Carotid Artery Structural and Functional Evaluation in Relatives of Type 2 Diabetic Patients

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Summary

Background: In Western countries type 2 diabetes mellitus (DM2) is the leading cause of morbidity and mortality, particularly from cardiovascular causes. Since a family history of diabetes, even in non-diabetic subjects, is regarded as an increased risk of coronary heart disease, the use of approved surrogate markers of early atherosclerosis, specially of ultrasonic measurements of the carotid arteries, is of vital importance.

Objective: To analyze the structural properties (intima-media thickness) and functional properties (distensibility measurement) of the carotid arteries in subjects with (FH+) a family history of type 2 diabetes, in comparison to subjects without (FH-) a family history of type 2 diabetes, both groups with no known cardiovascular risk factors.

Methods: 32 individuals (male and female, age range, 21-47 years; 19 FH+, 13 FH-) had their right and left common and internal carotid arteries measured, using high-resolution B-mode ultrasonography. Both groups had similar (P>0.05) age, BMI, blood pressure, and fasting blood glucose and insulin, leptin, and C-reactive protein (CRP) levels.

Results: The intima-media thickness (IMT) of the left common carotid artery (LCCA) in the FH+ group (0.568±0.107mm) was statistically greater (p=0.029) than in the FH- group (0.477±0.116mm). Multiple regression analysis identified age, overweight and obesity (determined by BMI), CRP, and LDL-cholesterol levels as independent predictors of the IMT in the LCCA.

Conclusion: FH+ individuals with no metabolic disorders presented greater IMT of the left common carotid artery (structural alteration) than FH- individuals, but normal vessel function. (Arq Bras Cardiol 2009;92(3): 186-192)

Key words: Carotid artery, common/anatomy & histology; diabetes mellitus type 2.

Introduction

Type 2 diabetes mellitus (DM2), a disease that is rapidly reaching pandemic proportions, is the leading cause of morbidity and mortality in Western countries[1,2]. The main etiology of death in patients with DM2 is cardiovascular. Therefore, DM2 is regarded as a "coronary risk equivalent"[1,3].

The presence of atherosclerosis is pronounced even before the clinical event, and much research has been conducted on early detection of atherosclerosis, both in DM2 and non-DM2 patients, over the last two decades[4,5].

High resolution carotid ultrasonography, initially described by Pignoli et al[6], corroborated later by the same author has proved to be very useful, as a real-time, non-invasive and reproducible method, with negligible risk and at reasonable cost, representing a true window to systemic atherosclerosis[7]. This method allows both structural (for example, intima-media thickness, which is considered a subclinical cardiovascular disease surrogate marker and a strong predictor of future vascular events) and functional (elasticity) vessel analysis[5,7-16].

Therefore, since a family history of diabetes, even in non-diabetics subjects with no known cardiovascular risk factors, is regarded as an increased coronary heart disease risk, *per se*, carotid ultrasonic measurements can be an important early atherosclerosis marker. The objective of this study is to analyze the structural and functional properties of the carotid arteries in subjects with and without a family history of type 2 diabetes, both groups with no other known cardiovascular risk factors[17-19].

Methods

Between July 2004 and January 2006, first and second-degree relatives of patients with type 2 diabetes mellitus (FH+) and controls (FH-) - individuals with no family history of type 2 diabetes mellitus - were recruited through an announcement published in Rio de Janeiro newspapers, or through direct invitation by diagnosed DM2 patients of the Endocrinology Outpatient Clinic of Hospital Universitário Antônio Pedro (HUAP). The following exclusion criteria were used: age < 18 years or > 50 years; smoking habit; competitive physical

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activities; history of cardiovascular, cerebrovascularand/ or peripheral arterial disease; medication use; and/or pregnancy. Due to the strictness of the selection criteria, only 31 FH+ and 15 FH- volunteers were selected. Out of these, 12 FH+ and 2 FH- volunteers were excluded, at the initial evaluation, due to: systolic blood pressure (SBP) > 140mmHg and/or diastolic blood pressure (DBP) > 90mmHg; capillary fasting glycemia equal to or greater than 110mg/dl; 2-h postchallenge glycemia (OGTT) with 75 g dextrose > 140mg/dl; total plasma cholesterol > 240mg/dL and/or plasma triglycerides > 150mg/dl. Ultimately, there were 19 FH+ volunteers (age range, 23 - 47 years; mean age, 37.94 years) and 13 FH- volunteers (age range, 21 - 46 years; mean age, 34 years), both men and women. All subjects signed a written informed consent to be included on a voluntary basis in the study, in accordance to the HUAP Human Research Ethics Committee Guidelines.

The protocol required an initial assessment, which consisted of a standard questionnaire (personal data and family health history); anthropometric measurements (body mass index [BMI], calculated as weight in kilograms divided by height in square meters; waist circumference in centimeters [according to the 2005 International Diabetes Federation definition] which corresponds to the minimum circumference below the xiphoid process and above the navel; and waist-hip ratio); capillary fasting (twelve hours) glycemia (ACCUTREND, ROCHE, Inc.); blood pressure measurement (Korotkoff stages I and V), in a sitting position, after a 10-minute rest in a quiet room, at a room temperature of 22ºC, using a standard mercury sphigmomanometer, with an appropriate cuff for arm size, at heart level position; and pulse pressure (SBP – DBP) in mmHg.

On the second assessment, after 12h-fasting, the individuals had a venous blood collection for biochemical analyses conducted in one sole clinical analysis laboratory. The laboratory tests included: fasting glucose (G) and 2h-postload of 75g of dextrose (DEXTROSOL) (automated method – glucose oxidase/peroxidase, ADVIA 1650, ADVIA 1650 BAYER KIT, Germany); plasma insulin (I) (chemiluminescence, DPC IMMULLITE 2000 KIT, USA); total cholesterol (automated enzymatic method – cholesterol oxidase/peroxidase, ADVIA 1650, ADVIA 1650 BAYER KIT, Germany); triglycerides (automated kinetic enzymatic method); HDL-cholesterol (automated enzymatic method, ADVIA 1650, ADVIA DASA KIT [direct method, conducted IN-HOUSE]), LDL-cholesterol (calculated by the Friedewald equation [LDL = Total cholesterol minus HDL minus {Triglycerides ÷ 5}]), CRP-us (turbidimetry – BORN, BN II DADE BERHING, DADE BERHING KIT, USA) – where values lower than 0.05 (due to method limitation) were considered equal to 0.05 - and leptin (radio immune assay, manual with Alto Delfio, IN-HOUSE KIT). The insulin resistance index was calculated from G and I values, using the mathematical model of the HOMA-IR ([G X I/22.5] X 0.0555, the glucose values expressed in mg/dl were transformed into mmol/l).

The third assessment was conducted in the Echocardiography Laboratory of the Cardiology Service at HUAP, in a climatized room (room temperature between 22 and 24ºC), with a high resolution echography machine (VIVID 3 - General Electric, measurement accuracy up to hundredths of millimeters) equipped with a 13MHz linear transducer. All studies were conducted by one of two trained physicians, blinded to the examinees' clinical condition, following the American Society of Echocardiography and the Society of Vascular Medicine and Biology guidelines. A second analysis of the measurements was subsequently performed by the other physician (not the examiner), and all data were recorded and stored in individual CD-Rs, for later off-line processing. As to the bilateral carotid study, the patients were examined in a supine position, and a preliminary transversal plane scanning was performed, to assess the anatomy, followed by a longitudinal scanning (for B-mode imaging and measurements), with the head turned 45°, for anterior oblique, lateral and posterior approaches (using only the posterior approach for measurements), on the opposite side of the artery to be studied. The intima-media thickness (IMT) obtained was defined as the distance between the lumen-intima interface and the media-adventitia interface of the posterior far wall of the common carotid artery (CCA) and internal carotid artery (ICA). The measurement reference points for the CCA were: point 0 (zero), immediately proximal to where the CCA dilates for its bifurcation; point 1, 5mm distal to point 0; point 2, 10mm distal to point 0; and point 3, 15mm distal to point 0. In the ICA, the measurement reference points were: point 0' at the carotid bulb; point 1', 2.5mm proximal to point 0'; and point 2', 5mm proximal to point 0'. The IMT measurements were performed on the R-wave peak of an electrocardiogram (ECG) - diastolic phase - recorded simultaneously to the echosonography. The statistical analysis values used were produced by one sole examiner based on the mean measurement for each studied arterial segment, namely, the left common carotid artery (LCCA), the right common carotid artery (RCCA), the left internal carotid artery (LICA) and the right internal carotid artery (RICA).

After obtaining the common carotid arteries IMT and the interadventitial radius (R) (at longitudinal approach, during the systolic phase - T wave end, in the ECG - and diastolic phase - R-wave peak, in the ECG), the LCCA measurements were used (as the IMT was relevant in both studied groups) as analysis source for the arterial distensibility parameters, using the following formulas, in consonance with the ARIC Study and corroborating the SMART Study[14-16]:

a) $CAS = [D(s) - D(d)]/D(d)$;

b) $SI = \ln [PAS / PAD]/CAS$;

c) $CAC = ([Ds - Dd]/Dd / (PAS - PAD)$;

d) $YEM = (R/EMI) \times \{[PAS - PAD] / CAS\}$,

where CAS corresponds to the circumferential arterial strain; D(s) is the carotid diameter at systole; D(d) is the carotid diameter at diastole; SI is the carotid artery stiffness index (or ß index); nl means natural logarithm; SBP is the systolic blood pressure; DBP is the diastolic blood pressure; CAC is the carotid artery compliance coefficient; YEM is Young's elastic modulus and IMT is intima-media thickness.

After both examiners' data had been obtained, the interobserver variability coefficient was calculated, using Spearman correlation analysis and Brand & Altman graphic method.

The Student's t-test and the Wilcoxon test were used for comparing the mean levels of the numerical variables.

Multiple linear regression, using all the clinical and laboratory measurements as independent variables in each model, was used to study the influence of several factors on the response variables, such as RCCA, LCCA, RICA and LICA. The statistical program used for the analysis was the S-Plus 6.0, considering *P* significant when lower than 0.05 ($p<0.05$).

The sample size estimation for the main objective of this study was based on the Panacciuli et al[17] study, which separated the volunteers in a similar pattern - DM2 patient relatives (FH+) and controls (FH-). In the performed Student's t-test, the expected difference between the measurements was 0.07, the standard deviation was 0.05, the statistical power was 0.8, the *p* value was 0.05, resulting in 10 individuals for each group[17].

Results

The general, laboratory and ultrasonographic data are shown in Table 1, 2 and 3.

The HF+ and HF- subjects had no significant difference concerning age, sex, BMI, waist, hip, waist-hip ratio, systolic blood pressure, PP (pulse pressure), HDL-cholesterol, LDL-cholesterol, triglycerides, fasting glycemia, glucose oral tolerance test, fasting insulin, leptin and CRP-us. The LCCE IMT in the FH+ group was significantly greater than in the FH- group ($p<0.03$). Conversely, despite the fact that the values found were within the limits of normality according to the applied methods, there was a significant difference ($p < 0.05$) in the HOMA-IR and the total cholesterol levels between the HF+ group and the HF- group, being greater in the HF+ group, but with no correlation between the cholesterol levels and the LCCA IMT.

With regard to carotid artery distensibility parameters, no significant difference was observed between the FH+ group and the FH- group, as shown in the angular coefficients of the regression analyses (in *p*) performed between these parameters (in separate) and other variables (table 4). However, positive correlations were observed between age and the three indicators of arterial distensibility (SI, CAC and YEM) ($p<0.05$); between fasting glycemia and the values of CAC and SI ($p<0.05$); and between SBP and the values of YEM and CAC ($p<0.04$). However,no correlation of statistical significance was detected between the radius and the distensibility and the artery structural measurements (table 5).

Table 1 - Basic clinical and anthropometric characteristics of the volunteers, according to family history of DM2 (n=32)

	FH+	FH-	p
Total	19	13	
Age (years)	38±8	34±9	0.184
Gender			
Women (%)	14 (73.68)	9(69.23)	0.901
Men (%)	5(26.32)	4(30.77)	
BMI (kg/m²)	25.040±3.240	24.244±3.660	0.347
Waist (cm)	85.640±7.460	83.510±11.270	0.525
Waist-Hip Ratio	0.868±0.080	0.825±0.072	0.135
SBP (mmHg)	120.260±11.590	115.000±8.440	0.172
Pulse Pressure (mmHg)	45.420±12.340	47.538±10.870	0.621

Values are mean ± standard deviation; BMI - body mass index; SBP - systolic blood pressure; FH+, positive family history of diabetes mellitus type2 (DM2); FH-, negative family history familiar of diabetes mellitus type2.

Table 2 - Laboratorial analysis of the volunteers, according to family history of DM2 (n=32)

	FH+	FH-	p
Total Cholesterol (mg/dl)	190.150±29.940*	162.850±34.900	0.024
Fasting Glycemia (mg/dl)	88.260±6.930	84.770±5.890	0.148
HOMA-IR	1.420±0.670*	0.929±0.540	0.037
Fasting Insulin (mcU/ml)	6.460±2.939	4.460±2.650	0.058
HDL-cholesterol (mg/dl)	56.680±8.370	54.920±8.780	0.571
LDL-cholesterol (mg/dl)	118.740±29.190	102.080±26.040	0.109
Triglycerides (mg/dl)	77.630±24.550	64.460±25.820	0.155
OGTT (mg/dl)	92.220±14.330	92.460±22.080	0.971
Leptin (mg/dl)	17.950±11.860	13,980±6,680	0.284
CRP-us†	0.358±0.250	0.240±0.180	0.159

*Values are mean ± standard deviation; DM2 - diabetes mellitus type 2;FH+, positive family history of diabetes mellitus type 2 (DM2); HF-, negative family history familiar of diabetes mellitus type 2; CRP–us, ultra sensitive C reactive protein; HDL - high density lipoprotein; LDL - low density lipoprotein; VLDL - very low density lipoprotein; OGTT - oral glucose tolerance test; * P<0.05; † Values lower than 0.05 obtained in the laboratory were converted to 0.05, for calculation purposes.*

Table 3 - Echographic analysis of the volunteers according to family history of DM2

	FH+	FH-	p
RCCA IMT(mm)†	0.561±0.115	0.521±0.117	0.604
RICA IMT(mm)	0.464±0.126	0.421±0.076	0.275
LCCA IMT(mm)*	0.568±0.107*	0.477±0.116	0.030
LICA IMT(mm)	0.480±0.125	0.404±0.123	0.095
RCCA IMT + RICA IMT (mm)	1.026±0.061	0.941±0.230	0.247
LCCA IMT + LICA IMT (mm)	1.048±0.022*	0.881±0.312	0.025
LCCA IMT + LICAIMT + RCCA IMT + RICA IMT(mm)	2.075±0.015	1.822±0.520	0.641
SI	1.410±0.198	1.379±0.150	0.617
CAC (mm^2Kpa^{-1})	0.002±0.001	0.002±0.001	0.967
YEM (Kpa)†	471.240±305.960	327.620±145.270	0.305
Radius (mm)	3.537±0.300	3.482±0.370	0.650

*Values are mean ± standard deviation; M2 - diabetes mellitus type 2; FH+, positive family history of DM2; FH-, negative family history of DM2; LCCA - left common carotid artery; IMT - intima-media thickness; RCCA - right common carotid artery; RICA - right internal carotid artery; LICA - left internal carotid artery; SI - carotid artery stiffness index; CAC - carotid artery compliance coefficient; YEM - Young elastic modulus; * p<0.05; † The RICA and YEM values have been log transformed*

Table 4 - Influence of independent variables on the SI, CAC (in mm^2Kpa^{-1}) and YEM (in Kpa) using regression analysis, in studied subjects (n=32), results in *p* value

	SI	*CAC* ‖	YEM
Age (years)	<0.001*	0.002*	<0.001*
BMI (Kg/m²)	0.374	0.867	0.092
Overweight/obesity	0.193	0.720	0.090
Altered waist measure†	0.888	0.614	0.938
SBP (mmHg)	0.406	0.034*	0.006*
DBP (mmHg)	0.433	0.062	0.803
TC(mg/dl)	0.109	0.233	0.068
HOMA–IR	0.029*	0.076	0.443
LDL-cholesterol (mg/dl)	0.058	0.264	0.040*
TG (mg/dl)	0.295	0.241	0.559
HDL-cholesterol (mg/dl)	0.944	0.704	0.786
Fasting glycemia (mg/dl)	0.043*	0.016*	0.129
OGTT (mg/dl)	0.768	0.818	0.929
CRP-us	0.775	0.798	0.348
Fasting insulin (mcU/ml)	0.070	0.166	0.610
Leptin (mg/ml)	0.114	0.328	0.395
Radius (mm)	0.075	0.110	0.118
To be a relative of DM2 patient	0.617	0.967	0.186

*SI - carotid artery stiffness index; CAC - carotid artery compliance coefficient; YEM - Young elastic modulus; BMI - body mass index; Overweight/obesity - individuals with abnormal BMI (either overweight or obesity); SBP - systolic blood pressure; DBP - diastolic blood pressure; TC - total cholesterol; HOMA-IR - homeostasis model assessment – insulin resistance; LDL - low density lipoprotein; TG - triglycerides; HDL - high density lipoprotein; OGTT - oral glucose tolerance test; CRP-us - ultra-sensitive C reactive protein; DM2 - diabetes mellitus type 2; * p < 0.05; † either by the IDF or NCEP ATP III criteria; || angular coefficient multiplied by 10,000.*

Table 5 - Influence of the structural and functional carotid artery variables on the common carotid artery radius (in centimeters) in studied subjects (n=32)

	Angular coefficient	p
mean RCCA (mm)	0.119	0311
mean RICA (mm)	0054	0.380
mean LCCA (mm)	0.013	0.840
mean LICA (mm)	0.036	0.622
mean YEM (Kpa)	-0.488	0.118
mean CAC (mm^2Kpa^{-1})	0.0007	0.110
mean SI	-0.174	0.075

RCCA - right common carotid artery; RICA - right internal carotid artery; LCCA - left common carotid artery; LICA - left internal carotid artery; YEM - Young elastic modulus; CAC - carotid artery compliance coefficient; SI - carotid arterial stiffness index.

Table 6 - Influence of independent variables on the LCCA IMT, using regression analysis, in the studied subjects (n=32)

	Angular coefficient	p
Age (years)	0.008*	0.001
BMI (kg/m²)	0.020*	<0.001
Overweight/obesity	0.121*	0.002
Altered waist measure †	0.056	0.190
SBP(mmHg)	0.003	0.092
DBP (mmHg)	0.005*	0.045
TC (mg/dl)	0.001	0.143
HOMA–IR	0.060	0.061
LDL-cholesterol (mg/dl)	0.002*	0.019
TG (mg/dl)	0.0005	0.578
HDL-cholesterol (mg/dl)	-0.001	0.710
Fasting glycemia (mg/dl)	0.003	0.392
OGTT (mg/dl)	0.0005	0.681
CRP-us	0.269*	0.007
Fasting insulin (mcU/ml)	0.013	0.081
Leptin (mg/ml)	0.0003	0.882
Radius (mm)	0.013	0.840
To be a relative of DM2 patient	0.091*	0.030

*BMI - body mass index; Overweight/obesity - individuals with abnormal BMI (either overweight or obesity); SBP - systolic blood pressure; DBP - diastolic blood pressure; TC - total cholesterol; HOMA-IR - homeostasis model assessment–insulin resistance; LDL - low density lipoprotein; TG - triglycerides; HDL - high density lipoprotein; OGTT - oral glucose tolerance test; CRP-us - ultra-sensitive C reactive protein; DM2 - diabetes mellitus type 2; * p < 0.05; either by the IDF or NCEP ATP III criteria.*

The results of the regression analysis between the LCCA IMT and other independent variables in all the individuals (table 6) demonstrated a positive correlation with BMI ($p<0.001$), age ($p=0.001$), CRP-us ($p=0.007$), DBP ($p<0.05$), and HF+ ($p=0.03$).

Altered waist circumference refers to measurements that exceed normal parameters for Brazil, as defined by International Diabetes Federation (≤80cm in women, and ≤90cm in men) and by NCEP ATP III (≤88cm in women, and ≤102cm in men).

Concerning interobserver correlation, using LCCA measurements, it was observed that the levels measured by the two observers were similar ($p = 0.294$) and presented a significant correlation ($r=0.737$ and $p=0.0109$). As to LICA measurements, although it was observed that the mean measurements of both observers were also similar ($p=0.7637$), there was no significant correlation ($r=0.1641$ and $p=0.5761$).

Discussion

This is the first study with relatives of diabetic patients in

which the carotid arterial segments were separately analyzed, with an evaluation of the carotid arteries structural and functional parameters.

Concerning the carotid structural analysis, higher values of the common carotid IMT were observed in family members of diabetic patients (including young adults), in comparison to the control group, as in the study conducted by Nicola Pannacciulli et al[17]. Our study, however, demonstrated that the IMT and the BMI values (in both groups) were lower than in the mentioned study. The mean IMT values, in Pannaciulli's study, were 0.840±0.010 mm in the FH+ group, and 0.770±0.001 mm in the FH- group, compared to 0.560±0.107 mm in the FH+ group, and 0.477±0.110 mm in the FH- group, in our study. The mean BMI values (in Kg/m^2), in Pannaciulli's study, were 30.0±0.500 in the FH+ group, and 29.400±0.500 in the FH- group, compared to 25.040 ±3.240, and 24.240 ±3.660 kg/m^2, respectively, in our study. Corroborating the findings from Pannacciulli et al's study, we also found no correlation with fasting glycemia and/or OGTT.

The CCA assessment, therefore, demonstrates a high degree of reproducibility, since in that segment the atherosclerosis process manifests as a diffuse thickening caused by progressive proliferation of smooth muscle cells and matrix deposition, it is a superficial segment, of straight walls, running parallel to the skin surface, of easy analysis (less time and effort to study), differing from ICA, where the manifestation of the atherosclerosis process occurs by focal atherosclerotic injury (or plaques), it is located deep within the neck, with walls of non-parallel origin in relation to the cervical skin surface. Furthermore, the CCA IMT is strongly, consistently and independently associated with coronary arterial disease.

In our study, the significant correlation between the LCCA IMT values obtained by both observers confirms the reproducibility of the ultrasound imaging method in clinical practice, when performed by trained professionals, in agreement with previous studies. Nonetheless, such correlation was not observed in the ICA values, confirming the conclusion that its anatomic aspects limit the analysis.

However, in keeping with other studies, our study considers the LCCA as the earliest atherosclerotic injury site, because the LCCA arises directly from the aortic arch, with lower shear stress (frictional force exerted by the circulating blood column on the intimal surface of the arteries), and DM2 is associated with lower CCA shear stress[20-23].

Adipose tissue, a true endocrine organ, produces several peptides (angiotensin, interleukin-6, tumor necrosis factor-alpha, PAI-1, leptin and adiponectin), which affect vascular structure. Interleukin-6 induces hepatic production of CRP, an acute phase protein. In addition to that, a study conducted by Balletshofer et al. observed the association between endothelial dysfunction and insulin-resistance in the young offspring of diabetic parents, regardless of the presence or absence of the classic cardiovascular risk factors[24].

In several observational and clinical studies, except one which demonstrated a weak correlation, the IMT has been a marker of subclinical vascular cardiac disease, in association with traditional risk factors for coronary heart disease (according to our findings: CRP, LDL-cholesterol and DBP) and the coronary atheroma load, being a predictor of subsequent cardiovascular events and an early indicator of atherosclerosis, besides being a valuable risk marker in non-invasive assessments[5-7,10-11,14,20-27].

With regard to carotid structural properties, no comparison with previous studies with the same group profiles could be conducted because none was found in the medical literature. Nevertheless, with different study groups, the same findings were observed, concerning the correlation between SI and CAC values and fasting glycemia, confirming that arterial stiffness is not independent from the risk factors for vascular events, and has a direct relationship (only regarding arterial compliance and elastic modulus, in our study) with systolic blood pressure levels. It is important to stress that the individuals analyzed in both groups of our study presented no significant differences in blood pressure levels, and both groups had normal blood pressure levels (according to JOINT VII guidelines)[28]. Furthermore, our study groups (both FH+ and FH-) had no classic cardiovascular risk factors, according to the Framingham risk score, and in spite of having greater HOMA-IR and total cholesterol values (although within the normal range), these were not sufficient to produce vascular functional changes, with levels of distensibility parameters lower than those found in other relevant studies. To confirm the importance of the heredity influence, we removed the largest HOMA-IR and total cholesterol values from the FH+ group, and the difference between the LCCA IMT of the two groups remained significant.

Remodeling, another atherogenesis component that delays significant lumen compromise[29], was not detected either in the FH+ group or the FH- group. (Mean age range under 50 years? Low mean IMT values?), due to the fact that no significant differences were found in the radius measurement between the FH+ and FH- groups.

Many studies suggest that genetic factors influence the carotid IMT, which can be more discriminative than the arterial distensibility assessment in distinguishing patients of high and low cardiovascular risk, especially in relatives of diabetic patients. Ultrasonography is a valuable and practical tool for the study of the early evolution of atherosclerosis, transforming us from mere spectators into active inducers of a regression of the atherosclerotic process, even before its first clinical manifestations[30]. It is a proposed cardiovascular risk marker to estimate coronary heart disease susceptibility, since no existing risk score[26] includes altogether obesity, a positive family history of premature coronary heart disease, a family history of diabetes mellitus, sedentary life and/or psychological and economical factors, among others, focusing only on fatal and non-fatal coronary disease, but not ischemic stroke.

Our study has several limitations; for example, the use of physical measurements to determine active cardiovascular disease, and the participants' self-reported absence of corresponding symptoms (i.e., all volunteers were apparently healthy individuals). However, this same methodology has been used in many other relevant studies involving this kind of analysis[18]. The small number of studied individuals did not allow a separate evaluation by sex, but that does not limit the importance of the study, since it meets Boissel's three criteria for surrogacy (efficiency and convenience, as a noninvasive

method; linkage; and congruency). The advantages of the method range from a lower sample number to the confirmation of a direct association between the measurements and the disease process[8,30]. It is known that ultrasonography does not allow for differentiation between an intimal atherosclerotic process and medial hypertrophy, due to arterial blood pressure effects. At first, this can be viewed as a limitation of the method; however, there are data that corroborate the fact that IMT is more directly related to intimal atherosclerosis than to medial hypertrophy. Although automated measurements have not been used in our study, recent studies have shown that a standardized manual approach, conducted by well-trained technicians, can be as reliable as the automated approach. The echographic analysis has been limited to the far wall, differently from some previous studies which analyzed both near and far arterial walls. This was done because the accuracy would be reduced if the near wall was included, since the method does not allow for differentiation between intima and media measurements, which have been determined using excess gain. The blood pressure levels were assessed by indirect brachial measurements, despite the knowledge that the brachial blood pressure is usually 10 to 15% higher than the common carotid artery blood pressure. This was done based on Borow and Newburger[31] studies, which evidenced an excellent correlation between the systemic blood pressure levels measured by invasive methods and the noninvasive brachial blood pressure measurements[15]. We used indirect methods for evaluating insulin-resistance (IR) - fasting insulinemia, HOMA-IR and oral glucose tolerance test - as well as indirect IR markers - body mass index, abdominal circumference and triglycerides serum concentration - instead of the gold-standard of the American Diabetics Association - hyperinsulinemic euglycemic clamp - because the latter method, in spite of producing purer and more reproducible data on tissue insulin action, requires a long time, instant analysis instruments for measuring blood glucose levels, infusion bombs and highly specialized and trained personnel. Besides, a laboratory investigation method of IR analysis which fulfills all the universally accepted and used criteria is still not available. Therefore, according to many studies, indirect methods are more prone to be used in the clinical practice[32]. No analysis was separately conducted according to race. Brazil is a country of racial miscegenation and, therefore, it is very difficult to separate volunteers by race. We did not use random selection, and this may be viewed as a negative aspect of the study. Nevertheless, all our efforts to eliminate every factor that could have obscured our results turned us into the most obstinate searchers of "ideal volunteers".

It is noteworthy that, by the conclusion of the study, the normal reference values for intima-media thickness (IMT) had been ratified by The American Society of Echocardiography, in August 2006, and the new values were much higher than those in our findings[33]. Nevertheless, in February 2008, Lim et al[34] conducted a study with 137 healthy participants, and found normal values for the LCC IMT, in agreement with our results, in participants with no DM2 family history (age range, 35-39 years - 0.49±0.06; 40-45 years - 0.52±0.06 - in the Lim et al[34] study) separated according to age group. Those results placed our group of individuals with a family history of DM2 at an older age group (50-59 years - 0.56±0.08 - Lim et al[34] study), reiterating what Sir William Osler declared in 1897: "We are as old as our arteries"[34].

Conclusion

Individuals with a family history of DM2, even those with no glucose-intolerance and/or altered basal insulin levels, presented greater values of carotid intima-media thickness (IMT) (structural change), particularly in the left common carotid territory, when compared to the group with no family history of DM2, although no functional changes were observed in the blood vessels.

The consequences resulting from a genetic predisposition to DM2, probably associated with insulin resistance, on the carotid IMT values, particularly in the left common carotid territory, preceding the vascular functional alteration, needs to be further investigated in order to elucidate this association.

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Potential Conflict of Interest

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

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Study Association

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