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Clinical, anthropometric, and biochemical factors associated with carotid intima media thickness in mexican subjects with hyper- or hypoalphalipoproteinemia

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ABSTRACT

Introduction: Assessments of the relationship between HDL cholesterol and atherosclerosis have rendered conflicting results due to the coexistence of multiple confounding variables. A comparison of subjects at the extremes of the HDL cholesterol distribution may permit the appraisal of more homogeneous groups. **Objective:** To identify clinical, anthropometric, and biochemical factors associated with carotid intima media thickness, a surrogate marker for atherosclerosis, in persons with hyperalphalipoproteinemia (HDL cholesterol > 60 mg/dl) or hypoalphalipoproteinemia (HDL cholesterol < 40 mg/dl). **Methodology:** A case control design. The hyperalphalipoproteinemia group consisted of 111 subjects while the hypoalphalipoproteinemia group contained 93 subjects. Participants with one or more of the following conditions were excluded: any acute disease within six weeks of inclusion and the use of medications or any condition that may modify serum lipids. Carotid intima media thickness

RESUMEN

Introducción: Las evaluaciones de la relación entre el colesterol HDL y la aterosclerosis han rendido resultados contradictorios debido a la coexistencia de múltiples variables de confusión. Una comparación de los sujetos en los extremos de la distribución del colesterol HDL puede permitir la evaluación de grupos más homogéneos. **Objetivo:** Identificar los factores clínicos, antropométricos y bioquímicos asociados con el grosor de la capa íntima-media de la carótida, un marcador de la aterosclerosis, en personas con hiperalfalipoproteinemia (colesterol HDL > 60 mg/dl) o hipoalfalipoproteinemia (colesterol HDL< 40 mg/dl). **Metodología:** Estudio de casos y controles. Los casos con hiperalfalipoproteinemia fueron 111 sujetos, mientras que el grupo hipoalfalipoproteinemia contenía 93 participantes. Los individuos con una o más de las siguientes condiciones fueron excluidos: cualquier enfermedad aguda en las seis semanas previas a la inclusión y el uso de medicamentos o cualquier

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measurement was carried out in all subjects utilizing PACS v. 11.3.2 Carestream software. Clinical, biochemical, anthropometric and dietetic factors were evaluated. **Results:** There was no difference in the mean carotid intima media thickness between groups: hyperalphalipoproteinemia 0.60 mm (0.50-0.70) and hypoalphalipoproteinemia 0.55 mm (0.50-0.65); p = 0.366. Both age and diastolic blood pressure were independently associated with carotid intima media thickness in the total population (r^2 = 22.4%). In hyperalphalipoproteinemia, the predictors of carotid intima media thickness were age and diastolic blood pressure (r^2 = 28.9%). In hypoalphalipoproteinemia, the predictors were age and systolic blood pressure (r^2 = 20.1%). The HDL cholesterol and apolipoprotein A-I did not influence carotid intima media thickness. **Conclusions:** In Mexican subjects with hyperalphalipoproteinemia or hypoalphalipoproteinemia, the carotid intima media thickness was associated with different factors. A large percentage of the variability in carotid intima media thickness was explained by age. This study did not find a direct relation between HDL cholesterol and carotid intima media thickness. (REV MEX ENDOCRINOL METAB NUTR. 2015;2:118-27)

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condición que puedan modificar los lípidos séricos. La medición del espesor íntima-media carotídeo se realizó en todos los sujetos que utilizan el programa PACS v. 11.3.2 Carestream. Se compararon datos clínicos, bioquímicos y antropométricos y se evaluaron los factores dietéticos. **Resultados:** No hubo diferencia en el espesor de la carótida entre los grupos: hiperalfalipoproteinemia 0.60 mm (0.50-0.70) vs. hipoalfalipoproteinemia 0.55 mm (0.50-0.65); p = 0.366. Tanto la edad y la presión arterial diastólica fueron de forma independiente asociados con el espesor carotídeo en la población total (r^2 = 22.4%). En el grupo con hiperalfalipoproteinemia, los predictores fueron la edad y la presión arterial diastólica (r^2 = 28.9%). En el grupo con hipoalfalipoproteinemia, los predictores fueron la edad y la presión arterial sistólica (r^2 = 20.1%). El colesterol HDL y la apolipoproteína AI no influyeron en el espesor carotídeo. **Conclusiones:** En sujetos mexicanos con hiperalfalipoproteinemia o hipoalfalipoproteinemia, el grosor carotídeo se asoció con diferentes factores. Un gran porcentaje de la variabilidad en el espesor de las capas íntima-media de la carótida se explica por la edad. Este estudio no encontró una relación entre el colesterol HDL y el espesor carotídeo.

Palabras clave: Hiperalfalipoproteinemia. Hipoalfalipoproteinemia. Espesor carotídeo.

INTRODUCTION

Cardiovascular disease (CVD) constitutes the first cause of death in occidental countries[1-3]. Measurement of carotid intima media thickness (cIMT) by B mode ultrasound is a non-invasive, sensitive, and reproducible technique for the identification and quantification of coronary heart disease and assessment of CVD risk. This surrogate marker of subclinical atherosclerosis may reclassify patients by intermediate risk, distinguish patients with and without prevalent CVD, and predict adverse major cardiovascular events[4]. It allows observing changes in the carotid artery walls while the measurement is being made[5].

Some cross-sectional and epidemiological studies indicate a relation between cIMT and the prevalence of CVD or coronary heart disease risk factors[6-8]. There are numerous reported risk factors that modify carotid thickness, mainly age, gender, race, diabetes mellitus, total cholesterol and high-density lipoprotein cholesterol (HDL-C), smoking, and high blood pressure. These are known as "traditional risk factors". These are positively associated with the incidence of vascular events (stroke and infarction). Clinical trials with lipid-lowering, antihypertensive, and antidiabetic drugs have confirmed that modification of these risk factors significantly reduces the progression of atherosclerotic plaque and leads to a decrease of cardiovascular risk in the future[9].

A cross-sectional study done in Mexico City assessed the frequency of carotid atherosclerosis and its relation to cardiovascular risk factors in the elderly. The prevalence of atherosclerotic lesions was high (males 61.9%, females 66.0%). Carotid atherosclerosis was significantly associated with age (p < 0.0001), high blood pressure (p < 0.001), isolated systolic hypertension (p = 0.01), hypercholesterolemia (p = 0.04), and diabetes mellitus (p = 0.06)[10]. Another case-control study in Japan reported a significant positive linear correlation between thickness of the intima media complex and age in healthy people; the mean cIMT was 0.59 ± 0.15 mm[11].

For over four decades, it has been described that high HDL-C levels or hyperalphalipoproteinemia (hyper HDL-C) are associated with a lower risk of CVD and its outcomes[12]. The plasma concentration of HDL-C and the risk of having ischemic heart disease assume a causal relation between both[13]. However, controversy remains. Multiple coexisting confounding variables (e.g. triglycerides, gender, sedentary activities, body mass index, ethnicity, etc.) have precluded authors from discerning the individual contribution of HDL-C to the incidence or prevalence of atherosclerosis-related events. To reach this goal, large sample sizes are needed. As a result, discordant conclusions are common in this issue. Here, we propose that a comparison of the extremes of the HDL-C distribution and a case-control design could be an alternative to have homogeneous groups for comparison. Hypoalphalipoproteinemia (hypo HDL-C) and hyper HDL-C are markers that identify remarkably different sets of individuals that differ between them in many sociodemographic and metabolic features. The comparison of the cIMT determinants in the low or high HDL-C groups may eliminate the confounding effect of several variables associated with HDL-C concentrations.

Thus, we aim to identify the clinical, anthropometrical, and biochemical factors that are associated with carotid thickness in subjects with hypo or hyper HDL-C.

METHODOLOGY

We designed a cross-sectional study in which we included Mexican men and women from ages 18 to 80 years old. The participants received medical care at the "Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán" (INCMNSZ, Mexico City), either at its lipid or diabetes clinic. We invited 361 patients to participate in the study, mainly living in Federal District and the central region of the country, from which we selected 204. Of them, 93 participants had a confirmed diagnosis of hypoalphalipoproteinemia (HDL-C < 40 mg/dl) and 111 had hyperalphalipoproteinemia (HDL-C > 60 mg/dl) (Fig. 1). The HDL-C levels were categorized according the ATPIII guideline. An abnormal HDL-C concentration should be present in at least two spaced apart measures for a period no greater than six months in order to avoid transitory abnormalities in HDL-C. The 204 included participants completed the study. The 157 other patients were not included due to the presence of one or more of the following conditions: any acute disease six weeks previous to their participation in the study, use of medications or any condition that modify serum lipids like pregnancy, smoking, diabetes, and HIV, among others.

We obtained sociodemographic information (Table 1), lifestyle characteristics, and data about risk factors for CVD through the implementation of a standardized and validated questionnaire for a Mexican population. Diet components were analyzed through a 24-hour dietary recall. Each person had anthropometric measures (weight, height, waist and hip perimeter) taken and a medium was taken from the two measurements. Body mass index (BMI) was calculated by dividing the subject's weight in kilograms by height in meters squared. Blood pressure was measured twice with a period of one minute between measurements; it was taken from the left arm using a mercurial sphygmomanometer after the patient had been sitting down for 10 minutes before the blood sample was drawn. To measure biochemical variables, samples were taken in the morning, with patients in a previous fasting of 9-12 hours; we took a sample of 40 ml of blood from each subject, which was analyzed in the Endocrinology Laboratory of the INCMNSZ (certified by the College of American Pathologists). Serum glucose concentrations were measured by the glucose-oxidase method (Boehringer Mannheim). Determination of cholesterol and triglycerides was done by enzymatic commercial methods (Boehringer Mannheim). Total HDL-C was measured after precipitating the lipoproteins that contained apolipoprotein B with phosphotungstate (Boehringer Mannheim) (CV2.5%). The low-density lipoprotein (LDL) determination was estimated through the Friedwald formula. The chief of the radiology department of the INCMNSZ evaluated the thickness of the carotid intima and media layer. A

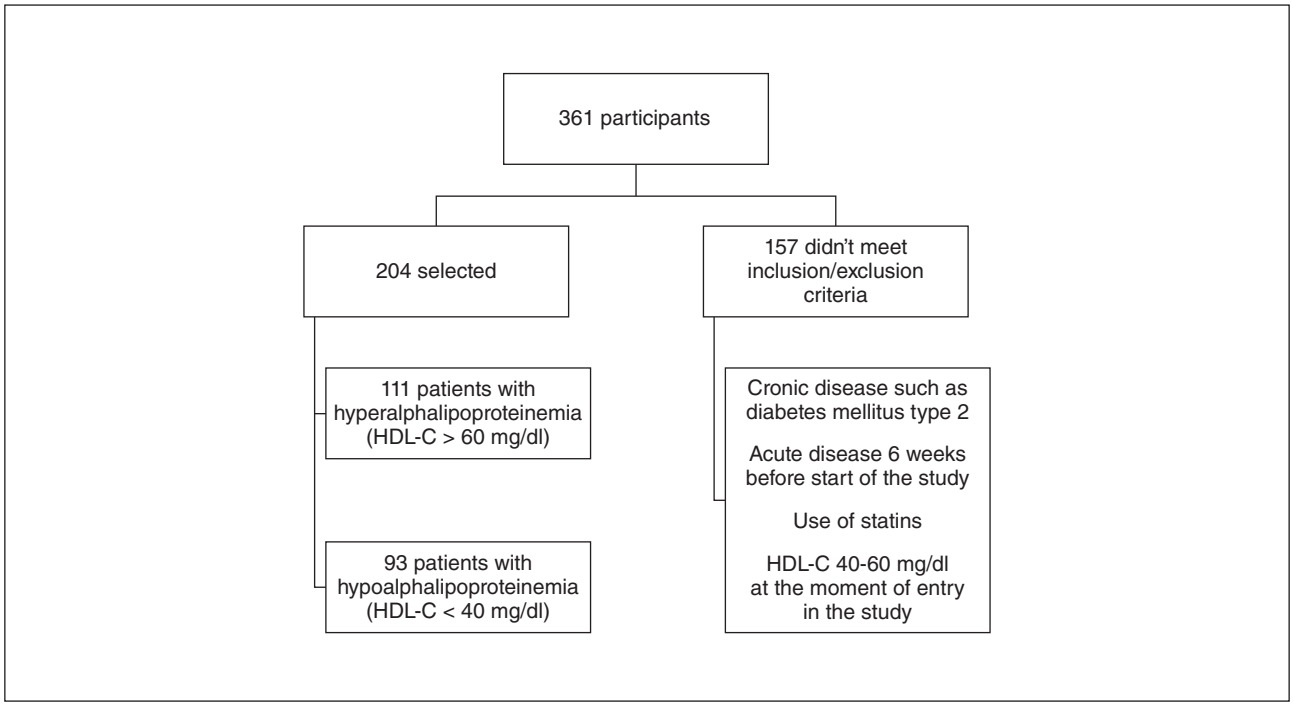

Figure 1. Selection of subjects.

Table 1. Characteristics of subjects

Variable	Women n = 149	Men n = 55	(p)
Age (years)	52 (39-61)	48 (35-62)	0.52
BMI (kg/m^2)	26.8 ± 4.7	27.13 ± 4.1	0.66
Waist circumference (cm)	86.9 ± 11.7	94.6 ± 11.1	**< 0.001**
Total cholesterol (mg/dl)	201.0 ± 40.4	184.7 ± 35.3	**0.008**
HDL-C (mg/dl)	67 (37.5-66.5)	36 (34-62)	**< 0.001**
– Hyperalpha/Hypoalpha	97/52	14/41	**< 0.001**
Non HDL-C (mg/dl)	141.5 ± 33.8	140.6 ± 34.15	0.86
Triglycerides (mg/dl)	109 (81.0-160.5)	89 (140-185)	0.09
LDL cholesterol (mg/dl)	114.0 ± 31.7	111.3 ± 27.13	0.58
Apo-A (mg/dl)	168 (135-200)	128 (113-153)	**< 0.001**
Apo-B (mg/dl)	80 (76.1-106.0)	97.4 (81.6-115.0)	0.13
Glucose (mg/dl)	93 (89-102)	97 (91-102)	0.12
Serum creatinine	0.63 (0.57-0.72)	0.88 (0.77-0.97)	**< 0.001**
SBP (mm/Hg)	110 (109-127)	120 (110-125)	0.141
DBP (mm/Hg)	76 (70-80)	80 (70-80)	0.09
cIMT (mm)	0.60 (0.50-0.65)	0.60 (0.50-0.70)	0.51

(n = sample numbers). Values are expressed as means ± standard deviations, or medians and interquartile ranges using Mann-Whitney, and Qui-Square for hyperalphalipoproteinemia versus hypoalphalipoproteinemia. Statistical differences are in bold.
BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL: low-density lipoprotein; Apo-A: apolipoprotein A; Apo-B: apolipoprotein B; cIMT: carotid artery intima media thickness.

standardized protocol was followed according to the international recommendations in force. Doppler ultrasounds from the patients were done by the same experienced radiologist, blinded to the clinical information. The cIMT was measured using a Siemens ultrasound with a lineal transducer of 10 MHz. The software used was PACS system v. 11.3.2 Carestream brand. The measurements were taken in a suitable room with dim light to achieve a better appreciation of details, with the patient in a supine position on a stretcher, neck extended and slightly rotated, with both arms next to the body. Both common carotid arteries of each patient were measured in a supine position, and the posterior wall of the common distal carotid artery was evaluated, one centimeter below the bifurcation[4]. To measure cIMT, an ultrasonic beam was directed towards a perpendicular axis in the carotid artery and amplified to clearly distinguish two lines, one corresponding to the intima-blood interface and the other one to the intima-adventitious interface. The cIMT was measured as the maximum distance (millimeters) between these two lines[15]. The parameter taken for disease was ≥ 1.0 mm, and validation was done by obtaining two measures and calculating the mean value.

An informed consent was signed by every participant. The study was performed in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the INCMNSZ.

Statistical analysis

Statistical evaluations were performed using SPSS software v. 21. A Kolmogorov-Smirnov normality test was carried out to determine the distribution of the data. Skewed values underwent logarithmic transformation. Sex- and age-adjusted data were used in most analyses. Group differences for parametric variables were determined using the students *t* test, and for non-parametric variables with the Mann Whitney U tests. The Chi-squared test was used by categorical variables. Partial Spearman correlation coefficients assessed the association between the parameters and cIMT. Stepwise linear regression analyses were performed using the mean cIMT as the dependent variable. The independent variables were: age, BMI, waist circumference, blood pressure (systolic and diastolic), total cholesterol, triglycerides, HDL cholesterol, non-HDL cholesterol, LDL cholesterol, apo-AI, apo-B, and diet composition (Kcal/day, carbohydrates, proteins, fat, and fiber in grams per day).

RESULTS

The sample was composed of 204 subjects: 149 women (73%) and 55 men (27%). The characteristics of all subjects are shown in tables 1 and 2. The study sample was composed mainly of middle-aged adults with a mean BMI of 27 kg/m^2.

The hyper HDL-C group included 97 women and 14 men. The hypo HDL-C group was composed of 52 women and 41 men (Table 2). The low HDL-C group was younger, heavier, and had lower total cholesterol concentrations compared to the hyper HDL-C patients. In addition, they had lower apo-AI concentrations. The remaining metabolic parameters were not statistically different between groups, after adjusting for age and gender. In relation with other cardiovascular risk factors, 55 (27%) of 204 patients had a diagnosis of hypertension, 30 in the hyper HDL-C group and 25 in the hypo HDL-C group. Former smokers (at least 100 cigarettes over the life of the participant) were more common in the hyper HDL-C group. As expected, we found more individuals with triglycerides ≥ 150 mg/dl in the hypo HDL-C group ($p < 0.001$).

The median value of the cIMT was 0.60 (0.50-0.70) in hyper HDL-C and 0.60 (0.55-0.72) in hypo HDL-C ($p = 0.366$). No significant differences were found in the value of cIMT when it was analyzed by gender.

Table 3 shows the correlation coefficients between cIMT and the clinical anthropometric and biochemical variables in each group. Age was strongly associated with cIMT in both groups. In addition, several variables correlated positively with cIMT: glucose, triglycerides, BMI, waist circumference, and systolic and diastolic blood pressure in the hyper HDL-C

Table 2. Clinical and biochemical characteristics of hyper HDL-cholesterol and hypo HDL-cholesterol by sex

	Hyperalphalipoproteinemia			Hypoalphalipoproteinemia			
Variable	Women n = 52	Men n = 41	(p)	Women n = 97	Men n = 14	(p)	p values between HDL-C groups n = 111, n = 93 (adjusted for age and weight by ANCOVA)
Age (years)	55 (44-62)	53 (33-71)	0.85	46.5 (34.2-58.75)	48 (36-60)	0.62	**< 0.001^t**
BMI (kg/m^2)	25.3 ± 4.3	24.4 ± 3,1	0.42	29.4 ± 4.3	28.0 ± 4.0	0.10	0.225^t
Waist circumference (cm)	83.2 ± 10.9	88.0 ± 9.4	0.98	93.9 ± 10.13	96.8 ± 10.9	0.187	0.439^t
Total cholesterol (mg/dl)	216.3 ± 34.8	202.0 ± 33.9	0.15	173.2 ± 35.13	178.0 ± 34.2	0.44	0.033
HDL-C (mg/dl)	72 (67.0-84.5)	70 (66.0-77.7)	0.33	35 (33-38)	35 (32.5-37.5)	0.86	Definition difference
Non HDL-C (mg/dl)	143.1 ± 33.7	129.7 ± 35.3	0.17	133.4 ± 33.9	144.3 ± 33.38	0.40	0.976^t
Triglycerides (mg/dl)	92 (72.5-127.0)	77.5 (55.7-126.7)	0.23	162 (114.7-223.2)	152 (108.5-195.5)	0.46	< 0.001
LDL cholesterol (mg/dl)	121.4 ± 30.2	110.8 ± 28.9	0.22	100.2 ± 29.9	111.5 ± 26.8	0.62	< 0.001
Apo-A (mg/dl)	192 (169-207)	174 (161.0-194.2)	0.06	125 (112.2-138.5)	119 (109-132)	0.28	< 0.001
Apo-B (mg/dl)	90 (76.1-106.0)	87.5 (73.8-108.2)	0.62	90 (75.4-106.0)	102 (82.5-121.0)	0.71	0.331
Glucose (mg/dl)	93 (88.0-101.5)	95 (87.2-97.2)	0.77	93 (90.0-104.7)	100 (91.5-103.0)	0.40	**0.825^t**
Serum creatinine	0.66 (0.59-0.73)	0.82 (0.72-0.93)	**0.001**	0.60 (0.51-0.69)	0.88 (0.79-0.98)	< 0.001	**0.502^t**
SBP (mm/Hg)	110 (110-125)	113 (107.5-121.5)	0.776	110 (100-130)	120 (110.0-126.5)	0.94	**0.283^t**
DBP (mm/Hg)	78 (70-80)	78.5 (70-80)	0.59	72 (70-80)	80 (70-80)	0.13	**0.427^t**
Hypertension diagnosis*	25 (83.3%)	5 (16.7%)	0.521	11 (44.0%)	14 (56%)	0.23	**1.0**
Ex-smokers*	19 (76%)	6 (24%)	0.051	19 (46.3%)	22 (53.7%)	0.099	**< 0.001**
Familial CHD*	40 (81.6%)	9 (18.4%)	0.104	18 (46.2%)	21 (53.8%)	0.107	**0.751**
Triglycerides ≥ 150 mg/dl*	11 (84.6%)	2 (15.4%)	0.749	32 (60.4%)	21 (39.6%)	0.318	**< 0.001**
Total cholesterol ≥ 200 mg/dl*	61 (89.7%)	7 (10.3%)	0.355	12 (52.2%)	11 (47.8%)	0.677	**< 0.001**
Combined dyslipidemias*	11 (84.6%)	2 (15.4%)	0.749	9 (47.4%)	10 (52.6%)	0.400	**0.088**
Physical activity ≥ 150 min/week	40 (85.1%)	7 (14.9%)	0.647	23 (53.5%)	20 (46.5%)	0.538	**0.529**
cIMT	0.60 (0.50-0.70)	0.60 (0.50-0.70)	1.0	0.55 (0.50-0.65)	0.60 (0.50-0.65)	0.231	**0.366**
cIMT n = 111	0.60 (0.50-0.70)			**cIMT n = 93** 0.55 (0.50-0.65)			0.366

(n=sample numbers). Values are expressed as means ± standard deviations, or medians and interquartile ranges for females and males in each group. ***p*** values in last column show differences between hyperalphalipoproteinemia and hypoalphalipoproteinemia adjusted for age and sex by ANCOVA^t.
*Cardiovascular risk factors.
Statistical differences in bold.
BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol; LDL: low-density lipoprotein; Apo-A: apolipoprotein A; Apo-B: apolipoprotein B; CHD: coronary heart disease; cIMT: carotid artery intima media thickness.

group. In contrast, only the diastolic blood pressure correlated positively with cIMT. None of the dietary variables showed an association with cIMT. We also investigated the association between the cIMT and metabolic variables in both groups stratified by gender (Tables 4 and 5). The associations found in the hyper HDL-C group were replicated in women, but not in men. On the other hand, the association between diastolic blood pressure and cIMT was limited to men in the hypo HDL-C group.

No association was found between cIMT and apo-AI in the whole population and by study group (hyper HDL-C: r = 0.002, p=0.983; and hypo HDL-C: r = –0.04, p = 0.972). We found no significant correlation with cIMT and HDL-C among the groups

Table 3. Correlation coefficients of carotid artery intima media thickness with clinical, anthropometric, and biochemical parameters

Group	Variable	(r)	(p)
Hyperalphalipoproteinemia (> 60 mg/dl)	Age	0.499	**< 0.001**
	Glucose	0.216	**0.023**
	Triglycerides	0.258	**0.007**
	BMI	0.341	**< 0.001**
	WC	0.235	**0.013**
	SBP	0.280	**0.003**
	DBP	0.276	**0.003**
	Apo-A	0.002	0.983
	HDL-C	0.005	0.955
Hypoalphalipoproteinemia (< 40 mg/dl)	Age	0.463	**< 0.001**
	DBP	0.305	**0.003**
	Apo-A	–0.004	0.972
	HDL-C	0.055	0.603

Correlation coefficients, adjusted for weight and sex. Variables without correlation in both groups were serum creatinine, non high-density lipoprotein cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, apolipoprotein A, apolipoprotein B.
Statistical differences in bold.
HDL-C: high-density lipoprotein cholesterol; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; Apo-A: apolipoprotein A.

Table 4. Correlation coefficients of carotid artery intima media thickness with clinical, anthropometric, and biochemical parameters in hyper high-density lipoprotein cholesterol by sex

Group	Variable	(r)	(p)
Hyperalphalipoproteinemia (women)	Age	0.432	**< 0.001**
	Glucose	0.318	**0.002**
	Triglycerides	0.279	**0.006**
	BMI	0.278	**0.006**
	WC	0.214	**0.035**
	SBP	0.287	**0.004**
	DBP	0.315	**0.002**
	Apo-A	0.157	0.124
	HDL-C	0.178	0.080
Hyperalphalipoproteinemia (men)	Age	0.676	**0.008**
	Apo-A	0.224	**0.440**
	HDL-C	0.225	**0.439**

Correlation coefficients, adjusted for age.
Statistical differences in bold.
HDL-C: high-density lipoprotein cholesterol; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; Apo-A: apolipoprotein A.

(r = 0.005, p = 0.955 in hyper HDL-C and r = 0.055, p = 0.603 in hypo HDL-C).

A stepwise linear regression analysis (Table 6) was performed with mean cIMT as the dependent variable for the total population and subsequently for both groups. The variables considered in the models were age, glucose, triglycerides, total cholesterol, HDL-C, LDL-C, BMI, waist circumference, systolic blood pressure (SBP), and diastolic blood pressure (DBP). Only age ($p < 0.001$) and DBP ($p = 0.042$) were independently associated with cIMT ($r^2 = 0.22$; $p < 0.001$).

In the hyperalphalipoproteinemia group, including the same independent variables, again age

Table 5. Correlation coefficients of carotid artery intima media thickness with clinical, anthropometric, and biochemical parameters in hypo high-density lipoprotein cholesterol by sex

Group	Variable	(r)	(p)
Hypoalphalipoproteinemia (women)	Age	0.350	**0.011**
	Apo-A	0.102	0.471
	HDL-C	0.067	0.636
Hypoalphalipoproteinemia (men)	Age	0.478	**0.002**
	SBP	0.315	**0.045**
	Apo-A	-0.029	0.859
	HDL-C	-0.215	0.176

Correlation coefficients, adjusted for age.
Statistical differences in bold.
HDL-C: high-density lipoprotein cholesterol; SBP: systolic blood pressure; Apo-A: apolipoprotein A.

Table 6. Independent determinants of carotid artery intima media thickness in the study sample

Group	Independent variables	B (95% CI)	R^2 (%)	(p)
All subjects n = 204[1]	Age	0.44 (0.20-0.35)	0.22.	**< 0.001**
	DBP	0.12 (0.00-0.47)		**0.042**
Stratification by study group				
– Hyperalphalipoproteinemia (n = 111)[2]	Age	0.45 (0.17-0.36)	0.28	**< 0.001**
	DBP	0.12 (0.08-0.69)		**0.012**
	HDL-C	0.06 (–0.22-0.39)		0.584
	Apo-A	0.10 (–0.13-0.36)		0.353
– Hypoalphalipoproteinemia (n = 93)[3]	Age	0.37 (0.12-0.39)	0.20	**< 0.001**
	SBP	0.17 (–0.4-0.68)		0.088
	HDL-C	–0.11 (–0.80-0.27)		0.328
	Apo-A	0.10 (–0.18-0.51)		0.347
Stratification by gender				
– Women (n = 149)[4]	Age	0.38 (0.13-0-32)	23.3	**< 0.001**
	DBP	0.20 (0.09-0.62)		**0.008**
	HDL-C	–0.02 (–0.14-0.12)		0.872
	Apo-A	0.07 (–0.14-0.25)		0.57
– Men (n = 55)[5]	Age	0.52 (0.18-0.56)	29.3	**< 0.001**
	SBP	0.10 (–0.39-0.94)		0.417
	HDL-C	0.00 (–0.31-0.31)		0.999
	Apo-A	-0.13 (–0.61-0.32)		0.548

Independent variables and carotid artery intima media thickness were transformed logarithmically (log_{10}) for the several lineal regression models.
cIMT: carotid artery intima media thickness; DBP: diastolic blood pressure; HDL-C: high-density lipoprotein cholesterol
Models: [1]: model 1; [2]: model 2; [3]: model 3; [4]: model 4; [5]: model 5.

($p \leq 0.01$) and DBP ($p = 0.012$) r^2=0.289 were independently associated with cIMT. In the hypoalphalipoproteinemia group, only age was independently associated with cIMT. Neither HDL-C nor apo-AI reached statistical significance. The same was observed when the population was stratified by gender.

DISCUSSION

The purpose of this study was to measure the contribution of HDL-C to the atherogenic burden (assessed by cIMT) in homogenous sets of patients at the extremes of the HDL-C distribution curve, using

a case-control design. This approach was applied to avoid the confounding effect of the multiple variables associated with HDL-C. In our study sample, age and blood pressure were the only predictors of cIMT. No association was found between cIMT and HDL-C or apo-AI (its main apolipoprotein). Thus, this approach did not provide new evidence to support that HDL-C level is an independent predictor of atherosclerosis.

Solid observational studies have shown that HDL-C is a cardiovascular protection factor. It has been documented for over four decades that low HDL-C levels are an independent risk factor of CVD, even after adjusting for the confounding effect of various comorbidities[16,17,18]. However, several studies have shown that high HDL-C concentrations do not necessarily protect against coronary artery disease[19-22]. Conditions that may cause hyper HDL-C (i.e. nephrotic syndrome, glucocorticoid treatment, or hypothyroidism) are associated with a higher incidence of cardiovascular events. In addition, HDL-targeted drug therapy (i.e. niacin, cholesteryl ester transfer protein inhibitors) has failed to decrease the incidence of major cardiovascular events. The reasons for the controversial results are multiple. The HDL-C concentration is strongly associated with other metabolic, ethnic, and sociodemographic variables. The confounding effect of some of them (i.e. triglycerides, insulin sensitivity, or ethnicity) is remarkable and difficult to control using multivariate analyses.

The approach applied in this report is based in the contrasting phenotypic profile associated with the extremes of HDL-C concentrations. Hypo HDL-C is usually associated with the clinical features of the metabolic syndrome. In contrast, hyper HDL-C is usually seen in lean, active, premenopausal women. Our study design may eliminate the confounding effect of several features included in the hypo or hyper HDL states. A surrogate marker of atherosclerosis (cIMT) was used for this purpose. The clinical profile of the study groups was as expected. The hypoalphalipoproteinemia group was heavier and had higher triglycerides concentrations compared to the hyper HDL-C cases. Also, they were younger and had lower total cholesterol concentrations. Despite that, no difference in cIMT was found between the study groups. This finding is in agreement with previous reports[23-26]. Potential reasons for the negative results could be a relatively small sample size, the relatively healthy profile of the participants, and the lack of inclusion of cases with atherosclerotic events. Furthermore, it has been proposed that HDL functionality rather than HDL-C concentration is the main determinant of the HDL-related protection against atherosclerosis[27,28].

The study had several limitations. The cross-sectional design, a relatively small sample size, and the use of a surrogate marker of atherosclerosis are the main ones. Also, we did not measure all possible biomarkers of atherosclerosis, nor did we compare with patients that had an established diagnosis of coronary disease.

In conclusion, the assessment of the contribution of HDL-C to the atherosclerotic burden (assessed by cIMT, a surrogate marker) in patients with hypo- and hyperalphalipoproteinemia was not useful to confirm the protective effect of HDL-C against atherosclerosis.

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