ORIGINAL ARTICLE

CLINICAL-EPIDEMIOLOGICAL ANALYSIS OF HDL2 AND HDL3 SUBFRACTIONS IN ADULTS FROM MARACAIBO CITY, VENEZUELA

Sergia Linares[1,a,b], Valmore Bermúdez[2,b], Juan Salazar[1,c,d], Manuel Nava[1,e], Ángel Ortega[1,e], Luis Olivar[1,e], María Calvo[1,e], María Sofía Martínez[1,c], Alex Morales-Carrasco[3,c], Maricarmen Chacín[2,f], Joselyn Rojas[4,g]

[1] Centro de Investigaciones Endocrino-Metabólicas «Dr. Félix Gómez», Escuela de Medicina, Universidad del Zulia, Maracaibo, Venezuela.
[2] Universidad Simón Bolívar, Facultad de Ciencias de la Salud, Barranquilla, Colombia.
[3] Universidad Técnica de Ambato, Ambato, Ecuador.
[4] Pulmonary and Critical Care Medicine Department, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, Estados Unidos.
[a] Master in Nutrition; [b] Master in Human Metabolism, Doctor of Medical Sciences; [c] Medical Doctor; [d] Specialist in Internal Medicine; [e] Student of Human Medicine; [f] Master in Dermatology; [g] Master in Immunology.

ABSTRACT

Objective: To carry out a clinical-epidemiological analysis of high-density lipoprotein cholesterol subfractions (HDL-C) in adults from Maracaibo, Venezuela. **Materials and methods:** A descriptive and cross-sectional study of the database from the Metbolic Syndrome Prevalence in Maracaibo Study was carried out. HDL3 and HDL2 serum concentration, as well as the HDL2/HDL3 ratio, were determined in 359 individuals of both sexes, over 18 years of age. Values obtained were evaluated according to sociodemographic, clinical and biochemical characteristics. **Results:** Mean population age was 39.4 ± 15.2 years, and 51.5% were female. Differences in HDL-C subfraction levels were only observed in those subjects with low HDL-C levels. Women with hypertriglyceridemia showed significantly lower serum HDL3 and HDL2 concentrations than those with normal triglycerides (p=0.033), as well as a lower HDL3 level and HDL2 / HDL3 ratio in those with higher levels of ultra-sensitive C-reactive protein (us-CRP) (p<0.001). A significantly lower concentration of HDL2 was observed in men with some degree of hypertension (p=0.031), insulin resistance (p=0.050) and metabolic syndrome (p=0.003); while those with elevated us-CRP showed a lower concentration of HDL3 (p=0.011). **Conclusion:** HDL-C subfractions show varying clinical-epidemiological behavior in adults from Maracaibo. Lower serum levels are observed in men, differences only in those with low HDL-C; and no predominance of any subclass was observed according to sociodemographic, clinical and biochemical characteristics.

Keywords: Lipoproteins, HDL3; Lipoproteins, HDL2; Cholesterol, HDL; Risk Factors; Epidemiology (source: MeSH NLM).

Cite as: Linares S, Bermúdez V, Salazar J, Nava M, Ortega A, Olivar L, *et al*. Clinical-epidemiological analysis of hdl2 and hdl3 subfractions in adults from Maracaibo city, Venezuela. Rev Peru Med Exp Salud Publica. 2020;37(3):412-22. doi: https://doi.org/10.17843/rpmesp.2020.373.4787.

Correspondence to: Juan Salazar; Centro de Investigaciones Endocrino-Metabólicas «Dr. Félix Gómez», Escuela de Medicina, Universidad del Zulia. Maracaibo 4004, Venezuela; juanjsv18@hotmail.com

Received: 07/09/2019
Approved: 01/07/2020
Online: 25/08/2020

INTRODUCTION

High density lipoproteins (HDL) are macromolecular, pseudo-micellar complexes whose most well-known function is to transport cholesterol from peripheral tissues to the liver for metabolism and excretion, a process known as reverse cholesterol transport (RCT). These lipoproteins also have antithrombotic, anti-inflammatory, vasodilatory, immunosuppressive and antioxidant properties [(1)]. This has led to the consideration for years of HDL as protective molecules for cardiovascular disease (CVD), which is considered a global public health problem and the first cause of death worldwide; it caused 17.8 million deaths in 2017 [(2)]. In the population of Maracaibo, high prevalence of low levels of high-density lipoprotein cholesterol (HDL-C) has been previously observed [(3)]; however, the coronary risk seems to be lower than in other populations with lower frequency of this type of lipid alteration [(4)].

Although there is epidemiological evidence that HDL-C is an independent predictor of CVD, clinical pharmacological trials focused on its quantitative improvement have not demonstrated benefit in reducing cardiovascular events or mortality [5]. This paradoxical phenomenon has been justified due to the heterogeneity and complexity shown by these molecules, whose functionality is the fundamental characteristic in their role as cardiovascular "protector" [6]. In this regard, there seems to be a close relationship between the function and the subfractions or subspecies of HDL-C [7].

De Lalla *et al.* [8] described for the first time the distribution of HDL-C subfractions and identified two subclasses: HDL2, the least dense (1.063-1.125 g/mL) and richer in lipids; and HDL3, the most dense (1.125-1.21 g/mL) and relatively rich in proteins. HDL2 and HDL3 seem to have different functions. HDL3 has the ability to receive free cholesterol from the endothelium or macrophages, through the ATP-binding cassette transporter A-1 (ABCA- 1), increasing its size with the decrease of its density, thus transforming into HDL2, which is responsible for the RCT by binding to SR-B1 receptors. Therefore, the role of each subfraction in cardiometabolic health could be different [9].

Epidemiologic findings related to subfractions are diverse and controversial, that is why no international diagnostic and management guidelines for dyslipidemia currently recommend routine evaluation in patients with cardiovascular risk. However, several reports show the potential role that these subfractions would have as markers for HDL functionality, and the modulating effect that drugs would have on CVD [7,10].

In Latin America, especially in Venezuela, although low HDL-C is a frequent dyslipidemia, there are few or no reports describing the behavior of HDL subfractions or their relationship to other cardiovascular risk factors. Therefore, the aim of this study was to perform a clinical-epidemiological analysis of HDL2 and HDL3 subfractions in an adult population in the city of Maracaibo, Venezuela.

KEY MESSAGES

Motivation for the study: The epidemiological behavior of HDL-C subfractions and their relationship with other metabolic alterations in Latin American populations, especially in Venezuela, is unknown.

Main findings: The average of HDL-C subfractions was lower in men; differences were only evident in subjects with low HDL-C. Subfractions had variable behavior and lower levels were observed in men and women with clinical and metabolic disorders.

Implications: HDL-C subfractions represent laboratory parameters of potential utility in individuals with low HDL-C. Their measurement in this context would allow early identification of subjects with risk factors, especially in those with intermediate or low cardiovascular risk.

MATERIALS AND METHODS

Population and sample

The data for this investigation came from the "Estudio de Prevalencia de Síndrome Metabólico de la Ciudad de Maracaibo (EPSMM)", a cross-sectional, descriptive, analytical, multi-stage random sampling study. It included 2,230 adults of both sexes, residents of Maracaibo, and it was designed to identify and evaluate risk factors for metabolic syndrome and cardiovascular disease. The main study sample was calculated on the basis of census estimates from the National Institute of Statistics for the city of Maracaibo in 2007; it included a population of 1,428,043 inhabitants over 18 years; the calculated sample was 2,230 subjects selected randomly and stratified in the 18 parishes that make up the city. The protocol was described above [11].

A sub-analysis with data obtained from EPSMM was performed to study HDL subfractions. Due to the lack of resources to identify subfractions in all subjects with low HDL-C, 359 individuals were randomly selected from the database using the SPSS program's random number tool, maintaining an equitable distribution by sex, age groups and presence of low HDL-C. For this subsample of individuals, HDL subfraction data were collected and processed as part of the study in 2012.

Procedure

Evaluation of the subjects

Previously trained personnel completed the participants' medical record with information related to age, sex, race, employment and socioeconomic status, educational level, family, and pathological history of endocrine-metabolic or cardiovascular disease. Socioeconomic status was classified as, stratum I (upper class), stratum II (upper-middle class), stratum III (middle class), stratum IV (working class), and stratum V (extreme poverty). Educational level was classi-

 https://doi.org/10.17843/rpmesp.2020.373.4787

fied as, up to primary school (those who did not have any knowledge of reading or writing or those who completed primary education), secondary (all those who completed secondary education), and higher (all those who completed some degree of higher education).

Regarding psychobiological habits, smoking was categorized into 1) current smokers, 2) non-smokers, and 3) ex-smokers (at least one year after quitting). Alcohol consumption was defined as the intake of >1 g/day. Physical activity levels were determined using the long version of the International Physical Activity Questionnaire (IPAQ) and were classified into two large groups: 1) those individuals with MET's = 0 (none) and 2) those with MET's > 0 (some degree of physical activity). This last group was later divided into quintiles, resulting in the following classification: Q1 or very low (men: <296.9; women: <230.9), Q2 or low (men: 297.0-791.9; women: 231.0-445.499), Q3 or moderate (men: 792.0-1,532.3; women: 445.5-742.4), Q4 or high (men: 1,532.4- 2,879.9; women: 742.5-1,798.4), Q5 or very high (men: ≥2,880.0; women: ≥1798.5).

Clinical evaluation

A calibrated and validated sphygmomanometer was used to measure the participants' blood pressure. The criteria proposed in the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) were used to classify the population into normotensive, prehypertensive, and hypertensive [12].

In addition, an anthropometric evaluation was carried out to determine weight, by using a digital scale (Tanita, TBF-310 GS Body Composition Analyzer, Tokyo-Japan); and height, with a calibrated measuring rod. The body mass index (BMI) was calculated using Quetelec's formula [weight/height^2] and the subjects were classified according to the criteria from the World Health Organization (WHO): underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), obesity (≥30.0 kg/m^2) [13]. The abdominal circumference was measured using a metric measuring tape, taking as an anatomical reference an equidistant point between the costal ridge and the anterior superior iliac crest. The values for abdominal obesity were ≥90 cm for men and ≥80 cm for women according to the criteria of the IDF/NHLBIAHA/WHF/IAS/IASO consensus [14], as well as the diagnosis of metabolic syndrome (MS).

Laboratory analysis

After 8 hours of fasting, serum glucose, total cholesterol and triacylglyceride levels were calculated using commercial enzymatic-colorimetric kits (Human Gesellshoft Biochemica and Diagnostica MBH, Hessen, Germany) and specialized computerized equipment. LDL-C levels were calculated using Friedewald's formula. Serum levels of ultra-sensitive C-reactive protein (us-CRP) were quantified by immunoturbodimetric assays (Human Gesellshoft Biochemica and Diagnostica MBH, Hessen, Germany), using ≥0.765 mg/L as a cut-off point to define elevated levels [15]. The basal insulin concentration was determined using a commercial kit based on the ELISA method (DRG International, Inc., USA, New Jersey), with <1 mU/L as a detection limit. On the other hand, the lipoprotein values (Lp[a]) were estimated through the turbidimetric latex method (Human Gesellschaft für Biochemica and Diagnostica, Hessen, Germany). The cut-off point for considering elevated Lp(a) values was ≥30 mg/dL [16]. HOMA2-IR values to define insulin resistance (IR) were calculated with software (HOMA-Calculator v2.2.3) supplied by the Oxford Centre for Diabetes Endocrinology and Metabolism, available at http://www.dtu.ox.ac.uk/homacalculator/index.php. The cut-off point used for HOMA2-IR was 2.0, previously established for the study population [17].

HDL were isolated according to the sequential ultracentrifugation method, dialyzed against a 0.09M Tris - 0.08M boric acid - EDTA$Na_2$3Mm, pH=8.35 buffer solution (TBE). Then, polyacrylamide gel gradient electrophoresis under non-denaturing conditions was carried out, with protein markers calibrated for molecular diameter (thyroglobulin 17.0 nm; ferritin 12.2 nm; catalase 10.4 nm; lactate dehydrogenase 8.1 nm, and albumin 7.1 nm; Amersham Pharmacia Biotech, Buckimghamshire, United Kingdom). Total protein was quantified by a modification of Lowry's method, HDL protein bands were stained with Coomassie blue R-250 and the average diameter was determined by optical densitometry.

Estimation of the relative apolipoprotein content was carried out by polyacrylamide 4-21% gradient electrophoresis of HDL, staining the apolipoproteins with Coomassie blue R-250, then the gel was analyzed by optical densitometry. The results are expressed as the percentage that represents the area under the curve for each apolipoprotein according to the sum of the areas of the HDL apolipoproteins. The Quantolip® HDL (HDL2/HDL3) kit (Technoclone, Vienna, Austria) was used to isolate HDL subfractions. The HDL2/HDL3 index was also calculated to evaluate the relationship between these variables.

Statistical analysis

Nominal and ordinal variables are presented as absolute and relative frequencies. Association between qualitative

variables was determined using the Chi-square test, and the normality of the quantitative variables was evaluated with the Kolmogorov-Smirnov Z test. These variables were presented by means ± standard deviation (SD). When comparing averages between groups, the t-Student test was used to compare two groups and the one-factor variance analysis (ANOVA) for more than two groups. A value of $p < 0.05$ was considered significant, and a statistical analysis was performed with the SPSS 20.0 program (IBM, USA).

Ethical aspects

The ethics committee of the Centro de Investigaciones Endocrino-Metabólicas (CIEM) "Dr. Félix Gómez" of the Universidad del Zulia, Venezuela, approved the study and authorized the use of the database for this sub-analysis. All participants signed an informed consent form prior to any intervention, interrogation, and physical examination.

RESULTS

Characteristics of the population

Table 1 shows general characteristics of the studied sample, made up of 359 individuals, of which 51.5% (n = 185) were female. The average age of the population was 39.4 ± 15.2 years. Regarding HDL-C behavior according to sex (Figure 1), it was shown that concentration of this lipoprotein was significantly higher in women than men (47.2 ± 13.4 mg/dL vs. 40.1 ± 9.4 mg/dL; $p < 0.001$). Also, statistically significant differences were found in the concentration of HDL3 (women: 28.9 ± 11.9 mg/dL vs. men: 25.1 ± 8.6 mg/dL; $p = 0.004$) and HDL2 (women: 18.2 ± 9.2 mg/dL vs. men: 15.0 ± 8.3 mg/dL; $p = 0.001$).

HDL-C subfraction percentiles

Table 2 shows the percentile distribution of HDL-C subfractions by sex. The average HDL3 in women was 26.7 mg/dL (P_{25}-P_{75}: 19.9-36.9); 17.6 mg/dL (P_{25}-P_{75}: 10.7-24.6) for HDL2; and 0.66 (P_{25}-P_{75}: 0.35-1.02) for the HDL2/HDL3 ratio. While the HDL3 average in men was 24.8 mg/dL (P_{25}-P_{75}: 18.9-30.5); 13.8 mg/dL (P_{25}-P_{75}: 9.2-18.1) for HDL2; and 0.56 (P_{25}-P_{75}: 0.35-0.83) for the HDL2/HDL3 ratio.

HDL-C subfractions according to socio-demographic characteristics and habits

Table 3 shows the behavior of HDL2 and HDL3 in individuals with low HDL-C according to sociodemographic variables. Women from socioeconomic strata IV-V presented significantly lower values of HDL3 compared to those from

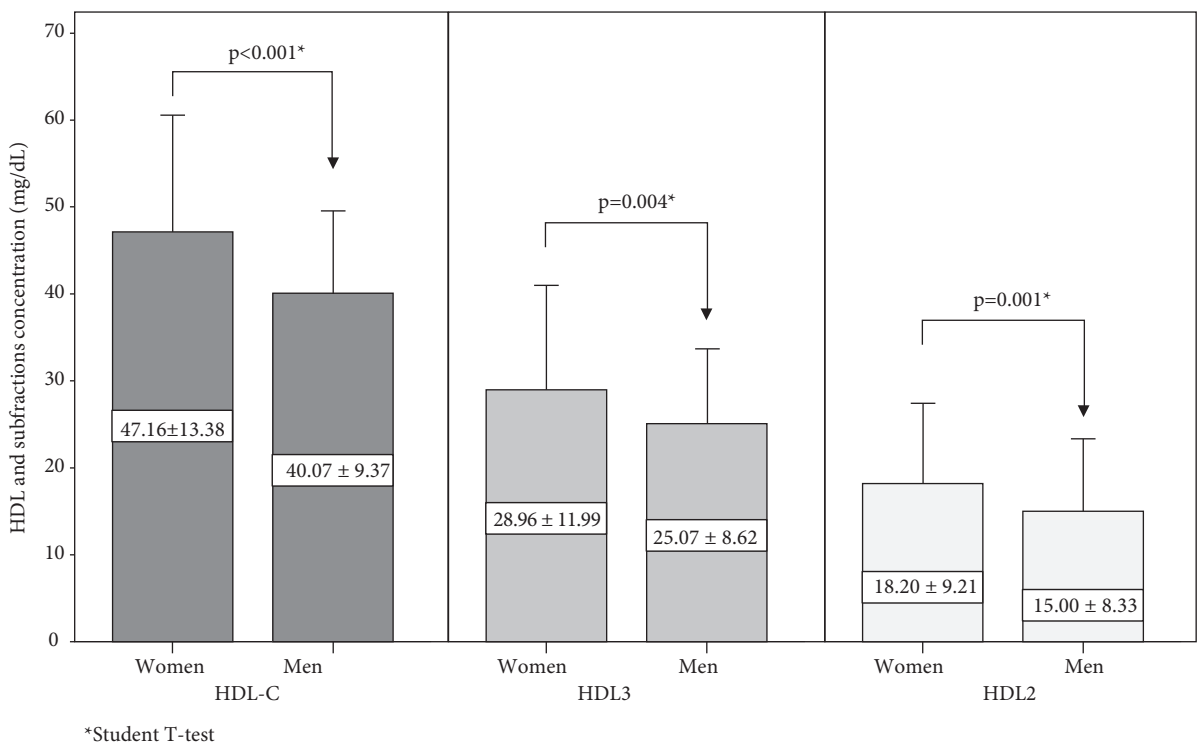

Figure 1. Epidemiological behavior of HDL-C concentration and subfractions according to sex

 https://doi.org/10.17843/rpmesp.2020.373.4787

Table 1. General characteristics of the studied population

Characteristics	Women (n = 185)		Men (n = 174)		Total (n = 359)	
	n	%	n	%	n	%
Age group (years)						
<30	54	29.2	67	38.5	121	33.7
30-49	71	38.4	63	36.2	134	37.3
≥50	60	32.4	44	25.3	104	29.0
Marital status						
Single	95	51.4	82	47.1	177	49.3
Married	90	48.6	92	52.9	182	50.7
Employment status						
Employed	81	43.8	135	77.6	216	60.2
Unemployed	104	56.2	39	22.4	143	39.8
Educational level						
Up to primary school	38	20.5	20	11.5	58	16.2
Secondary	78	42.2	88	50.6	166	46.2
Higher	69	37.3	66	37.9	135	37.6
Socioeconomic stratum						
I-II	34	18.4	35	20.1	69	19.2
III	87	47.0	77	44.3	164	45.7
IV-V	64	34.6	62	35.6	126	35.1
Race						
Mestizo	141	76.6	134	77.0	275	76.8
White-Hispanic	24	13.0	26	14.9	50	14.0
Afro-Venezuelan	3	1.6	5	2.9	8	2.2
American Indian	16	8.7	9	5.2	25	7.0
Alcohol consumption *						
Yes	33	17.8	100	57.5	133	37.0
No	152	82.2	74	42.5	226	63.0
Tobacco smoking habits						
No	153	82.7	111	63.8	264	73.5
Smoker	14	7.6	26	14.9	40	11.1
Ex-smoker	18	9.7	37	21.3	55	15.3
Leisure physical activity						
None	127	68.6	82	47.1	209	58.2
Extremely low	7	3.8	19	10.9	26	7.2
Low	19	10.3	15	8.6	34	9.5
Moderate	11	5.9	21	12.1	32	8.9
High	10	5.4	14	8.0	24	6.7
Extremely high	11	5.9	23	13.2	34	9.5
Low HDL-C						
No	86	46.5	84	48.3	170	47.4
Yes	99	53.5	90	51.7	189	52.6

AF: physical activity.
*Consumer of >1 g/day

Table 2. Percentiles of HDL subfractions in the population studied

Variable	p25	p33.3	Median	p66.6	p75	p95
Women (n = 185)						
HDL3 (mg/dL)	19.9	22.7	26.7	33.1	36.9	49.8
HDL2 (mg/dL)	10.7	13.0	17.6	20.8	24.6	36.0
HDL2/HDL3	0.35	0.43	0.66	0.86	1.02	1.89
Men (n = 174)						
HDL3 (mg/dL)	18.9	21.0	24.8	28.9	30.5	42.2
HDL2 (mg/dL)	9.2	10.9	13.8	16.1	18.1	33.6
HDL2/HDL3	0.35	0.41	0.56	0.74	0.83	2.47

p: percentile

higher socioeconomic strata ($p < 0.001$). Likewise, women from strata IV-V presented a higher HDL2/HDL3 ratio with respect to those from strata I-II (0.9 ± 0.5 vs. 0.7 ± 0.6, respectively; $p = 0.012$).

Regarding race, a higher concentration of HDL2 and a higher HDL2/HDL3 ratio were evidenced in American Indians compared to white-Hispanic individuals (HDL2: 17.8 ± 5.0 mg/dL vs. 9.8 ± 4.2 mg/dL; $p = 0.040$ and HDL2/HDL3: 1.1 ± 0.45 vs. 0.5 ± 0.3; $p = 0.007$, respectively). On the contrary, in men with low HDL-C, no significant differences were found in the subfraction averages with respect to the sociodemographic variables.

As for psychobiological habits, non-smoker women presented higher levels of HDL3 with respect to ex-smokers (22.4 ± 6.6 mg/dL vs. 17.1 ± 7.6 mg/dL, respectively, $p = 0.034$). On the contrary, men with low HDL-C only showed significant differences between the level of leisure physical activity and the HDL2/HDL3 ratio ($p = 0.038$), without observing important differences between the ratio values for each activity type.

HDL-C subfractions according to clinical-metabolic characteristics

Regarding clinical-metabolic alterations, it was observed (Table 4) that women with low HDL-C and hypertriglyceridemia showed significantly lower HDL3 and HDL2 serum concentrations compared to those with normal triglycerides (TG) ($p = 0.033$ and $p = 0.034$, respectively). Similar findings have been reported in women with elevated us-CRP, in whom lower HDL3 concentration and HDL2/HDL3 ratio were observed, compared to those with normal us-CRP ($p < 0.001$ and $p = 0.011$, respectively). In men with hypertension a significantly lower concentration of HDL2 ($p = 0.031$), HDL3 ($p = 0.028$) and ratio HDL2/HDL3 ($p = 0.028$) was evidenced. Likewise, these individuals showed a significantly lower concentration of HDL2 in the presence of IR ($p = 0.050$) and MS ($p = 0.003$) and men with elevated us-CRP also showed a lower concentration of HDL3 ($p = 0.011$).

DISCUSSION

HDL are molecules that can exist in multiple isoforms, and heterogeneity is one of their main properties. Although the HDL-C concentration has been inversely correlated to the risk of CVD and atherosclerosis, heterogeneity confers additional effects to its anti-atherogenic properties, which are attributed to the various molecules are part of the HDL-C [6].

For decades it has been suggested that HDL subfractions could be more directly related to the occurrence of cardiovascular events; however, despite positive results, there is no conclusive evidence about which subclass is the most important in this context [10,18,19]. This probably influences the absence of international recommendations for using these biochemical parameters. In fact, HDL subfractions are not mentioned in the most recent North American or European guidelines for diagnosis and management of dyslipidemia.

Given this controversial scenario and the lack of reports evaluating HDL subclasses in Latin American populations, especially in Venezuela, this study describes the main clinical and epidemiological characteristics of HDL subfractions in the population of the city of Maracaibo, the second most important city in Venezuela in terms of population and economy. In fact, during the sample collection period, Maracaibo presented a high frequency of cardiovascular risk factors and deleterious psychobiological habits, such as sedentarism and high consumption of saturated fats [20,21].

https://doi.org/10.17843/rpmesp.2020.373.4787

Table 3. HDL subfractions according to sociodemographic characteristics and habits in subjects with low HDL-C

Characteristics	Women							Men						
	n	HDL2		HDL3		HDL2/HDL3		n	HDL2		HDL3		HDL2/HDL3	
		Mean (SD)	p value*	Mean (SD)	p value*	Mean (SD)	p value*		Mean (SD)	p value*	Mean (SD)	p value*	Mean (SD)	p value*
Age group (years)														
<30	27	15.7 (8.0)	0.801	21.9 (6.6)	0.978	0.9 (0.7)	0.899	34	11.6 (4.9)	0.414	22.6 (5.3)	0.092	0.6 (0.3)	0.160
30-49	40	14.4 (5.8)		21.6 (6.4)		0.8 (0.5)		35	13.1 (5.3)		20.3 (6.9)		0.8 (0.5)	
≥50	32	15.8 (6.6)		21.8 (7.1)		0.8 (0.5)		21	11.1 (3.9)		22.9 (5.5)		0.5 (0.3)	
Marital status														
Single	38	15.5 (7.3)	0.926	21.9 (6.8)	0.935	0.8 (0.6)	0.978	38	11.7 (4.7)	0.381	21.5 (6.1)	0.613	0.7 (0.5)	0.700
Married	38	14.9 (6.1)		21.7 (6.5)		0.8 (0.5)		36	12.4 (4.9)		22.1 (6.1)		0.6 (0.3)	
Employment status														
Employed	44	15.0 (6.2)	0.989	22.4 (6.7)	0.453	0.8 (0.5)	0.718	69	12.4 (4.9)	0.235	21.5 (5.9)	0.429	0.7 (0.4)	0.235
Unemployed	55	15.4 (7.0)		21.3 (6.6)		0.9 (0.6)		21	10.9 (4.6)		22.9 (6.7)		0.6 (0.4)	
Educational level														
Up to primary school	23	16.3 (5.1)	0.332	19.0 (5.8)	0.020	0.9 (0.5)	0.062	12	9.9 (3.9	0.052	21.3 (5.6)	0.986	0.5 (0.3)	0.254
Secondary	42	15.7 (7.8)		21.4 (6.1)		0.9 (0.6)		41	13.5 (5.1)		21.9 (6.7)		0.7 (0.4)	
Higher	34	13.9 (6.1)		24.2 (7.1)		0.7 (0.5)		37	11.2 (4.5)		21.8 (5.7)		0.6 (0.4)	
Socioeconomic stratum														
I-II	17	13.8 (8.3)	0.231	25.5 (6.6)	<0.001	0.7 (0.6)	0.012	23	12.4 (4.5)	0.170	21.22 (5.31)	0.585	0.7 (0.5)	0,204
III	44	14.9 (6.6)		23.0 (6.7)		0.8 (0.5)		35	11.0 (4.8)		22.69 (6.27)		0.6 (0.3)	
IV-V	38	16.2 (6.0)		18.7 (5.1)		0.9 (0.5)		32	13.0 (5.0)		21.22 (6.40)		0.7 (0.4)	
Race														
Mestizo	74	15.4 (6.9)	0.040	22.23 (6.6)	0.108	0.8 (0.5)	0.007	69	12.3 (4.6)	0.197	21.3 (5.8)	0.156	0.7 (0.4)	0,130
White-Hispanic	10	9.8 (4.2)		23.5 (7.5)		0.5 (0.3)		16	11.9 (6.1)		24.0 (6.8)		0.6 (0.4)	
Afro-Venezuelan	1	-		-		-		2	8.4 (8.5)		28.4 (4.4)		0.3 (0.4)	
American Indian	3	17.8 (5.0)		18.1 (4.7)		1.1 (0.5)		3	10.2 (1.8)		17.8 (2.7)		0.6 (0.1)	
Alcohol Consumption[§]														
Yes	13	12.7 (6.4)	0.125	24.2 (7.0)	0.171	0.6 (0.5)	0.082	47	12.1 (4.9)	0.904	22.0 (6.0)	0.614	0.61 (0.32)	0.897
No	86	15.6 (6.7)		21.4 (6.5)		0.9 (0.5)		43	12.1 (4.8)		21.5 (6.2)		0.67 (0.46)	
Tobacco smoking habits														
No	80	14.9 (6.6)	0.496	22.4 (6.6)	0.034	0.8 (0.6)	0.124	61	12.1 (5.3)	0.916	22.0 (6.5)	0.735	0.65 (0.43)	0.837
Smoker	11	14.9 (6.6)		20.5 (4.9)		0.8 (0.4)		12	12.2 (4.2)		20.2 (4.7)		0.64 (0.25)	
Ex-smoker	8	17.2 (4.6)		17.1 (7.6)		1.1 (0.5)		17	11.8 (3.8)		22.1 (5.4)		0.60 (0.34)	
Leisure physical activity														
None	67	15.0 (6.3)	0.245	21.1 (5.9)	0.571	0.8 (0.5)	0.326	45	11.6 (4.7)	0.142	22.5 (6.2)	0.077	0.6 (0.4)	0.038
Extremely low	3	7.5 (1.1)		23.9 (6.9)		0.3 (0.2)		11	14.2 (5.5)		19.2 (6.8)		0.8 (0.4)	
Low	12	16.4 (6.0)		22.5 (7.2)		0.9 (0.6)		6	13.2 (4.6)		19.2 (4.9)		0.7 (0.3)	
Moderate	8	14.5 (8.1)		26.7 (9.9)		0.8 (0.8)		9	9.8 (5.9)		26.7 (6.0)		0.4 (0.3)	
High	3	17.3 (6.8)		22.1 (6.5)		0.91 (0.7)		8	10.9 (3.3)		21.0 (3.1)		0.5 (0.2)	
Exceedingly high	6	18.9 (10.3)		19.6 (6.8)		1.2 (0.9)		11	13.9 (4.0)		19.7 (4.8)		0.8 (0.4)	

SD: standard deviation.
[§] Consumer >1 g/day.
*t-Student test to compare between two categories, or one-factor ANOVA test to compare between three or more categories. In patients with normal HDL, no significant differences were observed between group means.

Table 4. HDL Subfractions according to clinical-metabolic characteristics and sex of subjects with low HDL-C

Characteristics		HDL 2		HDL 3		HDL2/HDL3	
	n	Mean (SD)	p value*	Mean (SD)	p value*	Mean (SD)	p value*
Mujeres							
TG (mg/dL) †							
<150	75	16.2 (6.9)	0.033	22.5 (6.5)	0.034	0.8 (0.6)	0.608
≥150	24	12.3 (4.8)		19.4 (6.4)		0.7 (0.5)	
BMI (kg/m²)							
≤24,9	27	15.5 (7.9)	0.976	23.2 (6.0)	0.041	0.8 (0.6)	0.516
25-29,9	32	15.0 (5.8)		22.9 (6.5)		0.8 (0.5)	
≥30	40	15.2 (6.6)		19.9 (6.8)		0.9 (0.5)	
AC†							
Normal	18	14.7 (8.4)	0.448	23.7 (5.5)	0.110	0.7 (0.6)	0.203
Increased	81	15.3 (6.3)		21.4 (6.8)		0.8 (0.5)	
Hypertension classification							
Normal	36	15.3 (7.0)	0.186	22.5 (6.1)	0.237	0.8 (0.6)	0.116
Prehypertension	40	14.6 (6.7)		21.5 (6.7)		0.8 (0.5)	
Stage 1	16	14.2 (6.1)		22.7 (7.8)		0.7 (0.4)	
Stage 2	7	20.5 (4.8)		17.2 (5.0)		1.3 (0.6)	
IR¶							
Absent	55	16.2 (6.7)	0.090	21.5 (6.0)	0.773	0.9 (0.5)	0.181
Present	44	13.9 (6.5)		22.2 (7.3)		0.8 (0.6)	
us-CRP (mg/L)							
<0,765	38	14.2 (7.3)	0.342	25.8 (6.0)	<0.001	0.6 (0.5)	0.011
≥0,765	25	15.3 (6.4)		19.1 (5.0)		0.9 (0.5)	
Lp (a)							
<30	54	15.3 (6.9)	0.956	22.9 (7.2)	0.128	0.8 (0.6)	0.499
≥30	43	14.9 (6.2)		20.5 (5.7)		0.8 (0.5)	
MS†							
Absent	49	16.2 (6.9)	0.190	21.9 (6.2)	0.634	0.9 (0.6)	0.481
Present	50	14.2 (6.3)		21.6 (7.0)		0.8 (0.5)	
Men							
TG (mg/dL) †							
<150	49	12.8 (5.0)	0.188	21.9 (5.9)	0.712	0.7 (0.4)	0.450
≥150	41	11.3 (4.6)		21.6 (16.2)		0.6 (0.4)	
BMI (kg/m²)							
≤24.9	15	13.4 (3.9)	0.334	19.8 (4.7)	0.230	0.8 (0.4)	0.308
25-29.9	37	12.2 (4.9)		23.0 (6.3)		0.6 (0.3)	
≥30	38	11.5 (5.1)		21.4 (6.2)		0.6 (0.5)	
AC†							
Normal	19	13.5 (4.1)	0.119	20.1 (4.8)	0.261	0.7 (0.4)	0.110
Increased	71	11.7 (4.9)		22.2 (6.3)		0.6 (0.4)	
Hypertension classification							
Normal	33	13.0 (4.4)	0.031	20.7 (5.4)	0.028	0.7 (0.4)	0.028
Prehypertension	32	11.4 (4.3)		20.4 (5.8)		0.7 (0.5)	
Stage 1	18	12.9 (5.8)		24.4 (7.1)		0.6 (0.3)	
Stage 2	7	8.3 (4.9)		26.5 (3.3)		0.3 (0.2)	
IR¶							
Absent	45	12.8 (4.2)	0.050	21.7 (5.9)	0.991	0.7 (0.3)	0.171
Present	45	11.3 (5.3)		21.9 (6.3)		0.6 (0.4)	
us-CRP (mg/L)							
<0.765	58	11.2 (5.2)	0.472	23.2 (6.3)	0.011	0.6 (0.4)	0.103
≥0.765	19	11.9 (4.0)		18.9 (4.9)		0.7 (0.5)	
Lp (a)							
<30	44	10.7 (5.0)	0.082	22.9 (4.9)	0.167	0.5 (0.3)	0.064
≥30	31	12.8 (4.7)		21.5 (7.7)		0.7 (0.5)	
MS†							
Absent	31	13.7 (3.9)	0.003	20.1 (4.8)	0.122	0.8 (0.4)	0.011
Present	59	11.2 (5.1)		22.7 (6.5)		0.6 (0.4)	

TG: triacylglycerides; BMI: body mass index; AC: abdominal circumference; IR: insulin resistance; us-CRP: ultra-sensitive C-reactive protein; MS: metabolic syndrome.
† Criteria according to IDF/NHLBI/AHA consensus; ¶ Criteria according to EPSMM (HOMA2-IR≥2).
* t-Student test to compare between two categories, or single-factor ANOVA test to compare between three or more categories in patients with normal HDL; no significant differences between group means were observed.

https://doi.org/10.17843/rpmesp.2020.373.4787

It is important to show average values and dispersion measures for any epidemiological analysis of a laboratory measurement with scarce reports from a region or country. When comparing the HDL-C subfraction results from this study, with other populations, we found different figures. For example, in a European population, Kim *et al.* [22] found a higher HDL3 average (women: 49 ± 11 mg/dL and men: 39 ± 11 mg/dL), and the HDL2 average was higher in women (women: 14.7±7.8 mg/dL and men: 8.5±5.3 mg/dL). In a study by Koumaré *et al.* [23], the HDL3 average in Burkina Faso was the same for men and women (26.2 mg/dL), while the HDL2 average in men was 15.4 mg/dL, and 18.9 mg/dL in women, similar values to those obtained by this research.

It is important to mention that there are differences in the averages of HDL subfractions in subjects with low HDL-C. In relation to the sociodemographic data, American Indians presented higher levels of HDL2 and HDL2/HDL3 compared to white-Hispanic individuals; this data differs from the study carried out by Martin *et al.* [24], where there was no evident relation between ethnic groups and the different subfractions of HDL-C.

As for the clinical-metabolic characteristics, it has been described that dyslipidemias represent a frequent cardiovascular risk factor and that high levels of triglycerides frequently coexist with low HDL-C and alterations in the subfraction distribution [25]. Our findings show that the levels of both subfractions were significantly lower in women with hypertriglyceridemia, which differs from the results of Jia *et al.* [26], who observed that hyperlipidemic subjects presented lower levels of HDL2 but higher levels of HDL3 when compared with normolipidemic subjects. Similarly, Gou *et al.* [27] found that HDL2 levels were significantly lower in women with hypertriglyceridemia than in those with normal triglyceride levels, while the HDL3 average was higher in the latter group. Such differences could be explained by the method used for obtaining HDL-C subfractions and by the size of the sample; however, these results suggest an inverse relationship between hypertriglyceridemia and HDL2.

Also, low HDL-C levels have been associated with low-grade inflammation, IR, and visceral obesity. In this regard, the results obtained show decreased HDL3 levels in subjects with elevated us-CRP in both sexes, while those with MS and IR showed lower HDL2 levels in men. These results match with the findings of the ELSA-Brazil study, one of the few Latin American reports that shows characteristics of HDL-C subclasses in our region, in which the concentration of HDL-C and its subfractions were inversely associated with low grade inflammation, IR and MS [27]. Similarly, another Brazilian study that evaluated the impact of IR on the metabolism of different lipoproteins showed lower percentages of the largest subfractions (HDL2 and HDL3) in subjects with IR [28], these findings evidence the role that the structure and function of HDL-C would have as cardioprotective lipoproteins.

The molecular mechanisms involved in the inverse relationship between HDL-C subfractions and inflammatory mediators, such as us-CRP, have not yet been clarified. However, the lower HDL3 levels in subjects with elevated us-CRP could be interpreted as a smaller number of nascent lipoproteins available to mobilize and externalize cholesterol from endothelial foam cells, which would lead to the activation and accumulation of the triggers of the inflammatory phenomenon. Another Latin American study shows lower levels of HDL2 and HDL3 specifically in Peruvian diabetic subjects [29], an association that was not significant in our study.

The values observed in this study for the HDL2/HDL3 ratio only showed relation with the socioeconomic status, race, and us-CRP in women; while in men, relations were observed with physical activity, blood pressure and MS. In this regard, Moriyama *et al.* [30] carried out several investigations in Japanese population and showed that changes in the HDL2/HDL3 ratio correlate inversely with abdominal circumference and IR, and positively with healthy lifestyle habits, which is why it is used as a useful marker for MS and atherogenic conditions in that population.

Regarding the limitations to this study, the sample size does not allow the generalization of the results to the entire population; the cross-sectional design makes it impossible to establish relationships of causality or recommendation of clinical use of HDL-C subfractions, and the socioeconomic changes that affect the study population during recent years could influence the results shown.

In conclusion, HDL-C subfractions have variable clinical-epidemiological behavior in adult individuals of Maracaibo's population, lower averages in men, differences in socioeconomic levels only in those with low HDL-C, and no predominance of any subclass according to sociodemographic, clinical and biochemical characteristics. Therefore, it is suggested to deepen the study of these lipoproteins in the Latin American population, emphasizing their relationship with different disorders that lead to a higher cardiometabolic risk.

Authors' contributions: SL and VB conceived and designed the study. JS, JR, MC, and MM conducted the analyses. MN, LO, AO, AM, and MC contributed to the data acquisition, interpretation and writing of the manuscript. All authors contributed to the discussion, review, and approval of the final manuscript and assume responsibility for its contents.

Sources of funding: This study was funded by the Consejo de Desarrollo Científico, Humanístico y Tecnológico CONDES (CC-0437-10-21-09-10) and by Fundacite-Zulia (FZ-0058- 2007).

Conflicts of Interest: The authors have no conflict of interest to declare.

REFERENCES

1. Besler C, Lüscher T, Landmesser U. Molecular mechanisms of vascular effects of High-density lipoprotein: alterations in cardiovascular disease. EMBO Molecular Medicine. 2012;4(4):251-268. doi: 10.1002/emmm.201200224.
2. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392:1736–88. doi: 10.1016/S0140- 6736(18)32203-7.
3. Bermudez V, Salazar J, Rojas J, Martínez MS, Bello L, Añez R, *et al*. Prevalence, Lipid Abnormalities Combinations and Risk Factors Associated with Low HDL-C Levels in Maracaibo City, Venezuela. J J Commun Med. 2015;1(2):009.
4. Bermúdez V, Salazar J, Bello L, Rojas J, Añez R, Roque W, *et al*. Coronary Risk Estimation According to a Recalibrated Framingham-Wilson Score in the Maracaibo City Metabolic Syndrome Prevalence Study. The Journal for Cardiology. Photon 2014;107:160-170.
5. Salazar J, Cabrera M, Ramos E, Olivar L, Aguirre M, Rojas J, *et al*. HDL-C y riesgo de aterosclerosis. Diabetes Internacional. 2013;5(2):42-54.
6. Salazar J, Olivar LC, Ramos E, Chávez-Castillo M, Rojas J, Bermudez V. Dysfunctional High-Density Lipoprotein: An Innovative Target for Proteomics and Lipidomics. Cholesterol. 2015;2015:296417. doi: 10.1155/2015/296417.
7. Martin S, Jones S, Toth P. High-density lipoprotein subfractions: current views and clinical practice applications. Trends Endocrinol Metab. 2014;25(7):329-36. doi: 10.1016/j.tem.2014.05.005.
8. De Lalla OF, Gofman JW. Ultracentrifugal analysis of serum lipoproteins. Methods Biochem Anal.1954;1:459-478. doi: 10.1002/9780470110171.ch16.
9. Pirillo A, Norata G, Catapano A. High-Density Lipoprotein Subfractions - What the Clinicians Need to Know. Cardiology. 2013;124(2):116-125. doi: 10.1159/000346463.
10. Davidson M. The Battle of the HDL Subfractions. Cardiovasc Revasc Med. 2019;20(11):943-944. doi: 10.1016/j.carrev.2019.09.013.
11. Bermúdez V, Marcano R, Cano C, Arráiz N, Amell A, Cabrera M, *et al*. The Maracaibo City Metabolic Syndrome Prevalence Study: Design and Scope. Am J Ther. 2010;17(3):288-294. doi: 10.1097/MJT.0b013e3181c121bc.
12. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, *et al*. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. JAMA. 2003;289:2560-2571. doi: 10.1161/01. HYP.0000107251.49515.c2.
13. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser [Internet]. WHO; 2000 [cited on August 1, 2019]. Available at: https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/.
14. Alberti K, Eckecl R, Grundy S, Zimmer PZ, Cleeman JI, Donato KA, *et al*. Harmonizing the Metabolic Syndrome: A Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention: National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Circulation. 2009;120:1640-45. doi: 10.1161/CIRCULATIONAHA.109.192644.
15. Bermúdez V, Cabrera M, Mendoza L, Chávez M, Martínez M, Rojas J, *et al*. High-sensitivity c-reactive protein epidemiological behavior in adult individuals from Maracaibo, Venezuela. Revista Latinoamericana de Hipertensión. 2013;8(1):22-29.
16. Bermúdez V, Rojas J, Salazar J, Bello L, Áñez R, Toledo A, *et al*. Variations of Lipoprotein(a) Levels in the Metabolic Syndrome: A Report from the Maracaibo City Metabolic Syndrome Prevalence Study. J Diabetes Res. 2013;2013:416451. doi: 10.1155/2013/416451.
17. Bermúdez V, Rojas J, Martínez M, Apruzzese V, Chávez-Castillo M, Gonzalez R, *et al*. Epidemiologic Behavior and Estimation of an Optimal Cut-Off Point for Homeostasis Model Assessment-2 Insulin Resistance: A Report from a Venezuelan Population. Int Sch Res Notices. 2014;2014:616271. doi: 10.1155/2014/616271.
18. Williams P. Fifty-three year follow-up of coronary heart disease versus HDL2 and other lipoproteins in Gofman's Livermore Cohort. J Lipid Res. 2012;53(2):266-272. doi: 10.1194/jlr.M019356.
19. Chaudhary R, Kinderytė M, Chaudhary R, Sukhi A, Bliden K, Tantry U, *et al*. HDL3-C is A Marker of Coronary Artery Disease Severity and Inflammation in Patients on Statin Therapy. Cardiovasc Revasc Med. 2019;20:1001-6. doi: 10.1016/j.carrev.2018.12.019.
20. Bermudez V, Rojas J, Salazar J, Calvo MJ, Morillo J, Torres W, *et al*. The Maracaibo city metabolic syndrome prevalence study: primary results and agreement level of 3 diagnostic criteria. Revista Latinoamericana de Hipertensión. 2014;9(4):20-31.
21. Souki A, Sandoval M, Sánchez G, Andrade U, García-Rondón D, Cano C, *et al*. Intake of saturated fatty acids and sensitivity to insulin in obese young adults in Maracaibo. Revista Latinoamericana de Hipertensión. 2007;3(5):159-165.
22. Kim DS, Burt AA, Rosenthal EA, Ranchalis JE, Eintracht JF, Hatsukami TS, *et al*. HDL-3 is a superior predictor of carotid artery disease in a case-control cohort of 1725 participants. J Am Heart Assoc. 2014;3(3):e000902. doi: 10.1161/JAHA.114.000902.
23. Koumaré AT, Sakandé LP, Kabré E, Sondé I, Simporé J, Sakandé J. Reference ranges of cholesterol sub-fractions in random healthy adults in Ouagadougou, Burkina Faso. PLoS One. 2015;10(1):e011642. doi: 10.1371/journal.pone.0116420.
24. Martin SS, Khokhar AA, May HT, Kulkarni KR, Blaha MJ, Joshi PH, *et al*. HDL cholesterol subclasses, myocardial infarction, and mortality in secondary prevention: the Lipoprotein Investigators Collaborative. Eur Heart J. 2015;36(1):22-30. doi: 10.1093/eurheartj/ehu264.
25. Quintanilla-Cantú A, Peña-de-la-Sancha P, Flores-Castillo C, Mejía-Domínguez AM, Posadas-Sánchez R, Pérez-Hernández N, *et al*. Small HDL subclasses become cholesterol-poor during postprandial period after a fat diet intake in subjects with high triglyceridemia increases. Clin Chim Acta. 2017;464:98-105. doi: 10.1016/j.cca.2016.11.018.
26. Jia L, Fu M, Tian Y, Xu Y, Gou L, Tian H, *et al*. Alterations of high-density lipoprotein subclasses in hypercholesterolemia and combined

 https://doi.org/10.17843/rpmesp.2020.373.4787

hyperlipidemia. Int J Cardiol. 2007;120(3):331-7. doi: 10.1016/j.ijcard.2006.10.007.

27. Generoso G, Bensenor IM, Santos RD, Santos IS, Goulart AC, Jones SR, *et al.* Association between high-density lipoprotein subfractions and low-grade inflammation, insulin resistance, and metabolic syndrome components: The ELSA-Brasil study. J Clin Lipidol. 2018;12(5):1290-1297. doi: 10.1016/j.jacl.2018.05.003.
28. Cartolano FDC, Dias GD, Freitas MCPde, Figueiredo Neto AM, Damasceno NRT. Insulin Resistance Predicts Atherogenic Lipoprotein Profile in Nondiabetic Subjects. J Diabetes Res. 2017;2017:1018796. doi: 10.1155/2017/1018796.
29. Jo N, Garmendia F, Pando R. Sub-clases de lipoproteínas de alta densidad en diabéticos. Rev méd peru.1994;66(351):68-70.
30. Moriyama K, Takahashi E. HDL2/HDL3 ratio changes, metabolic syn- drome markers, and other factors in a Japanese population. J Atheroscler Thromb. 2016;23(6):704-712. doi: 10.5551/jat.32896.