Impaired HDL-mediated macrophage cholesterol efflux in patients with abdominal aortic aneurysm

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The concept of circulating human high-density lipoproteins (HDL) includes a highly complex and heterogeneous array of particles that are continuously remodeled by plasma factors. The ability of HDL particles to promote macrophage cholesterol efflux is the most extensively reported cardioprotective function associated with HDL. ATP-binding cassette (ABC) A1 mediates cholesterol efflux to lipid-poor preβ-HDL and small HDL particles, whereas ABCG1 stimulates cholesterol efflux to large HDL particles. The stimulation of the HDL-dependent cholesterol efflux from J774 macrophage cells mediated by the ABCA1 has been found to be inversely associated with the incidence of atherothrombotic cardiovascular disease events.[1, 2] Abdominal aortic aneurysm (AAA) may be considered a form of atherothrombosis. A recent meta-analysis has demonstrated **a potential role of lipoproteins** in the etiology of AAA.[3] We have previously reported in an epidemiological study that HDL cholesterol levels in AAA patients are lower than in patients with aortoiliac occlusive disease.[4] HDL cholesterol levels also predicted aneurysmal growth rate in a population-based prospective cohort study of AAA detected by mass screening.[4] However, **HDL cholesterol levels do not necessarily reflect the dynamic of HDL to promote macrophage cholesterol efflux [1, 2]. Cholesterol crystals and macrophage infiltration are associated to a local immune-inflammatory response of the media layer of human AAA;[5] furthermore, cholesterol accumulation as a result of an impaired cholesterol efflux sensitizes macrophages to a more pro-inflammatory state.** In the present study, we evaluated **the composition of circulating** HDL particles and their potential for promoting macrophage cholesterol efflux in AAA subjects (aortic size > 30mm, confirmed with abdominal ultrasound) and in normolipidemic control subjects (aortic size < 30 mm) **from Spain and Denmark.** All samples were obtained from the biobank of Fundación Jimenez Diaz (Spain) **and from the VIVA trial (Denmark).** The study was performed in accordance with the ethical principles set forth in the Declaration of Helsinki **and approved by the institutional review committee of each Institution; the subjects studied gave informed consent.**

AAA patients in the Spanish cohort showed lower apoAI levels which were concomitant with low levels of plasma HDL cholesterol and preβ-HDL particles (Table 1). Preβ-HDL particles were kept at low levels, even upon incubation of plasma at 37 ºC in the presence of a lecithin-cholesterol acyltransferase (LCAT) inhibitor, as compared with the control group (Table 1). However, the activities of the

main HDL lipid transfer proteins and enzymes involved in HDL remodeling, phospholipid transfer protein (PLTP), cholesteryl ester transfer protein (CETP) and LCAT were similar in both groups (Table 1). **Pearson correlation test did not show association between these plasma HDL remodeling proteins and apoAI or preβ-HDL levels.** The analysis of mature α-mobile HDL composition only revealed a moderate enrichment of triglycerides in HDL derived from the Spanish AAA patients (Table 1). The lack of association among the plasma factors involved in HDL remodeling and preβ-HDL particle levels in AAA patients indicate that the low preβ-HDL levels may be attributable to an impaired *de novo* formation of preβ-HDL or an enhanced clearance of lipid-free apoAI, thereby reducing the potential to form **apoAI-containing** mature HDL

We also determined macrophage cholesterol efflux to apoB-depleted plasma, which contains mature HDL, preβ-HDL particles and HDL regulatory proteins, thereby permitting optimal HDL remodeling and cholesterol flow to HDL particles. ApoB-depleted plasma from **Spanish** AAA patients displayed an impaired ability to promote macrophage cholesterol efflux (Table 1). **These differences were also observed under experimental settings which stimulated mainly ABCA1-dependent cholesterol efflux by treating the cells with 0.3mM of cyclic adenosine monophosphate (14.23 ± 0.51% in controls vs 10.80 ± 0.62% in AAA patients, p<0.001) or, alternatively, stimulated concerted ABCA1/ABCG1-dependent efflux pathways by treating the cells with 2μM of the liver X receptor agonist T090137 (25.29 ± 0.56% in controls vs 21.39 ± 1.00% in AAA patients, p=0.002).**

Danish AAA patients also showed lower apoAI levels, but HDL cholesterol levels were similar in both groups. Preβ-HDL particles levels tended to be lower in Danish AAA patients; however, this trend did not reach statistical significance (Table 1). More importantly, Danish AAA patients also showed impaired macrophage cholesterol efflux, thereby indicating that this major HDL cardioprotective function was altered independently of HDL cholesterol levels, age and statins use.

We also performed a logistic regression model taking into account potential confounders in both populations. An inverse association between efflux and AAA

was observed, even after adjusting for age, statin intake and lipid and lipoprotein levels ($p \leq 0.016$).

In conclusion, the present study shows that AAA patients have low plasma apoAI levels and impaired macrophage cholesterol efflux. This major HDL functional alteration in AAA patients could be mechanistically linked to AAA, at least in experimental animal models, since the injection of apoAI mimetics, **which promotes macrophage cholesterol efflux**, inhibited experimental AAA progression.[4]

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Table 1. Plasma and HDL parameters

	Spain			Denmark		
	Controls (N=20)	**AAA patients (N=20)**	**p value**	**Controls (N=21)**	**AAA patients (N=21)**	**p value**
General information						
Age (years)	65 ± 0.02	70.6 ± 1.36	<0.001	**68.7 ± 0.52**	**69.5 ± 0.73**	**0.373**
Gender (N / % of men)	20 / 100	18 / 90	0.487	**20/100**	**20/100**	**1.000**
Diabetes (N / %)	7 / 35	4 / 25	0.480	**1 / 4.8**	**2 / 9.5**	**1.000**
Hypertensive (N / %)	13 / 65	11 / 55	0.748	**8 / 38.1**	**11 / 52.4**	**0.536**
Smokers (N / %)	8 / 40	7 / 35	1.000	**5 / 23.8**	**7 / 33.3**	**0.734**
Statins (N / %)	5 / 25	14 / 70	0.010	**7 / 33.3**	**11 / 52.4**	**0.350**
Plasma lipid, lipoprotein profile and HDL parameters						
Total cholesterol (mM)	5.35 ± 0.20	3.03 ± 0.21	<0.001	**5.55 ± 0.16**	**4.45 ± 0.12**	**<0.001**
Triglycerides (mM)	1.62 ± 0.18	1.02 ± 0.12	0.009	**1.41 ± 0.16**	**1.27 ± 0.12**	**0.505**
LDL cholesterol (mM)	3.35 ± 0.19	1.78 ± 0.16	<0.001	**3.17 ± 0.20**	**2.24 ± 0.14**	**<0.001**
HDL cholesterol (mM)	1.27 ± 0.06	0.79 ± 0.06	<0.001	**1.74 ± 0.09**	**1.64 ± 0.05**	**0.331**
Mature HDL ApoAI (g/L)	1.29 ± 0.04	0.89 ± 0.04	<0.001	**1.63 ± 0.07**	**1.38 ± 0.05**	**0.008**
Preβ-HDL apoAI (g/L)	0.29 ± 0.02	0.09 ± 0.01	<0.001	**0.33 ± 0.03**	**0.27 ± 0.02**	**0.081**
Generation of preβ-HDL apoAI (g/L 6h)	0.50 ± 0.03	0.14 ± 0.01	<0.001	**0.64 ± 0.04**	**0.56 ± 0.03**	**0.112**

Macrophage cholesterol efflux (%)	12.39 ± 0.39	9.26 ± 0.29	<0.001	**12.71 ± 0.68**	**10.41 ± 0.46**	**0.008**
Master HDL remodeling lipid transfer proteins and enzymes						
PLTP activity (µM/h)	6387 ± 380	6549 ± 311	0.743			
CETP activity (µM/h)	29.31 ± 1.20	31.94 ± 2.43	0.285			
LCAT activity (µM/h)	34.13 ± 4.90	34.39 ± 5.49	0.972			
Mature HDL composition (mass %)						
Triglycerides	4.01 ± 0.23	5.13 ± 0.41	0.023			
Phospholipids	33.39 ± 0.48	33.26 ± 0.51	0.857			
Free cholesterol	3.50 ± 0.07	3.54 ± 0.11	0.743			
Esterified cholesterol	16.50 ± 0.25	16.42 ± 0.51	0.886			
ApoAI	42.61 ± 0.35	41.66 ± 0.49	0.120			

Data are presented as mean ± SEM for continuous variables and as frequencies and percentages for categorical variables. Fisher's exact test was used to compare the categorical data between groups. The student’s unpaired t-test was used to compare the continuous variables. Plasma lipid and apoAI were determined enzymatically and by nephelometry, respectively, using commercial kits adapted to a COBAS 501c autoanalyzer. Phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP) activities were measured with radiometric assays based on the use of radiolabeled liposome vesicles-HDL and discoidal HDL systems. Lecithin-cholesterol acyltransferase (LCAT) activity was measured using the lipoproteins of plasma as substrate and expressed as molar esterification rates. The amount of preß-HDL apoAI levels were quantified with two-dimensional crossed immunoelectrophoresis in the plasma samples and in the plasma samples incubated for 6h at 37C in the presence of an LCAT inhibitor (1 mM iodoacetate). Mature HDL was isolated by sequential ultracentrifugation at density 1.063-1.21 g / kg, and lipids and apoAI were determined. The cholesterol efflux capacity of apoB-depleted plasma samples (equivalent to 5% of plasma) was determined by using

J774 [^{3}H]-cholesterol-labeled mouse macrophages. **After 4 h of incubation with apoB-depleted plasma, efflux of cholesterol was determined and expressed as [^{3}H]-cholesterol medium / ([^{3}H]-cholesterol cells + medium) x 100. In each population, AAA cases and controls were run on the same plates/batches. Body mass index did not differ significantly between Danish AAA and control groups (27.09 ± 0.62 vs 25.81 ± 0.41, P=0.092).**