Plasma distribution of non-cholesterol sterols precursors of cholesterol synthesis and phytosterols depend on HDL concentration

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Abstract

Background

Because several sterols are carried by serum lipoproteins that are pivotal in cholesterol metabolism and atherosclerosis this study aimed at investigating their lipoprotein distribution in individuals differing according to HDL-C plasma levels.

Methods

Healthy participants were selected with plasma HDL-C concentrations < 40 mg/dL (Low HDL, n = 12), above 40 mg/dL and below 60 mg/dL (Controls, n = 10), or above 60 mg/dL (High HDL, n = 15).

Plasma lipoproteins were separated by sequential ultracentrifugation. [27-OHC]), noncholesterol sterols precursors of cholesterol synthesis (NCSPCS: desmosterol and lathosterol) and phytosterols (campesterol and sitosterol) were measured by GC-MS analysis. Differences between groups were compared by Kruskall-walys and Dunn's post-test with correction by Bonferroni.

Results

The groups were similar in about BMI, age, gender distribution and blood glucose, total cholesterol and LDL-C. The High HDL group had lower concentrations of triglycerides and VLDL-C compared to others. Percentage distribution of plasma 24-OHC and 27-OHC in lipoproteins did not differ among investigated groups, but plasma concentration of 24-OHC was lower in High HDL than in Low HDL (27-OHC significance difference was borderline). NCSPCS and phytosterols predominate in LDL, which carries approximately 50% of these molecules. Approximately 30% of desmosterol and lathosterol are present in HDL, the High HDL group carrying higher percent of both sterols than the Low HDL and Control groups. Less than 20% of NCSPCS are transported in VLDL. Concentration of plasma campesterol and sitosterol did not differ among groups. However, LDL sitosterol percent distribution is lower in High HDL than in Low HDL and Controls. Campesterol and sitosterol percent distributions are higher in High HDL than in the Low HDL group.

Conclusions

Elevated NCSPCS in the High HDL group suggests HDL facilitates the export of these sterols from cells but not the export of the cholesterol metabolite 24-OHC which is lower in the High HDL than in the Low HDL group. Percent campesterol and sitosterol elevated in HDL suggests phytosterols absorbed in the enterocytes are incorporated mostly into the nascent HDL.
Introduction
Noncholesterol sterols precursors of cholesterol synthesis (NCSPCS: desmosterol and lathosterol, and squalene) are carried by serum lipoproteins and reflect cholesterol synthesis rates, whereas plant sterols, campesterol and sitosterol, and cholestanol, a metabolite of cholesterol, reflect the efficiency of cholesterol absorption in normal and hyperlipidemic populations [1–3]. Furthermore, Nunes and collaborators demonstrated that individuals with high HDL-C plasma levels have greater plasma concentrations of cholesterol absorption markers (campesterol and β-sitosterol) and lower plasma concentration of lathosterol which is a marker of body cholesterol synthesis [4]. Oxysterols, in addition to contributing directly to hepatic cholesterol excretion through bile acid formation, also have anti-atherosclerotic effects contributing to eliminate the excess of cellular cholesterol via ABCA1 or passive diffusion. 24-hydroxycholesterol (24-OHC) and 27-hydroxycholesterol (27-OHC) are transported mainly in LDL and HDL. Babiker et al. investigated the distribution of 24-OHC, 27-OHC and 3β-hydroxy-5-cholestenoic acid in lipoprotein fractions and lipoprotein-free plasma of seven healthy non-smokers volunteers showing that 24-OHC and 27-OHC have similar pattern of distribution in lipoproteins (40% being present in LDL and 40–50% in HDL). The 27-OHC/cholesterol and 24-OHC/cholesterol ratios were higher in the HDL fraction, indicating that HDL may be important for the transport of these oxysterols [5]. In this regard, increased plasma 27-OHC/cholesterol ratio of individuals with low HDL-C when compared to those with high HDL-C, suggests cellular cholesterol excretion higher via this pathway, thus protecting cells from the cholesterol accumulation [6].

Due to the importance of HDL in the reverse cholesterol transport pathway the present study aimed at investigating the lipoprotein distribution of oxysterols, noncholesterol sterol precursors of cholesterol synthesis (NCSPCS) and phytostrols in individuals that differed according to HDL-C plasma levels.

Materials And Methods
Subjects
Volunteers of both genders were recruited from primary health care centers in Campinas (SP-Brazil) and Ambulatório de Dislipidemia do Serviço de Endocrinologia e Metabologia do Hospital das Clínicas
da Faculdade de Medicina da Universidade de São Paulo. The study was approved by the Research Ethics Committee of UNICAMP School of Medicine under nº 120/2007 and Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo under nº 149/7. All participants were informed of the objectives of the protocol and signed an informed written consent according to research protocols approved by the Ethics Committee of HCFMUSP and UNICAMP.

The study included nonsmokers, asymptomatic individuals with body mass index (BMI) lower than 30 kg/m², without regular use of any medications that interfere with lipid metabolism and daily intake of alcohol lower than 14 g, as previously described [7].

Subjects were selected with plasma HDL-C concentrations below 40 mg/dL (Low HDL, 7 males and 5 females), above 40 mg/dL and below 60 mg/dL (Control, 4 males and 6 females), or above 60 mg/dL (High HDL, 7 males and 8 females) corresponding to values below the 10th percentile and above the 90th percentile of the Brazilian population. The subjects’ ages were between 20–74 years and body mass index (BMI) below 30 kg/m². The exclusion criteria were obesity, diabetes mellitus, metabolic syndrome, thyroid function disorders, liver and kidney failures, smoking, alcohol abuse and use of medications that might interfere with the metabolism of cholesterol.

Study Procedures
Blood was drawn after 12 h fasting period into tubes containing ethylenediamine tetraacetic acid (EDTA) (10%). Plasma was immediately separated and added benzamidine 2 mM (5 µL/mL), gentamycin + cloranphenicol 15 mM (20 µL/mL), phenylmethyl sulfonyl fluoride 0,5 mM (0,5 µL/mL) and aprotinin 10 mg/mL (5 µL/mL) and butylated hydroxytoluene (BHT). Plasma lipoproteins very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL) and lipid free fraction (LFF) were separated by sequential ultracentrifugation using a Beckman Model L-8 ultracentrifuge 50 Ti rotor (Beckman Instruments, Palo Alto, CA, USA) [8]. Plasma and lipoprotein NCSPCS (desmosterol, lathosterol) and phytosterols (campesterol and sitosterol) were measured in samples (100 µL) added 5α-cholestane (1 µg) as the internal standard, hydrolyzed with KOH in ethanol (1 mol/l, 1 ml) at 60 °C (1 h) and extracted with hexane. Sterols were derivatized with a sylilating solution (pyridine and BSTFA (N,O-bis (trimethylsilyl) trifluoroacetamide) + 1% TMCS
Plasma and lipoproteins oxysterols (24-OHC and 27-OHC) were measured according S. Dzeletovic et al. (modified) [9]. Internal standard (100 ng of 24-hydroxycholesterol-d7 and 27- hydroxycholesterol-d7 in chloroform; Avanti Polar Lipids, Alabaster, USA) was added in samples and oxysterols measurements were performed after alkaline hydrolysis adding a mixture of 10 mL of absolute ethanol and 0.4 M of potassium hydroxide overnight, at room temperature. The pH was adjusted to 7 with phosphoric acid followed by 20 mL of chloroform and 6 mL of water. After vigorous shaking and centrifugation at 4°C, the aqueous phase was removed and the organic phase evaporated. The lipid extract was dissolved in toluene (1 mL). Oxysterols were separated from cholesterol by solid phase extraction. Briefly, the sample was applied into the column (Sigma-Aldrich Supelclean LC-Si SPE Tubes SUPELCO, Bellefonte, USA) previously conditioned with 8 mL of hexane. Cholesterol were eluted with 1.5% isopropanol in hexane (8 mL), and oxysterols were further eluted with 30% isopropanol in hexane (6 mL). Finally, the solvent was evaporated and samples were derivatized with 100 µL of pyridine and 100 µL of N,O-bis (trimethylsilyl) trifluoroacetamide with trimethylchlorosilane (BSTFA; Sigma-Aldrich, St. Louis, USA), for 1 h at 60°C. The derivatized sample (1 µL) was injected into a gas chromatograph coupled to a mass spectrometer (Shimadzu GCMS-QP2010, Kyoto, Japan) by automatic injector and analyzed in selected ion monitoring. The separation was performed on a Restek capillary column (100% dimethyl polysiloxane-RxiR − 1 ms. Cat. #13323), 30 m, internal diameter 0.25 mm, for 30 min, using helium as mobile phase, with constant linear velocity of 44.1 cm/sec. The oven started at 240°C with increment of 5°C/min, for 7 min up to 290°C. The mass spectrometer operated in impact electron mode at an ionization voltage of 70 eV with the temperature of the ion source at 300°C.

The quantification was performed comparing the peak areas of the standard curve and corrected for internal standards [10]. Plasma non-cholesterol sterols (µg) and oxysterols (ng) were expressed as ratio of plasma total cholesterol (mg).

**Statistical analysis**
The results were expressed as mean ± SD or the median (variation). Differences between groups were
compared by Kruskal Wallis \( p < 0.05 \) and Dunn's multiple comparison with correction by Bonferroni post hoc \( p < 0.017 \) was performed when appropriate. Different letters represent statistically significant in the post-test. Gender distribution were compared by Chi-squared test \( p < 0.05 \)

**Results**

Anthropometric data, glucose, lipids and plasma lipoproteins concentrations, characterizing the samples are presented in Table 1. Characterizing each group HDL-C plasma concentrations showed significant differences. Triglycerides and VLDL-C plasma levels are lower in the High HDL group when compared to the Low HDL and the Control group. Triglycerides were within the normal range (< 150 mg / dL) in all participants.

| Table 1. Anthropometric data, glucose, lipids and plasma lipoprotein concentrations. Results are expressed as the mean ± SD or median (variation) |
|--------------------------------------------------|
| **Low HDL** | **High HDL** | **Control** | **p** |
| n           | 12           | 15          | 10    |
| Gender (men/women) | 7/5         | 7/8          | 4/6    |
| Age (years)  | 46 ± 14       | 52 ± 14      | 47 ± 11 |
| BMI (kg/m²)  | 24 ± 2        | 22 ± 2       | 23 ± 3  |
| Glucose (mg/dL) | 89 ± 11      | 89 ± 7       | 89 ± 5  |
| Cholesterol (mg/dL) | 163 ± 28    | 198 ± 37     | 173 ± 44 |
| HDL-C (mg/dL)         | 34 (25–39) a | 74 (62–95) b | 46 (42–49) c | < 0.001 |
| LDL-C (mg/dL)          | 104 ± 17     | 108 ± 31     | 107 ± 39 |
| VLDL-C (mg/dL)         | 21 ± 6 a     | 14 ± 6 b     | 22 ± 9 a  | 0.009 |
| Triglycerides (mg/dL)  | 107 ± 30 a   | 71 ± 29 b    | 108 ± 44 a | 0.011 |

BMI (body mass index), VLDL-C (very-low-density lipoproteins cholesterol), LDL-C (low-density lipoproteins cholesterol), HDL-C (high-density lipoproteins cholesterol). Kruskal Wallis \( P < 0.05 \), Dunn's post-test with correction by Bonferroni. Different letters represent statistically significant in the post-test.

To eliminate the influence of cholesterol concentrations between individuals, as well as in their lipoproteins, plasma oxysterols were expressed in relation to cholesterol [11].

24-OHC plasma levels were higher in the Low HDL group \( p = 0.024 \) compared to High HDL, but the percentage distribution in lipoproteins did not differ between groups (Table 2). 27-OHC plasma levels and the percentage distribution in lipoproteins did not differ between groups (Table 3) \( p = 0.07 \).

| Table 2 |
|----------|
| 24-OHC (ng) to cholesterol (mg) ratio in plasma and its percentage distribution among lipoproteins. The results were expressed as the median (variation). |
|----------|
| n                  | Low HDL | High HDL | Control | **p** |
| Plasma (ng/mg)     | 12      | 15       | 10      |       |
| (46–198) a         | 75      | 52       | 63      | 0.024 |
| (34–100) b         |         |          | (9 -120) ab |
| Percentage distribution (%) |
| VLDL              | 14      | 10       | 20      |
| (9-19)            | 10 (6-39)| 6 (6-40)| |
| LDL               | 27      | 35       | 28      |
| (16-70)           | 35 (14-49)| 11 (14-54)| |
| HDL               | 32      | 35       | 38      |
| (11-66)           | 35 (16-68)| 15 (12-64)| |
| LFF               | 11      | 13       | 10      |
| (0-49)            | 13 (3-7)| 10 (6-31)| |

VLDL (very-low-density lipoproteins), LDL (low-density lipoproteins), HDL (high-density lipoproteins) and LFF (lipid free fraction). Kruskal Wallis \( P < 0.05 \), Dunn's post-test with correction by Bonferroni. Different letters represent statistically significant in the post-test.
Table 3
27-OHC (ng) to cholesterol (mg) ratio in plasma and its percentage distribution among lipoproteins. The results were expressed as the median (variation).

|            | Low HDL       | High HDL      | Control      | p   |
|------------|---------------|---------------|--------------|-----|
| Plasma (ng/mg) | 113 (54–141) | 86 (48–151)   | 123 (69–323) | 0.07|
| Percentage distribution (%) | | | | |
| VLDL       | 19 (14–47)    | 12 (0–26)     | 19 (0–35)    |     |
| LDL        | 36 (23–54)    | 36 (19–64)    | 41 (21–42)   |     |
| HDL        | 36 (30–50)    | 42 (28–64)    | 40 (26–60)   |     |
| LFF        | 0 (0–0)       | 0 (0–38)      | 0 (0–17)     |     |

VLDL (very-low-density lipoproteins), LDL (low-density lipoproteins), HDL (high-density lipoproteins) and LFF (lipoprotein free fraction). Kruskal Wallis (P < 0.05), Dunn's post-test with correction by Bonferroni. Different letters represent statistically significant in the post-test.

NCSPCS (desmosterol and lathosterol) and phytosterols (campesterol and sitosterol) plasma levels properly corrected cholesterol concentration (expressed as µg/mg cholesterol) did not differ among groups (Tables 4, 5, 6 and 7).

Percentage of NCSPCS and phytosterols are predominant in LDL, which carries about 50% of these molecules (Tables 4, 5, 6 and 7), but did not differ between groups. 30% of desmosterol and lathosterol are present in HDL, with the High HDL group having significantly higher percentage than the Low HDL and Control group (Tables 4 and 5). VLDL transports less than 20% of NCSPCS. Percent lathosterol was lower in the High HDL group than in the Low HDL and Control groups (Table 5). 30% of campesterol and sitosterol are present in the High HDL group being significantly higher than in the Low HDL and Control groups. Conversely, the sitosterol LDL distribution favored the Low HDL and Control groups (Tables 6 and 7).

Table 4. Desmosterol (ng) to cholesterol (mg) ratio in plasma and its percentage distribution among lipoproteins. The results were expressed as the median (variation).

|            | Low HDL       | High HDL      | Control      | p   |
|------------|---------------|---------------|--------------|-----|
| Plasma (µg/mg) | 0.770 (0.250–1.715) | 0.633 (0.053–1.431) | 0.508 (0.209–1.321) |     |
| Percentage distribution (%) | | | | |
| VLDL       | 17 (9–39)     | 12 (0–38)     | 15 (3–23)    |     |
| LDL        | 54 (24–77)    | 55 (22–92)    | 64 (43–77)   |     |
| HDL        | 26 (7–40)     | 33 (8–64)     | 24 (14–40)   | 0.039|
| LFF        | 0 (0–0)       | 0 (0–38)      | 0 (0–17)     |     |

VLDL (very-low-density lipoproteins), LDL (low-density lipoproteins), HDL (high-density lipoproteins). Kruskal Wallis (P < 0.05), Dunn's post-test with correction by Bonferroni. Different letters represent statistically significant in the post-test.

Table 5. Lathosterol (ng) to cholesterol (mg) ratio in plasma and its percentage distribution among lipoproteins. The results were expressed as the median (variation).

|            | Low HDL       | High HDL      | Control      | p   |
|------------|---------------|---------------|--------------|-----|
| Plasma (µg/ mg) | 0.188 (0.043–0.467) | 0.159 (0.068–0.327) | 0.272 (0.082–0.534) |     |
| Percentage distribution (%) | | | | |
| VLDL       | 19 (11–55) a | 14 (4–37) b   | 18 (11–31) a, b | 0.041|
| LDL        | 56 (29–76)   | 56 (17–69)    | 62 (49–68)   |     |
| HDL        | 16 (5–56) a  | 29 (20–59) b  | 20 (11–38) a, b | 0.006|

VLDL (very-low-density lipoproteins), LDL (low-density lipoproteins), HDL (high-density lipoproteins). Kruskal Wallis (P < 0.05), Dunn's post-test with correction by Bonferroni. Different letters represent statistically significant in the post-test.
Table 6. Campesterol (ng) to cholesterol (mg) ratio in plasma and its percentage distribution among lipoproteins. The results were expressed as the median (variation).

|                  | Low HDL (µg/mg) | High HDL (µg/mg) | Control (µg/mg) | p     |
|------------------|-----------------|------------------|----------------|-------|
| Plasma n         | 12              | 15               | 10             |       |
| Percent distribution (%) |       |                  |                |       |
| VLDL             | 20 (12–50)      | 18 (3–33)        | 17 (8–35)      |       |
| LDL              | 58 (37–70) a    | 54 (22–61) b     | 56 (47–63)     |       |
| HDL              | 31 (9–43) a     | 39 (26–51) b     | 38 (16–44) a, b| 0.003 |

VLDL (very-low-density lipoproteins), LDL (low-density lipoproteins), HDL (high-density lipoproteins). Kruskal Wallis (P < 0.05), Dunn’s post-test with correction by Bonferroni. Different letters represent statistically significant in the post-test.

Table 7. Sitosterol (ng) to cholesterol (mg) ratio in plasma and its percentage distribution among lipoproteins. The results were expressed as the median (variation).

|                  | Low HDL (µg/mg) | High HDL (µg/mg) | Control (µg/mg) | p     |
|------------------|-----------------|------------------|----------------|-------|
| Plasma n         | 12              | 15               | 9              |       |
| Percent distribution (%) |       |                  |                |       |
| VLDL             | 15 (10–51)      | 10 (2–65)        | 15 (9–35)      |       |
| LDL              | 60 (34–71) a    | 47 (21–55) b     | 51 (36–65) a, b| 0.028 |
| HDL              | 23 (9–50) a     | 43 (11–67) b     | 33 (19–42) a, b| 0.002 |

VLDL (very-low-density lipoproteins), LDL (low-density lipoproteins), HDL (high-density lipoproteins). Kruskal Wallis (P < 0.05), Dunn’s post-test with correction by Bonferroni. Different letters represent statistically significant in the post-test.

Discussion

The percentage distribution of oxysterols among lipoproteins in High HDL, Low HDL and Controls was reported in healthy volunteers with similar plasma HDL-C [5, 12]. Our results agree with the study showing 24-OHC/cholesterol and 27-OHC/cholesterol ratios higher in the HDL fraction, indicating the importance of HDL for the transport of these oxysterols [5]. However, we found lower 24-OHC (0.024), (borderline 27-OHC, p = 0.07) plasma level in subjects with High HDL, which suggests that the increase in HDL does not provide greater efficiency for cellular export of cholesterol metabolites.

On the other hand, we found no percent differences between groups regarding the distribution of NCSPCS among lipoproteins. This is probably due to the fact that this distribution is likely dependent on other factors such as their esterification rate by the enzyme lecithin-cholesterol acyltransferase (LCAT) and transfer between lipoproteins mediated by cholesteryl ester transfer protein (CETP) [13].

In this regard LCAT activity is higher in individuals with low HDL-C plasma concentration and the CETP activity does not differ between individuals with low and high plasma HDL-C levels [14]. VLDL transports less than 20% of NCSPCS. Approximately 30% of desmosterol and lathosterol are present in HDL. The High HDL group carried higher NCSPCS percent than the other groups. Although the fraction of these sterols transported in LDL is the largest, we noted that participation of HDL increase suggesting its role in the cellular removal of cholesterol synthesis precursors.

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In this regard LCAT activity is higher in individuals with low HDL-C plasma concentration and the CETP activity does not differ between individuals with low and high plasma HDL-C levels [14]. VLDL transports less than 20% of NCSPCS. Approximately 30% of desmosterol and lathosterol are present in HDL. The High HDL group carried higher NCSPCS percent than the other groups. Although the fraction of these sterols transported in LDL is the largest, we noted that participation of HDL increase suggesting its role in the cellular removal of cholesterol synthesis precursors.
Similarly to cholesterol, phytosterols and phytostanols are carried in lipoproteins, being 70–80% in low density lipoprotein (LDL) and 20–30% in high density lipoprotein (HDL) [15, 16]. Simonem et al. (2007) showed in normal weight, good to moderate glucose balance, no insulin therapy, mild to moderate hypercholesterolemia, and normotriglyceridemia type 2 diabetics campesterol and sitosterol concentrations 7%-9% in VLDL, 3%-4% in IDL, 59%-61% in LDL, and 27%-30% in HDL [17]. We found similar distributions of phytosterols as shown by Björkhem et al. [15, 16] and Simonem et al.[17], however our High HDL-C group presented higher percent campesterol and sitosterol in HDL, suggesting that phytosterols absorbed in the enterocytes are incorporated into the nascent HDL.

Similar observations of higher plasma phytosterol levels have been made in patients with high HDL-C levels (matched for similar LDL-C levels) [4] and patients with high HDL-C levels due to exercise [18], although in the PROCAM study (Prospective Cardiovascular Münster), patients with low HDL-C levels displayed decreased plasma phytosterol and a direct correlation occurred between low HDL-C and decreased plasma phytosterol [19]. However, PROCAM dealt with metabolic syndrome, not with normal cases.

It is worth noting that the increase in phytosterols in cases of elevated HDL-C caused by the CETP inhibitor dalcetrapib is similar to that seen with statin treatment [20] and different from that measured in ABCG5/G8 mutation leading to atherogenic phytosterolemia [21]. Based on these results it was proposed that phytosterols not returned to the intestinal lumen via ABCG5/G8 activity are absorbed via chylomicrons with trace amounts absorbed via an HDL pathway and very likely efficiently excreted by the liver [22]. However, since measurable amounts of cholesterol are absorbed at the intestinal level through the ABCA1/ApoA1 efflux system [23] it has been hypothesized that the absorption of phytosterol - which cannot be synthetized by animals - via the HDL pathway could be used as a marker of intestinal ABCA1/ApoA1 activity [24]. Accordingly, Niesor E.J et al. showed that plant sterols, which offer the advantage of being strictly of dietary origin, are absorbed at the intestinal level via an HDL pathway [25], very likely due to ApoA1 lipidation with cholesterol, and related to pre-beta-1 HDL levels [26]. The original observation [25] was made both in hamsters and healthy human volunteers treated with the CETP modulator dalcetrapib, which affects HDL
metabolism in both species. High percent of HDL campesterol and sitosterol suggests phytosterols absorbed in the enterocytes mostly are incorporated into the nascent HDL and corroborates the hypothesis of Niesor EJ et al. [24] that phytosterol absorption via the HDL pathway could represent a marker of intestinal ABCA1/ApoA1 activity. An indirect proof of this concept is the lack of HDL-increase in plasma phytosterol on CETP inhibition with dalcetrapib in patients with mutations in ApoA1 and/or ABCA1 [27].

Conclusion
Elevated percentage HDL desmosterol and lathosterol in the High HDL group suggests HDL facilitates the export of these NCSPCS from cells but not the export of the cholesterol metabolite 24-OHC which was lower in the High HDL than in the Low HDL group. A high percentage of campesterol and sitosterol in HDL suggests that phytosterols are absorbed by enterocytes, incorporated mainly into nascent HDL and corroborates the hypothesis of Niesor EJ et al. [24] that the absorption of phytosterol via the HDL pathway could be used as a marker of the intestinal activity of ABCA1 / ApoA1.

Abbreviations
24-OHC
24-hydroxycholesterol
27-OHC
27-hydroxycholesterol
ABCA1
ATP Binding Cassette Subfamily A Member 1
ABCG5/G8
ATP-Cassette Binding Proteins G5/G8
ApoA1
Apolipoproteína A-I
BHT
butylated hydroxytoluene
BMI
body mass index
BSTFA
N,O-bis (trimethylsilyl) trifluoroacetamide
CETP
Cholesteryl Ester Transfer Protein
EDTA
ethylenediamine tetraacetic acid
HDL-C
High-Density Lipoproteins-Cholesterol
LCAT
Lecithin-Cholesterol Acyltransferase
LDL
low-density lipoproteins (LDL), high-density lipoproteins (HDL)
LFF
lipid free fraction
NCSPCS
Noncholesterol Sterols Precursors of Cholesterol Synthesis
PROCAM
Prospective Cardiovascular Münster
TMCS
trimethylchlorosilane
VLDL
very-low-density lipoproteins

Declarations

Ethics approval and consent to participate
This study was approved by the Research Ethics Committee of UNICAMP School of Medicine under nº 120/2007 and Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo under nº 149/7. All participants were informed of the objectives of the protocol and signed an informed written consent according to research protocols approved by the Ethics Committee of HCFMUSP and UNICAMP.

Consent for publication
All the authors have consented for the publication of this study.

Availability of data and materials
The data used to support the findings of this study are included within the article. Additional data or information can be requested by contacting the corresponding author.
Competing interests

The authors declare that they have no competing interests

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Authors' contributions

VSN: Conceptualization, Methodology, Funding acquisition, Writing - Review & Editing. EJS: Investigation, Writing - Original Draft. GSF: Formal analysis. SISA: Investigation. VHSZ: Data Curation. PMC: Investigation, Writing - Original Draft. ERN: Writing - Review & Editing. ECF: Writing - Review & Editing, Data Curation. ECRQ: Supervision, Writing - Review & Editing

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