Altered HDL remodeling and functionality in Familial Hypercholesterolemia

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Current research is focused on improving high-density lipoprotein (HDL) function rather than simply targeting to increase HDL-cholesterol (HDL-C) levels (1). The results of several recent studies indicate that stimulation of the HDL-dependent cholesterol efflux from macrophage-foam cells mediated by the ATP-binding cassette (ABC) A1 transporter, considered to be the major atheroprotective property of HDL, is inversely associated with the incidence of cardiovascular disease (CVD) events (1). Familial hypercholesterolemia (FH) is an inherited autosomal dominant disorder mainly caused by mutations in the low-density lipoprotein receptor (LDLR) gene and characterized by high low-density lipoprotein cholesterol (LDL-C) levels, which often are associated with low HDL-C levels (2). Several studies have reported that FH patients display qualitative abnormalities in the HDL particles that may be critical for HDL remodeling and macrophage cholesterol efflux (2). Furthermore, recent evidence indicates that hypercholesterolemia alters HDL lipidome and protein cargo in pigs, thereby impairing its ability to promote macrophage cholesterol efflux (3). The potential of HDL particles to be remodeled in non-treated FH patients and the effect of such remodeling on the HDL-mediated macrophage cholesterol efflux have not been investigated.

In the present study, we evaluated the HDL-associated master remodeling lipid transfer proteins and enzymes and their potential to alter HDL composition and macrophage cholesterol efflux in non-treated FH subjects with an identified LDLR mutation and in normolipidemic subjects of similar age (51.0 ± 1.21 years of age and 49.8 ± 1.98 years of age in adult subjects, respectively; 15.9 ± 0.64 years of age and 16.1 ± 0.75 years of age in adolescent subjects, respectively) and sex distribution (Table 1). All studied subjects were recruited from the Vascular Medicine and Metabolism Unit of the Sant Joan University Hospital and the Department of Biochemistry of the Hospital de la Santa Creu i Sant Pau. The study was performed in accordance with the ethical principles set forth in the Declaration of Helsinki.

As expected (2), adult FH patients showed higher levels of LDL-C and apolipoprotein (apo) B, whereas HDL-C and apoA-I levels were lower than those of controls. Importantly, non-treated FH patients were also characterized by higher cholesteryl ester transfer protein (CETP) (4, 5) and phospholipid transfer protein (PLTP) activities but reduced lecithin-cholesterol acyltransferase (LCAT) activity. The increased lipid transfer activities of CETP and PLTP may explain the higher potential for plasma to convert mature HDL into preβ-HDL particles (Table 1). LCAT activity is essential for converting

unesterified cholesterol into cholesteryl esters, and via this mechanism LCAT induces the transformation of preβ-HDL into mature spherical HDL. Reduced LCAT may thus contribute to the higher contents of unesterified cholesterol and apoE and the reduced content of apoA-I in mature FH HDL (Table 1). We also determined macrophage cholesterol efflux to apoB-depleted plasmas, which contains mature HDL, the HDL regulatory proteins and preβ-HDL particles, thereby permitting optimal HDL remodeling and cholesterol flow to HDL. ApoB-depleted plasmas from FH patients displayed an impaired ability to promote macrophage cholesterol efflux under experimental settings which stimulated mainly ABCA1-dependent cholesterol efflux by treating the cells with cyclic adenosine monophosphate or which stimulated concerted action of ABCA1/ABCG1-dependent efflux pathways by overloading the cells with acetylated-LDL (Table 1). Notably, most of these HDL alterations were also found in non-treated FH adolescents (Table 1). The higher CETP facilitated lipid transfer along with the reduced LCAT activity in FH adolescents indicates that these functional alterations may be primarily responsible for the altered HDL remodeling and functionality in early-aged FH patients.

In conclusion, our findings in FH patients, together with those found in hypercholesterolemic pigs (3), indicate that high LDL particle concentration is linked to dysfunctional HDL particles characterized by their altered remodeling and an impaired capacity to promote cholesterol removal from macrophages. These findings also suggest a potential role for LCAT activating and CETP silencing therapies to improve HDL function in FH patients.

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Table 1. Plasma and HDL parameters

Plasma parameters	Adults (40-60 years old)			Adolescents (12-19 years old)		
	Control (7 men and 8 women)	FH patients (7 men and 8 women)	p value	Control (6 men and 6 women)	FH patients (6 men and 7 women)	p value
LDL cholesterol (mM)	3.24 ± 0.75	10.19 ± 2.10*	<0.001	2.50 ± 0.96	5.80 ± 1.22*	<0.001
HDL cholesterol (mM)	1.83 ± 0.46	1.31 ± 0.30*	0.001	1.60 ± 0.34	1.28 ± 0.27*	0.005
ApoA-1 (g/L)	1.71 ± 0.28	1.41 ± 0.26*	0.006	1.50 ± 0.22	1.28 ± 0.20*	0.01
ApoB (g/L)	0.94 ± 0.19	2.51 ± 0.54*	<0.001	0.79 ± 0.19	1.49 ± 0.29*	<0.001
PLTP activity (µM/h)	5509 ± 1633	7222 ± 1545*	0.006	5293 ± 1364	4530 ± 1043	0.13
CETP activity (µM/h)	25.09 ± 2.71	36.67 ± 4.57*	<0.001	30.23 ± 5.01	36.85 ± 3.70*	0.001
LCAT activity (µM/h)	30.05 ± 3.30	24.98 ± 2.39*	<0.001	32.64 ± 5.43	26.63 ± 4.17*	0.004
Generation preβ-HDL (%/6h)	13.83 ± 2.46	32.83 ± 5.18*	<0.001	15.80 ± 3.03	23.29 ± 3.41*	<0.001
HDL composition (%)						
Triglycerides	2.86 ± 0.65	2.74 ± 0.86	0.67	2.85 ± 1.16	2.86 ± 0.85	0.98
Phospholipids	29.76 ± 1.67	29.51 ± 1.51	0.67	30.64 ± 0.83	29.68 ± 1.37*	0.03
Free cholesterol	3.38 ± 0.41	3.82 ± 0.41*	0.007	3.52 ± 0.63	3.87 ± 0.66	0.20
Esterified cholesterol	14.21 ± 0.41	14.77 ± 1.23	0.10	15.20 ± 1.00	15.51 ± 1.40	0.53
ApoA-I	37.26 ± 1.45	34.64 ± 1.99*	<0.001	36.45 ± 1.56	34.57 ± 1.28*	0.003
ApoA-II	11.69 ± 1.55	12.56 ± 1.25	0.10	9.31 ± 1.33	11.14 ± 1.94*	0.012
ApoE	0.83 ± 0.62	1.96 ± 0.76*	<0.001	2.03 ± 0.31	2.37 ± 0.32*	0.006
Macrophage cholesterol efflux						
+ cAMP	1.00 ± 0.17	0.87 ± 0.10*	0.01	1.00 ± 0.12	0.90 ± 0.07*	0.01
+ Ac-LDL	1.00 ± 0.11	0.86 ± 0.12*	0.002	1.00 ± 0.12	0.90 ± 0.07*	0.01

Values are mean ± SD. * $p<0.05$ versus the control group. Plasma lipid and apolipoproteins were determined by using commercial kits. PLTP, CETP, and LCAT activities were measured with radiometric assays. The amount of preß-HDL generated by plasma in the presence of an LCAT inhibitor was quantified with two-dimensional crossed immunoelectrophoresis. HDL was isolated by sequential ultracentrifugation and the lipids and apolipoproteins were determined. The cholesterol efflux capacity of 2.5% apoB-depleted plasma samples (%/4h) was determined by using J774 [^{3}H]-cholesterol-labeled mouse macrophages in the presence of 0.3 mM cAMP or after loading the cells with 0.05 g/L ac-LDL. The efflux of controls was set at a normalized value of 1 arbitrary unit. ac-LDL = acetylated low-density lipoprotein; apo = apolipoprotein; cAMP = cyclic adenosine monophosphate; CETP = cholesteryl ester transfer protein; FH = familial

hypercholesterolemia; HDL = high-density lipoprotein; LCAT = lecithin-cholesterol acyltransferase; LDL = low-density lipoprotein; PLTP = phospholipid transfer protein.