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Reference values for high-density lipoprotein particle size and volume by dynamic light scattering in a Brazilian population sample and their relationships with metabolic parameters

F. Alexandre [a], V.H.S. Zago [a], N.B. Panzoldo [a], E.S. Parra [a], D.Z. Scherrer [a], F. Vendrame [a], V.S. Nunes [b], E.I.L. Gomes [a], P.D. Markato [c], E.R. Nakandakare [b], E.C.R. Quintão [b], E.C. de Faria [a,*]

[a] Department of Clinical Pathology, School of Medical Sciences, State University of Campinas-Unicamp, Campinas, Sao Paulo, Brazil
[b] Lipids Lab (LIM 10), Endocrinology and Metabolism Division of Clinical Hospital, Faculty of Medical Sciences of the University of São Paulo, Sao Paulo, Brazil
[c] Nanobiolab, School of Pharmaceutical Sciences of Riberão Preto, Universidade de São Paulo, Ribeirão Preto, Sao Paulo, Brazil

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ABSTRACT

Background: Current data indicate that the size of high-density lipoprotein (HDL) may be considered an important marker for cardiovascular disease risk. We established reference values of mean HDL size and volume in an asymptomatic representative Brazilian population sample ($n = 590$) and their associations with metabolic parameters by gender.
Methods: Size and volume were determined in HDL isolated from plasma by polyethyleneglycol precipitation of apoB-containing lipoproteins and measured using the dynamic light scattering (DLS) technique.
Results: Although the gender and age distributions agreed with other studies, the mean HDL size reference value was slightly lower than in some other populations. Both HDL size and volume were influenced by gender and varied according to age. HDL size was associated with age and HDL-C (total population); non- white ethnicity and CETP inversely (females); HDL-C and PLTP mass (males). On the other hand, HDL volume was determined only by HDL-C (total population and in both genders) and by PLTP mass (males).
Conclusions: The reference values for mean HDL size and volume using the DLS technique were established in an asymptomatic and representative Brazilian population sample, as well as their related metabolic factors. HDL-C was a major determinant of HDL size and volume, which were differently modulated in females and in males.

1. Introduction

Several epidemiological studies have demonstrated a strong, inverse and independent relationship between high-density lipoprotein (HDL-C) and atherosclerotic cardiovascular disease (CVD) [1–3].

HDL is a heterogeneous category of lipoproteins, consisting of a set of distinct subclasses of particles that vary in size, shape, density, surface charge, number and chemical composition. These physicochemical properties of HDL influence its metabolism and functionality through mechanisms involving lecithin-cholesterol acyl transferase, lipases and lipid transfer proteins [4].

Experimental evidence indicates that the cholesterol concentration of HDL is not the only atheroprotective determinant of the lipoprotein [5,6]. It has been proposed that a more precise characterization of the physicochemical properties of HDL particles may provide better information about the relationship between HDL-C and CVD risk [1–3], and even more information about the relationship between HDL and its atheroprotective functions [4,7]. Among these characteristics, increased HDL particle number and size have been inversely associated with CVD [5,6].

Epidemiologic studies described the mean HDL size measured by different methods. Some of them, using gradient gel electrophoresis, have reported that CVD patients have smaller HDL particles and that large HDL particles (subfractions HDL_{2a} and HDL_{2b}) may protect against the development of atherosclerosis [8,9]. Ion mobility studies have revealed that larger HDL particles were highly associated with CVD risk [10]. Studies using proton nuclear magnetic resonance (NMR) have reported that higher levels of small HDL particles were associated with more severe atherosclerosis and that the mean HDL size was inversely correlated with CVD [6,11].

Total HDL isolated from plasma or serum after apoB-containing lipoproteins precipitation can be analyzed using the dynamic light scattering (DLS) technique, which has been used for decades to determine the LDL particle size [12] and has been used more recently to quantify

* Corresponding author at: Department of Clinical Pathology, Faculty of Medical Sciences, University of Campinas-Unicamp, P.O. Box 6111, 13083-970, Campinas, São Paulo, Brazil. Tel.: +55 19 3521 7064; fax: +55 19 3521 9434.
E-mail addresses: cottadefaria@gmail.com, cotta@fcm.unicamp.br (E.C. de Faria).

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the mean HDL particle size [13,14]. DLS is simpler, faster and cheaper than other conventional methods.

The mean size represents an integrative measure of HDL heterogeneity. Lima et al. [13] was the first study to use the DLS method for HDL particle size determination; the authors obtained results similar to those described by other studies using NMR and gradient gel electrophoresis. O'Neal et al. [15] evaluated the comparison between DLS and the gradient gel electrophoresis technique in LDL particle size measurement and obtained similar results. Recently, more studies were performed using the DLS method to determine the HDL particle size [14,16]. HDL volume is another physicochemical measurement of HDL that can be determined based on size. We speculate that the HDL volume measurements could add information concerning modified HDL aggregation-dissociation [17] and its interactions during metabolic intravascular remodeling [4].

2. Methods

2.1. Study population

Among the individuals who spontaneously sought governmental primary care in the city of Campinas, SP, Brazil, from 2009 to 2012, we selected a random representative sample of 590 healthy individuals of both genders who identified themselves as white or non-white and who were between the ages of 18 and 70 years. The following exclusion criteria (determined after clinical examinations and laboratory test evaluations) were applied: dyslipidemia, diabetes mellitus, obesity, thyroid function disorders, liver, lung and/or kidney failure, high blood pressure, smoking, alcohol abuse, pregnancy, stress, excessive exercise, contraceptive use, hormonal replacement therapy and medication (any) use, including drugs that might interfere with lipid metabolism. The study was approved by the institutional ethics committee, and all patients provided signed, informed consent.

2.2. Laboratory analyses

Blood samples were collected after a 12-h overnight fasting and immediately centrifuged at 3000×*g* for 10 min. The following analyses were performed in an automatic chemical analyzer (Modular Evolution, Hitachi/Roche) using Roche Diagnostics® reagents: uric acid, alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, urea, glucose, total cholesterol (TC) (CHOD-PAP), triglycerides (TG) (GPO-PAP) and high-density lipoprotein cholesterol (HDL-C). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald equation [18]. Apolipoprotein B100 (apoB) and apolipoprotein A-I (apoAI) were determined by nephelometry in a BNII equipment and reagents from Dade-Boehringer. Thyroid stimulant hormone and free thyroxin were determined by electrochemiluminescence using the Elecsys System (Roche).

Plasma exogenous lecithin: cholesterol acyltransferase (LCAT) activity (nmol/mL/h) was determined using a recombinant HDL_3 [19] and endogenous activity (% CE) through the rate of esterification of ^{14}C-free cholesterol by LCAT in the subject's HDL [20].

Lipoprotein lipase (LPL) and hepatic lipase (HL) activities were measured in post-heparin plasma samples collected 15 min after the intravenous administration of heparin (100 U/kg body weight) on the basis of fatty acid released from a radiolabeled triolein emulsion as the substrate, and NaCl as LPL inhibitor [21].

Cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) activities were determined in plasma and serum, respectively, by exogenous radiometric assays [22,23] and PLTP mass was measured using an enzyme-linked immunosorbent assay, as described by Jauhiainen et al. [23]. The coefficients of variation were as LCAT (9.5%), LPL (10%), HL (9%), CETP (12%) and PLTP (14%) activities.

2.3. HDL particle size and volume analyses

2.3.1. HDL isolation

HDL was isolated through the precipitation of apoB-containing lipoproteins (VLDL and LDL) with polyethylene glycol (PEG) 8000 (Sigma-Aldrich) as previously reported [24]. Blood venous samples were collected after 12 h fasting, placed in tubes containing EDTA (1.5 mg/mL), and centrifuged (4 °C, 15 min, 1250×*g*) for plasma separation. To 200 μL of plasma, 200 μL of PEG 8000/glycine solution (400 g/L PEG in 0.2 mol/L glycine adjusted to pH 10 with NaOH) were added. The samples were homogenized for 30 s and centrifuged for 10 min (25 °C, 1800×*g*). After that, 200 μL of the supernatant containing the HDL subfraction was transferred to another tube.

Lipoprotein and protein gel electrophoresis were accomplished to confirm the purity of HDL samples after PEG precipitation, using SPIFE Lipoprotein Electrophoresis System (Helena Laboratories) following the manufacturer's instructions. In addition, apoAI levels was measured in 12 normolipidemic samples before and after PEG precipitation to determine, in quantitative terms, how much of HDL was present in the supernatant.

2.3.2. HDL size and volume analysis

The DLS technique was used to determine the mean diameter and volume of HDL particles, as earlier described [13] using the Nanotrac Particle Size Analyzer (Microtrac). After PEG8000 precipitation, HDL samples were transferred to another tube, homogenized with 600 μL of NaCl 10 mM, maintained at 25 °C in a heat block (Kacil) and immediately analyzed. The measurements were performed in triplicate, and each sample was analyzed 3 times with 30 s of running time. A 100 nm polymeric nanoparticle was used as the standard and a control sample obtained from the same individual plasma was used in all determinations. The intra-assay and inter-assay CVs were 0.32% and 0.41%, respectively.

The volume was also determined in the Nanotrac Particle Size Analyzer using the following equation: $MV = \Sigma Vidi/\Sigma Vi$, (where: M = mean volume; V = volume percent between sizes; d = size represented by center (geometric progression) between any 2 sizes and i = the intensity percent between sizes).

The DLS method was compared and correlated with the proton nuclear magnetic resonance (NMR) in 24 samples of HDL isolated by gradient ultracentrifugation as previously described [25]. The NMR analyses were performed using a 600-MHz proton NMR spectrometer (Agilent Inova) at the Brazilian National Biosciences Laboratory (LNBio/CNPEM), as previously described [26]. Additionally, the sizes of ($n = 32$) HDL isolated by gradient ultracentrifugation were compared and correlated with the ones of HDL obtained after the precipitation of plasma apoB-containing lipoproteins.

2.4. Statistics

We performed statistical analyses using SAS System for Windows, ver 9.2. The Kolmogorov–Smirnov test was used to determine the normality of the distributions and χ^2 and Mann–Whitney U tests were used to compare the genders. Kruskal–Wallis and ANOVA compared the age groups.

The reference distributions were analyzed by visually inspecting the frequency histograms. Nonparametric determinations of reference intervals were based on ranked data. Data are expressed as the mean (SD), median and reference intervals with 0.90 confidence limits. Gender and age were the partition criteria for subgrouping. Level of significance was assessed with regression models (ANOVA by ranks with transformation, followed by Tukey's test).

To determine the variation of HDL size and volume according to age, the total populations was categorized into three homogeneous age groups (18–30, 31–59 and ≥60 years). The correlations of apoAI levels

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