Moderate hyperalphalipoproteinaemia in a Brazilian population is related to lipoprotein lipase activity, apolipoprotein A-I concentration, age and body mass index

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ABSTRACT

We investigated 95 Brazilian adults, aged 21–79 years, who were divided into two groups defined as having high-density lipoprotein (HDL)-cholesterol concentrations above [hyperalphalipoproteinaemia (HALP); $n = 48$] or below (controls; $n = 47$) the 90th percentile of a local population. The activities of lipid transfer proteins and enzymes involved in the plasma reverse cholesterol transport and the prevalence of factors that modulate HDL metabolism (alcohol consumption, ponderosity, physical exercise, menopause and use of hormone replacement treatment in women and smoking) were measured, as well as the prevalence of cardiovascular disease and of its various risk factors. The two groups showed no differences in their frequencies of cardiovascular disease. The HDL_2/HDL_3-cholesterol and triacylglycerol (triglyceride) ratios and the activities of the phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) were similar in both groups. Lipoprotein lipase (LPL) and hepatic lipase (HL) activities were 35% higher ($P = 0.0002$) and 40% lower ($P = 0.0006$) respectively, in HALP compared with control subjects. In a multivariate analysis, HDL-cholesterol and its subfractions were influenced by LPL, apolipoprotein A-I, age (negative relationship) and body mass index (negative relationship). Use of alcohol and ponderosity, as well as the interaction of these factors, explained the LPL activity. HL activity was modulated by smoking, and hormone-replacement therapy influenced the apolipoprotein A-I concentration. CETP activity was influenced by race and PLTP by age. The unique phenotype found in this Brazilian HALP population, namely low HL and high LPL activities, could be determined mostly by genetic components, on which future work will focus.

Key words: atherosclerotic cardiovascular disease, cholesteryl ester transfer protein (CETP), hyperalphalipoproteinaemia, hepatic lipase, lipoprotein lipase, phospholipid transfer protein.

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; CVD, cardiovascular disease; HALP, hyperalphalipoproteinaemia; HDL, high-density lipoprotein; HDL-C, HDL-cholesterol; HL, hepatic lipase; HRT, hormone-replacement therapy; Lp(a), lipoprotein a; LDL, low-density lipoprotein; LDL-C, LDL-cholesterol; LPL, lipoprotein lipase; NCEPIII, National Cholesterol Education Program-Adult Treatment Panel III; NEFA, non-esterified fatty acid; PLTP, phospholipid transfer protein; RCT, reverse cholesterol transport; TAG, triacylglycerol.

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INTRODUCTION

Numerous epidemiological studies demonstrate the inverse relationship between high-density lipoprotein (HDL)-cholesterol (HDL-C) and coronary heart disease (CHD) [1].

The participation of HDL in the reverse cholesterol transport (RCT), an efficient mechanism to dispose of excess cholesterol from tissues to the liver for excretion, may be one of its protective actions. RCT involves free cholesterol efflux from cell membranes via ATP-binding cassette transporter A1 (ABCA1) [2] and the scavenger receptor type B class 1 (SR-B1) receptor to nascent HDL [3], lipoproteins containing apolipoproteins (apos) A-I and A-II. Cholesterol esterification by lecithin:cholesterol acyl transferase (LCAT) and the transfer of cholesteryl ester to triacylglycerol (TAG; triglyceride)-rich lipoproteins by cholesteryl ester transfer protein (CETP) are subsequent steps of RCT. Hepatic lipase (HL) and phospholipid transfer protein (PLTP) are involved in the interconversion of the two main HDL subfractions, regenerating acceptor lipoproteins for cell cholesterol efflux [4].

Besides its participation in the RCT, HDL has several pleiotropic effects that are cardioprotective [5], including anti-oxidative [6], anti-inflammatory [6] and antithrombotic effects [6] and also play a role in the reduction of platelet aggregation [7], monocyte adhesion and smooth muscle cell proliferation [6].

HDL-C levels in plasma are modulated by environmental factors such as age, sex, race, ponderosity, diet and sedentariness, in addition to the genetic factors. Genetic studies in different populations indicate that the contribution of the genetic component to the variation of HDL is large, for example, 44 % [8], 83 % [9] and 65 % [10].

Hyperalphalipoproteinaemia (HALP) is a metabolic state that has been associated with primary and/or secondary causes. In different studies it has been associated more frequently with familial CETP [11] or HL [12] deficiencies. It has also been found in a family with an increased production rate of apoA-I [13] or in its polymorphism. Alcoholism [14], primary biliary cirrhosis and emphysema are also secondary causes for HALP, as well as several drugs, including corticosteroids, insulin, oestrogen, lipid-lowering agents, phenytoin and chlorinated hydrocarbons, which modulate HDL plasma levels [15].

The specific objectives of the present study were to investigate which parameters determined the moderate HALP phenotype and what factors influenced HDL metabolism in this selected group of adults. To find out if HALP patients were more resistant to atherosclerosis, the frequencies of cardiovascular disease (CVD) and risk factors for CHD were carefully evaluated. To better understand HDL metabolism in HALP, the relative association of lipoprotein lipase (LPL), CETP, HL, PLTP and apoA-I with HDL and its main subfractions was measured using a multiple linear regression analysis that hierarchically determines which environmental factors could modulate the activities of these proteins.

Table 1 Anthropometric and clinical characteristics of HALP subjects and controls

Values are means ± S.E.M. Values in parentheses indicate the number of subjects. BP, blood pressure. Statistical comparisons between HALP and controls by Mann–Whitney test: $^{*}P = 0.0294$ and χ^2.

	HALP subjects		Controls	
Parameters	Values	Range	Values	Range
Age (years)	49 ± 2* (48)	23–77	44 ± 2 (47)	21–79
Gender (male/female)	10/38	–	10/37	–
BMI (kg/m^2)	24 ± 1 (45)	16–37	25 ± 1 (46)	18–36
Waist (cm)	79 ± 2 (44)	58–117	82 ± 2 (47)	65–108
Systolic BP (mmHg)	130 ± 32 (45)	80–210	123 ± 21 (46)	100–170
Diastolic BP (mmHg)	84 ± 19 (45)	50–120	82 ± 17 (46)	60–100

MATERIALS AND METHODS

Experimental protocol

We investigated 95 adults, native to the urban areas in the State of São Paulo, Brazil, defined as having HDL-C concentrations $\geqslant 68$ mg/dl (HALP; $n = 48$) or below (controls; $n = 47$) the 90th percentile of a local population presenting to the Clinical Chemistry Laboratory of Campinas State University Hospital ($n = 1700$). These 95 individuals were chosen after a preliminary selection of healthy subjects and were invited by a letter to take part in this study. The characteristics of the HALP subjects and controls is shown in Table 1. The participants had their blood pressure and physical data measured during a complete clinical examination in which blood samples were drawn after fasting for the requested baseline tests. They also answered a detailed medical questionnaire aimed at determining the presence of CVDs as well as factors related to CHD, such as dyslipidaemia, hypertension, cigarette smoking, positive family history of CHD, frequency of alcohol consumption, physical exercise, use of hormone-replacement therapy (HRT) or contraceptive pills and other drugs such as statins, fibrates and phenytoin. The questionnaire included questions on the presence of angina pectoris, myocardial infarction, coronary insufficiency, the history of coronary revascularization procedures, percutaneous transluminal coronary angioplasty (PTCA), coronary artery grafting bypass (CABG) and the presence and history of cerebral and peripheral artery diseases. Patients with metabolic diseases were excluded from the study. The presence of menopause was assumed among women aged $\geqslant 51$ years, in accordance with the criteria of the North American

Menopause Society [16]. Patients with hypertension and clinical suggestion of ischaemia or arrythmia had their ECG taken. The two groups were selected to be matched by race, sex, body mass indexes (BMI) and waist circumference and were roughly matched for their diets after answering a questionnaire [17]. The participants were defined as being overweight if BMI $\geqslant 25$ kg/m^2 (ponderosity) and classified as dyslipidaemic according to the recent standards of the National Cholesterol Education Program-Adult Treatment Panel III (NCEPIII) [18], which assigns optimal levels to individuals in which low-density lipoprotein (LDL)-cholesterol (LDL-C) $\geqslant 100$ mg/dl and TAGs $\geqslant 150$ mg/dl. Their CHD risk scores were also based on NCEPIII guidelines.

Heparin (Liquemine®; Roche) was injected intravenously (100 international units/kg of body weight) as described for measurements of lipases [19], and the samples were collected 15 min later. The plasma was isolated by centrifugation at 1000 *g* at 4°C for 10 min.

The Ethical Committee of the School of Medicine of the State University of Campinas approved all procedures and all participants gave written informed consent to participate in the study. The research was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association.

Biochemical measurements

Plasma glucose, urea, uric acid, alanine aminotransferase and γ-glutamyltransferase were assayed in an automated system (Mega-Bayer) using enzymic methods (Merck). Thyroid-stimulating hormone (TSH) was measured by electrochemiluminescence, using a commercial kit in the Elecsys (Roche).

Lipids, apo and lipoprotein analysis

Total cholesterol and TAGs were determined using enzymic methods and reagents provided by Merck in an automated system (Mega-Bayer). Phospholipids were measured by enzymic colorimetric assays (Wako Chemicals). Plasma HDL-C was measured in the supernatant after precipitation of apoB100-containing lipoproteins. HDL_2 and HDL_3 were separated by sequential microultracentrifugation (Airfuge; Beckman). LDL-C was estimated by Friedewald's formula [20]. ApoA-I, B100 and lipoprotein a [Lp(a)] were measured by nephelometric assays in the Array 360 system (Beckman) and the Dade Boehringer system (Boehringer).

Determination of lipases and transfer proteins

LPL and HL activities were measured [19] in post-heparin plasma samples on the basis of fatty acid release, using a radiolabelled triolein emulsion as the substrate and NaCl (1 M) as the LPL inhibitor. The results were expressed as nmol of non-esterified fatty acid (NEFA) $\cdot$ ml^{-1} $\cdot$ h^{-1}.

The activity of CETP was measured by an exogenous assay [21] that measures the transfer of radiolabelled cholesteryl ester between a normal donor pool of [^{14}C]cholesteryl ester-HDL and an unlabelled acceptor mixture of very-LDL plus LDL, with plasma being the CETP source. The results were expressed as a percentage of cholesteryl ester transferred from HDL to very-LDL plus LDL in 4 h.

The activity of PLTP was measured by an exogenous radiometric method using phospholipid liposomes as the substrate [22] and a HDL pool, obtained from healthy plasma donors, as the acceptor. The activity was expressed as the rate of phospholipid transferred to HDL/h.

The assays for CETP, PLTP and lipases were carried out in triplicate. The inter-assay coefficients of variation were 12 %, 2 %, 9 % and 8 % for CETP, PLTP, LPL and HL respectively.

Statistics

The data were analysed by the SAS statistical package utilizing the following tests to measure differences between the groups: Mann–Whitney, χ^2 and Fisher's exact, all at the significance level of 5 %; levels between 5 and 10 % were considered marginally significant. TAGs, HL and LPL values were log-transformed. Spearman's test was used to correlate the variables in both groups. A hierarchical multiple linear regression analysis was used in HALP to assess the influence of specific plasma proteins on HDL-C concentrations (and its subfractions) and HDL-TAG subfractions. The results are expressed as coefficients of determination R^2 that represent the percentage of variation in the dependent variables explained by the independent variables. The independent predictors of HDL were: gender (women/men), age (all ages), BMI (all BMI), race (white/non-white), HL (log-transformed), LPL (log-transformed), CETP, PLTP and apoA-I. The dependent variables were HDL-C, HDL_2-C and HDL_2-TAG, and HDL_3-C and HDL_3-TAG. Several independent variables were tested sequentially for their influence on these proteins, including sex, age, race, BMI, use of alcohol, physical activity, smoking, menopause and use of HRT or of contraceptive pills.

RESULTS

Clinical and biochemical characteristics

Table 1 summarizes the clinical and biochemical characteristics of the participants. HALP subjects were older than controls ($P = 0.0294$), although the difference was not large. The HALP group was comprised of 38 females and 10 males, with an age range of 23–69 years and 38–77 years respectively. Their HDL-C levels ranged from 68–100 mg/dl. The control group was comprised of

Table 2 Lipids, lipoproteins and apos in HALP subjects and controls

Values are means ± S.E.M. Values in parentheses indicate the number of subjects. Statistical comparisons between HALP and controls by Mann–Whitney test: $^{*}P = 0.0002$ and $\dagger P = 0.0001$.

	HALP subjects		Controls	
Parameters	Values	Range	Values	Range
Total cholesterol (mg/dl)	232 ± 6* (48)	142–329	196 ± 6 (47)	127–306
LDL-C (mg/dl)	134 ± 6 (47)	59–223	126 ± 5 (47)	66–215
HDL-C (mg/dl)	81 ± 2† (48)	68–110	50 ± 1 (47)	34–66
Total TAGs (mg/dl)	86 ± 6 (48)	30–267	100 ± 6 (47)	31–200
Phospholipids (mg/dl)	256 ± 11 (32)	120–375	246 ± 10 (29)	110–342
ApoA-I (mg/dl)	186 ± 4† (46)	141–271	141 ± 3 (47)	53–184
ApoB100 (mg/dl)	98 ± 4 (46)	47–164	100 ± 4 (47)	54–187
Lp(a) (mg/dl)	30 ± 5 (43)	2–188	37 ± 5 (46)	2–109

37 females and 10 males, with age ranges from 29–68 years and 21–79 years respectively. Their HDL-C levels ranged from 34–66 mg/dl.

No differences were observed for blood pressure, waist circumference and BMI. The results of the ECGs (positive/negative for ischaemia) were similar in the two groups: 4/21 (HALP group) and 3/31 (control group). No differences were found either in the baseline tests measuring metabolic variables, such as thyroid, renal and liver functions, or in plasma glucose, and all values were within the reference ranges (controls compared with HALP group; values are means ± S.E.M.): glucose (mg/dl), 94 ± 5 ($n = 50$) compared with 95 ± 5 ($n = 53$); thyroid-stimulating hormone (international units/ml), 1.66 ± 0.5 ($n = 20$) compared with 1.47 ± 0.5 ($n = 20$); uric acid (mg/dl), 4.6 ± 0.2 ($n = 28$) compared with 5.0 ± 0.3 ($n = 33$); urea (mg/dl), 28 ± 1 ($n = 29$) compared with 29 ± 2 ($n = 29$); alanine aminotransferase (units/l), 15 ± 1 ($n = 28$) compared with 23 ± 6 ($n = 34$); and δ-glutamyltransferase (units/l), 27 ± 5 ($n = 28$) compared with 54 ± 11 ($n = 31$).

Lipids, apos and lipoproteins

Table 2 shows the concentrations of plasma lipids, lipoproteins and apos. Values for cholesterol, HDL-C and apoA-I were higher in the HALP group compared with controls by 16 %, 38 % and 24 % respectively, which was as expected due to the selection criteria of the cases, but the participants were classified as moderately hyperalphalipoproteinaemic. LDL-C, apoB-100 and phospholipids were similar in both groups. Triglyceridaemia showed a trend towards lower values (14 %) in the HALP group ($P = 0.0578$). Lp(a) levels were not different between the groups. When the lipid composition of HDL subfractions was analysed, there were striking differences between the groups for cholesterol in both HDL subfractions, but not for TAG. HDL_2-C/HDL_3-C and HDL_2-TAG/HDL_3-TAG ratios were similar in both groups, and no bimodal distributions were found.

Lipases and transfer proteins and their metabolic relationships

Fasting post-heparin plasma LPL was higher in HALP subjects by 35 %, but the activity of HL was 40 % lower when compared with controls (Table 3). When tested, the same differences in LPL and HL activities were observed in three subpopulations: (i) non-smokers (individuals not smoking for the past year; $P = 0.001$ and 0.027 respectively); (ii) non-alcoholic individuals (individuals who are not dependent on alcohol; $P = 0.0001$ and 0.0151 respectively); and (iii) sedentary individuals (individuals who do not exercise as recommended by the guidelines of the American Heart Association; $P = 0.004$ and 0.006 respectively) (results not shown).

Table 4 shows the associations between several parameters in HALP subjects and controls.

Prevalence of CVD and risk factors for CHD

The prevalence of hypertension was higher in HALP subjects ($P = 0.0418$; results not shown). Smoking was lower in HALP subjects and statistically different from controls ($P = 0.0031$). The distribution of risk factors was similar in the two groups. CVD was highly prevalent in both groups, but HALP apparently did not show cardioprotection. The frequency of dyslipidaemia in this population was very high, owing, in part, to the more strict

Table 3 Lipases and transfer proteins activities in HALP subjects and controls

Values are means ± S.E.M. Values in parentheses indicate the number of subjects. Statistical comparisons between HALP and controls by Mann–Whitney test: $^{*}P = 0.0002$; $\dagger P = 0.0006$.

	HALP subjects		Controls	
Parameters	Values	Range	Values	Range
LPL (nmol of NEFA · ml^{-1} · h^{-1})	5301 ± 317* (43)	1679–9131	3444 ± 255 (43)	555–7099
HL (nmol of NEFA · ml^{-1} · h^{-1})	1410 ± 103† (43)	218–3602	2370 ± 231 (43)	404–6848
CETP (%)	10 ± 1 (45)	2–20	11 ± 1 (37)	5–18
CETP/TAG (% per mg/dl)	0.14 ± 0.01 (45)	0.02–0.36	0.12 ± 0.09 (37)	0.04–0.29
PLTP (%)	11 ± 1 (44)	2–23	12 ± 1 (34)	0–21

Table 4 Univariate linear regression coefficients for apoA-I, lipases and transfer proteins' activities in HALP subjects and controls

Spearman coefficients: $^{*}P < 0.05$, $\dagger P < 0.01$, $\ddagger P < 0.005$, $\S P < 0.0005$ and $\|P < 0.0001$. NS, not significant.

Parameters	Groups	LPL	HL	PLTP	CETP	ApoA-I
Total cholesterol	HALP			NS		NS
	Control			− 0.37*		0.30*
Total TAGs	HALP		− 0.33*			NS
	Control		NS			0.34*
LDL-C	HALP			NS		
	Control			− 0.39*		
HDL-C	HALP	0.42‡				0.55‖
	Control	NS				0.58‡
HDL_2-C	HALP	0.40†				0.42‡
	Control	NS				0.50§
HDL_3-C	HALP					0.54§
	Control					0.48*
HDL_3-TAG	HALP		NS		0.31*	
	Control		− 0.33*		NS	
Phospholipids	HALP			0.37*		
	Control			NS		

NCEPIII diagnostic criteria used in this study. The use of alcoholic beverages was high in both groups and included beer, wine and *cachaça* (a sugar-cane-derived spirit).

Distribution of secondary factors that modulate plasma HDL

The frequency of physical activity was higher in HALP subjects than in controls ($P = 0.013$) and comprised walking, swimming, biking and anaerobic exercises (results not shown). Ponderosity, physical activity and smoking frequencies were comparable with those described previously by us [23] in a Brazilian population.

No differences were observed between the groups in the use of hormones and contraceptives.

Multiple linear regression analysis

In order to evaluate if some of the observed relationships in the univariate analysis are true and independent of each other, the effects of age, sex, race, BMI, LPL, HL, PLTP, CETP and apoA-I on HDL-C and TAG and its subfractions in HALP subjects were studied by multiple linear regression analysis. In this analysis HDL_2-C, HDL_3-C, HDL_2-TAG and HDL_3-TAG were used as dependent variables and each one of the five proteins above (LPL, HL, PLTP, CETP and apoA-I) was used as an independent variable.

The results in Table 5 show that, in HALP subjects, HDL-C was independently determined by apoA-I, LPL and BMI (negative relationship) by 56 %. HDL_2-C was independently correlated with LPL plus apoA-I by 33 %. Age (negative relationship) and apoA-I influenced 37 % of the variation in HDL_3-C. HDL_2-TAG was independently determined by race (white) by 10 %. HL did not influence any of the dependent variables.

Table 5 Influence of the regulator proteins on plasma HDL-C and its subfractions in HALP subjects

Independent variables: LPL, HL, PLTP, CETP, apoA-I, sex, age, race and BMI. The significant associations are shown. Negative refers to a negative relationship between the variables.

Models	Independent variables (*P* values)	*P* values (Model)	Cumulative R^2
For HDL-C ($n = 35$)	BMI (negative) (0.0415)		
	Log LPL (0.0279)		
	ApoA-I (0.0008)	0.0001	0.56
For HDL_2-C ($n = 35$)	Log LPL (0.0044)		
	ApoA-I (0.0252)	0.0016	0.33
For HDL_2-TAG ($n = 46$)	Race (0.0314)	0.0314	0.10
For HDL_3-C ($n = 35$)	Age (negative) (0.0586)		
	ApoA-I (0.0004)	0.0006	0.37

Table 6 Influence of secondary factors on plasma lipases and transfer proteins in HALP subjects

Independent variables: use of alcohol, ponderosity, physical activity, menopause, HRT, use of contraceptive pills, gender, race and age. The significant associations are shown. Negative refers to a negative relationship between the variables.

Models	Independent variables (*P* values)	*P* values (model)	Cumulative R^2
For LPL ($n = 33$)	BMI (0.2055)	0.0271	0.27
	Alcohol (0.0192)		
	BMI + alcohol (negative) (0.0100)		
For HL ($n = 33$)	Smoking (0.0235)	0.0235	0.14
For CETP ($n = 30$)	Race (non-white) (0.0473)	0.0473	0.13
For PLTP ($n = 28$)	Age (negative) (0.0438)	0.0438	0.15
For apoA-I ($n = 28$)	HRT (0.0397)	0.0397	0.15

Hierarchically, the dependent variables, which were significant in the regression models above, were tested for secondary and environmental factors that most strongly correlated with them as shown in Table 6: age, sex, race, BMI, use of alcohol, physical activity, menopause, HRT and use of contraceptive pills.

In HALP, LPL was determined by BMI (negative relationship), use of alcohol (positive relationship) and BMI plus use of alcohol (negative relationship) ($R^2 = 0.27$). CETP was independently determined by race (non-white; $R^2 = 0.13$) and PLTP by age (negative relationship; $R^2 = 0.15$). ApoA-I was independently determined by HRT ($R^2 = 0.15$). None of the other secondary modulators determined HL or the other regulator proteins, but smoking was associated with HL in HALP subjects only ($R^2 = 14$ %).

DISCUSSION

Intriguing data from population and clinical studies show that the status of high levels of HDL is not always protective [24] and that patients with HDL deficiency do not necessarily present with premature atherosclerosis [25]. In the present study, different distributions of risk factors for CHD, but similar final CHD risk scores, were found: HALP subjects presented HDL-C levels $\geqslant 68$ mg/dl (a negative risk factor), but a higher hypertension frequency and older ages (75th percentiles: 50 years for controls and 60 years for HALP subjects). The controls were younger, not hypertensive, but smoked more and had HDL-C levels < 68 mg/dl. It is interesting to note that the prevalence of dyslipidaemia was equally high in both groups. The metabolic state presented by HALP subjects was not anti-atherogenic, since the groups are similar in terms of the frequency of CVD manifestations.

We showed that LPL, but not HL, apoA-I or BMI, explained 58 % of the HDL-C variation seen in HALP subjects. In contrast, De Oliveira e Silva et al. [26] proposed that HL, apoA-I, apoA-II and TAGs explained 80 % of the variation in HDL-C. The multiple regression models proposed in the present study have shown that 33 % of the variation of HDL_2-C was determined by LPL and apoA-I and 37 % of HDL_3-C variation was determined by apoA-I and age.

Low CETP and HL activities are the main causes of HALP described so far [15]. The phenotype of HALP present in the current study was characterized by a decrease in HL and an increase in LPL activities and increases in HDL_2-C, HDL_3-C and apoA-I levels. No difference in the HDL_2/HDL_3-C ratio was observed between HALP subjects and controls in the present study, in contrast with the results described previously by Sich et al. [27]. Taskinen et al. [28] also described increased LPL activity in HALP patients. A modest increase in plasma HDL-C and LPL activity was described by Wittrup et al. [29] for the LPL mutation Ser^{447}stop carriers. Rader et al. [30] have also described an increased production of apoA-I in HALP, which was associated with elevated plasma levels of HDL and apoA-I. They concluded that a selective up-regulation of apoA-I production rate is one cause of HALP and possibly this phenotype confers a protection against atherosclerotic disease.

CETP and PLTP were not altered in HALP subjects. All were normotriglyceridaemic, but presented a trend to lower TAG values (14 %). This fact could reflect an increased lipolysis rate of TAG-rich lipoproteins in HALP due to the increased LPL activity occurring simultaneously with the reduced HL activity.

Among the environmental factors evaluated, a higher frequency of physical activity was found in HALP but, when a multiple regression model was proposed, physical activity had no effects on these HDL modulators.

Another factor that accounted for the variation of the regulator proteins was the use of HRT in women, which influenced apoA-I (15 %). HRT suppresses the activity of HL but not LPL. Walsh et al. [31] showed increases of 8 % and 16 % in HDL-C and apoA-I respectively, after HRT.

LPL was influenced by alcohol in HALP subjects. Alcohol was shown to increase HDL-C by 3.99 mg/dl when consumed at $30\ g \cdot dl^{-1} \cdot day^{-1}$ in a meta-analysis of 42 studies [32]. The possible mechanism to account for this is an increase in LPL activity and a decrease in CETP activity [33]. However, Hartung et al. [14] did not show changes in the activities of the lipases. BMI and its association (negative relationship) with alcohol contributed to increased LPL activity in HALP subjects (27 %). BMI has been described as negatively related to HDL-C, and this effect may be due to HL modulation [12].

CETP was influenced (13 %) by the non-white skin colour [34] and PLTP (15 %) by age (negative relationship) [35] and HL by smoking (14 %).

Genetic studies in families [9] showed that the contribution of the genetic components is much larger than the environmental ones for several plasma lipids, lipoproteins, apos and regulator proteins. The genetic contribution to HDL-C concentration reaches 80 % [8].

We believe that in this group of HALP subjects the simultaneous changes in the activities of the regulator proteins, decreased HL and increased LPL may lead to compensatory mechanisms. HL deficiency generates HDL particles that are relatively rich in TAGs, and are not as good substrates for CETP. On the other hand, the increased LPL activity favours the generation of pre-β-HDL and leads to an increased RCT.

In conclusion, taken together, these results show that, in the carriers of the HALP syndrome presented in this Brazilian population, there were no differences in CHD frequency and HDL-C was influenced by LPL, apoA-I and BMI. Genetic components were probably the primary causes of HDL modulation, and genetic studies should aim at characterizing this population better.

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