interest relevant to this article was reported.

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