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Transfer of lipids to high-density lipoprotein (HDL) is altered in patients with familial hypercholesterolemia

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ABSTRACT

Objective. In familial hypercholesterolemia (FH), the metabolism and anti-atherogenic functions of HDL can be affected by the continuous interactions with excess LDL amounts. Here, lipid transfers to HDL, an important step for HDL intravascular metabolism and for HDL role in reverse cholesterol transport (RCT) were investigated in FH patients.

Methods. Seventy-one FH patients (39 ± 15 years, LDL-cholesterol = 274 ± 101; HDL-cholesterol = 50 ± 14 mg/dl) and 66 normolipidemic subjects (NL) (38 ± 11 years, LDL-cholesterol = 105 ± 27; HDL-cholesterol = 52 ± 12 mg/dl) were studied. In vitro, lipid transfers were evaluated by incubation of plasma samples (37 °C, 1 h) with a donor lipid nanoemulsion labeled with 3H-triglycerides (TG) and 14C-unesterified cholesterol (UC) or with 3H-cholesteryl ester (EC) and 14C-phospholipids (PL). Radioactivity was counted at the HDL fraction after chemical precipitation of apolipoprotein (apo) B-containing lipoproteins and the nanoemulsion. Data are % of total radioactivity measured in the HDL fraction.

Results. Transfer of UC to HDL was lower in FH than in NL (5.6 ± 2.1 vs 6.7 ± 2.0%, p = 0.0005) whereas TG (5.5 ± 3.1 vs 3.7 ± 0.9%, p = 0.018) and PL (20.9 ± 4.6 vs 18.2 ± 3.7 %, p = 0.023) transfers were higher in FH. EC transfer was equal. By multivariate analysis, transfers of all four lipids correlated with HDL-cholesterol and with apo A-I.

Conclusion. FH elicited marked changes in three of the four tested lipid transfers to HDL. The entry of UC into HDL for subsequent esterification is an important driving force for RCT and reduction of UC transfer to HDL was previously associated to precocious coronary heart disease. Therefore, in FH, HDL functions can be lessened, which can also contribute to atherogenesis.

Abbreviations: apo, apolipoprotein; CE, cholesteryl esters; CHD, coronary heart disease; FH, familial hypercholesterolemia; HDL-C, high-density lipoprotein-cholesterol; LCAT, lecithin-cholesterol-acyl-transferase; LDL-C, low-density lipoprotein-cholesterol; Lp(a), lipoprotein (a); PL, phospholipids; TC, total cholesterol; TG, triglycerides; UC, unesterified cholesterol.

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1. Introduction

Familial hypercholesterolemia (FH) is characterized by high concentrations of LDL-cholesterol (LDC-C) and premature coronary heart disease (CHD) [1]. In FH patients, LDL is slowly removed from the circulation, due to the LDL receptor defects. Since lipoprotein interacts with each other, these marked alterations in LDL catabolism may conceivably disturb the metabolism of HDL. Consequently, anti-atherogenic functions of HDL, such as cholesterol esterification and reverse cholesterol transport (RCT), may also be impaired [2,3].

HDL is continuously being remodeled and lipid transfers are an essential step to HDL metabolism and RCT [4]. Although the inverse correlation between HDL-cholesterol (HDL-C) and the incidence of CHD is well-known, the anti-atherogenic functions of HDL are only partially reflected by its concentration [5]. Thus, the acquisition of new HDL metabolism markers may contribute to the comprehension of the multifaceted functional aspects of this lipoprotein. Recently, an in vitro assay was validated, in which the simultaneous transfer of the four main lipoprotein lipids, unesterified cholesterol (UC), cholesteryl esters (CE), phospholipids (PL) and triglicerides (TG), from a donor lipid nanoemulsion to HDL was measured in plasma [6]. It was reported that the lipid transfers were changed with aging [7] and disturbed in patients with premature CHD [8] or with type 2 diabetes mellitus plus CHD [9] and in patients submitted to cardiac transplantation [10].

The aim of this study was to investigate lipid transfers to HDL in FH patients attempting to unravel disturbances of HDL metabolism and function consequent to accumulation of LDL in the plasma.

2. Material and methods

2.1. Study patients

Seventy-one adults with a clinical diagnosis of FH [11] and 66 age-matched normolipidemic individuals (LDL-C < 160 mg/dL) (NL) were evaluated at the Lipid Clinic Unit of the Heart Institute. The study protocol was approved by an institutional committee and informed consent was obtained from all patients. Exclusion criteria were previous atherosclerotic events, thyroid diseases, congestive heart failure, pregnancy, kidney diseases, and any current inflammatory disease. FH patients that were on statin therapy (n = 26, 37%) underwent a 6-week washout period before they were studied. Clinical and laboratory characteristics are shown in Table 1.

2.2. Laboratory methods

Blood samples were collected after 12-h overnight fasting. Serum total cholesterol (TC), TG, HDL-C and glucose levels were determined by enzymatic methods (Roche, Basel, Switzerland). LDC-C was calculated according to the Friedewald formula. Apo A-I, apo B, and lipoprotein (a) [Lp(a)] were measured by nephelometry (Dade Behring, Newark, NJ). The ratio of TG/HDL-C, a parameter associated with insulin resistance [12], was calculated.

Table 1 – Clinical and laboratory characteristics of patients with familial hypercholesterolemia (FH) patients and normolipidemic control subjects (NL).

	FH (n = 71)	NL (n = 66)	P
Age, years (ranges)	39 ± 15 (14–80)	38 ± 11 (15–53)	0.78
Male gender, n (%)	26 (36)	27 (40.9)	0.73
Body Mass Index (kg/m^2)	26 ± 5	24 ± 4	0.48
Waist (cm)	88 ± 12	87 ± 12	0.97
Smoking habit, n (%)	13 (18.3 %)	2 (8 %)	0.34
Hypertension, n (%)	8 (11.3 %)	0 (0 %)	0.11
SBP (mmHg)	119 ± 13	116 ± 9	0.75
DBP (mmHg)	76 ± 8	75 ± 6	0.69
Previous statin use, n (%)	14 (19.7 %)	0 (0%)	< 0.0001
Early Familial history of CHD	64 (90.1%)	3 (2%)	< 0.0001
Cholesterol (mg/dl)			
Total	355 ± 101	183 ± 35	< 0.0001
HDL	50 ± 14	52 ± 12	0.3949
LDL	274 ± 101	105 ± 27	< 0.001
Triglycerides (mg/dl)	134 ± 61	77 ± 27	< 0.001
Triglycerides/HDL-cholesterol	2.97 ± 1.88	1.90 ± 1.10	< 0.0001
Glucose (mg/dl)	94 ± 29	88 ± 11	0.14
Apolipoproteins (g/l)			
A-I	1.3 ± 0.3	1.5 ± 0.2	0.002
B	1.6 ± 0.5	0.8 ± 0.2	< 0.001
Lipoprotein(a) (mg/dl)	32 (1–185)	9 (1–127)	< 0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; CHD, coronary heart disease. Values are means ± SD. Values are means ± SD except for values of Lipoprotein(a), which are medians and range.

2.3. Lipid transfer from LDL-like nanoemulsion to HDL in vitro

The simultaneous transfer of radioactively labeled PL, TG, and UC and CE lipids from an artificial nanoemulsion to the HDL plasma fraction was measured in vitro, as described by Lo Prete et al. [6]. Briefly, the donor lipidic nanoemulsions containing 3H-cholesteryl oleate and 14C-phosphatidyl choline or 3H-triolein and 14C-cholesterol were incubated with plasma under agitation, during 1 h at 37 °C. After chemical precipitation of the nanoemulsion and the apo B lipoproteins, the supernatant containing the HDL fraction was counted for radioactivity in a scintillation solution for calculation of the % of each lipid transferred from the nanoemulsions to HDL.

2.4. Statistical analysis

Continuous variables are presented as mean ± standard deviation except for Lp(a) values that are as median (ranges). Data normality was tested by the Kolmogorov–Smirnov test. A two-tailed p value <0.05 was considered statistically significant. Continuous variables were compared by the Student t test or Mann–Whitney test for non-parametric variables. Categorical variables were analyzed by chi square, or Fisher's exact test if necessary. Initially, correlation among data was calculated by Pearson's or Spearman's test for non-parametric data. The correlation of lipid transfers with clinical and laboratory parameters was performed by multivariate analyses. Data were adjusted by gender, post-menopausal status,