Basic Research in Cardiology (2019) 114:27
https://doi.org/10.1007/s00395-019-0735-0

ORIGINAL CONTRIBUTION

Atrial fibrillation is associated with alterations in HDL function, metabolism, and particle number

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Received: 19 September 2018 / Accepted: 2 May 2019 / Published online: 8 May 2019

Abstract

Increased morbidity and mortality in atrial fibrillation (AF) are related to the pro-fibrotic, pro-thrombotic, and pro-inflammatory processes that underpin the disease. High-density lipoproteins (HDL) have anti-inflammatory, anti-oxidative, and anti-thrombotic properties. Functional impairment of HDL may, therefore, associate with AF initiation or progression. We studied indices of HDL quality and quantity of AF patients and healthy controls, including HDL-particle number, HDL cholesterol, apolipoprotein (apo) A–I levels, serum amyloid A (SAA) content and HDL-cholesterol efflux capacity, and paraoxonase activity of apoB-depleted serum. Serum samples were collected from AF patients ($n=91$) before catheter ablation and from age- and sex-matched control subjects ($n=54$). HDL-cholesterol efflux capacity was assessed in a validated assay using [^{3}H]-cholesterol-labeled J774 macrophages. Lecithin–cholesterol acyltransferase (LCAT) and paraoxonase activities were assessed using fluorometric assays, SAA levels were determined by ELISA, and total and subclass HDL-particle number was assessed by nuclear magnetic resonance spectroscopy. ApoA-I levels were determined by immunoturbidimetry. HDL-cholesterol efflux capacity, HDL-particle number, apoA-I levels, and LCAT activity were markedly reduced in AF patients when compared to healthy individuals (all $p<0.001$), whereas HDL-associated paraoxonase activity and SAA content were not altered ($p=0.578$, $p=0.681$). Notably, cholesterol efflux capacity, HDL-particle number, apoA-I levels as well as LCAT activity recovered following restoration of sinus rhythm (all $p<0.001$). We identified marked alterations in HDL function, HDL maturation, and HDL-particle number in AF patients. Assessing HDL-particle number and function in AF may be used as a surrogate marker of AF onset and progression and may help identifying patients at high risk.

Keywords Atrial fibrillation · HDL function · Cholesterol efflux capacity · HDL-particle number · PON

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia and is associated with increased mortality and morbidity [18]. AF onset is associated with increased age, male sex, hypertension, diabetes, obesity, or accompanies cardiac disease, e.g., valvular and/or coronary heart disease [18]. AF patients are at a fivefold increased risk for stroke and 1.5 to twofold increased risk of heart-related deaths [5]. AF is a progressive disease with advanced pro-fibrotic atrial remodeling, but the pathomechanisms that underpin AF onset and progression are incompletely understood, hindering the validation of new therapeutic concepts and treatment strategies.

Although dyslipidemia is a well-established risk factor for cardiovascular disease [34], its association with AF remains

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00395-019-0735-0) contains supplementary material, which is available to authorized users.

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controversial. In two community-based cohorts, high-density lipoprotein cholesterol (HDL-C) and triglycerides, but not low-density lipoprotein cholesterol (LDL-C) or total cholesterol were associated with the risk for AF [1]. Other studies have shown that AF patients depict a lipid profile in the normal range [5]. HDL exerts various atheroprotective functions, such as anti-inflammatory and anti-oxidative properties [3, 24], endothelium regenerative capabilities [6, 29], and anti-thrombotic activities [22], connecting it to known risk factors for AF. In this explorative study, we assessed several indices of HDL quality, such as cholesterol efflux capacity [17, 26], HDL content of serum amyloid A (SAA) [2, 35], and paraoxonase activity [7] in AF patients and healthy controls. In addition, we measured metrics of HDL quantity, including HDL-particle number (HDL-P) [25] and levels of HDL-C and apolipoprotein (apo) A-I.

Methods

Study populations

The AF cohort was established from October 2015 until April 2017 at the Department of Electrophysiology at Heart Center Leipzig (Germany) by recruiting consecutively selected AF patients undergoing first AF radiofrequency catheter ablation [9]. Exclusion criteria were pregnancy, age < 18 or > 75, valvular AF, cancer, acute or systemic inflammatory diseases, renal dysfunction with eGFR < 15 ml/min or dialysis, and the use of cholesterol lowering medication. When inflammation was questionable, additional C-reactive protein (CRP) measurements were done. Increased CRP was an exclusion criterion for catheter ablation procedure and study inclusion. The study was approved by the local Ethical Committee (Medical Faculty, University Leipzig, IRB no: 259-15-13072015) and all patients provided written informed consent for participation in accordance with the Declaration of Helsinki. Paroxysmal and persistent AF was defined according to current guidelines [18]. Paroxysmal AF was defined as self-terminating within 7 days after onset. Persistent AF lasted longer than 7 days or required drugs or direct current cardioversion for termination [18]. Blood samples were obtained in serum-gel test tubes (Sarstedt, Germany) in fasting state prior ablation procedure from the femoral vein and were processed within 30–60 min following collection. Blood serum was prepared (1800×*g* for 10 min at 4 °C) and aliquots were kept at − 80 °C until analysis.

The control cohorts were recruited at Leipzig, Germany ($n = 25$) and Graz, Austria ($n = 29$). Patients from the cardiology outpatient clinic at the Heart Center Leipzig were recruited in 2018 as control probands when none of the following criteria were present: cardiac disease (coronary artery disease, valve disease ≥ 2nd degree, cardiomyopathy, acute coronary syndrome, unstable angina pectoris, heart failure, any history of myocardial infarction, thromboembolic events, and supraventricular/ventricular arrhythmia), cancer, severe renal impairment (eGFR < 60 ml/min/1.73 m^2), liver dysfunction, acute or systemic infections, or autoimmune disease. Blood samples were obtained in serum-gel test tubes (Sarstedt, Nürmbrecht, Germany) and processed within 1 h of collection. Serum was prepared (1800×*g* for 10 min at 20 °C) and aliquots were stored at − 80 °C for subsequent analysis. This study was also approved by the ethics committee at the University of Leipzig. Furthermore, age- and sex-matched healthy controls were recruited at the Medical University of Graz and included after passing the following exclusion criteria: any history of AF, cardiovascular disease, chronic inflammatory disorders, history of diabetes types 1 and 2, intake of antihyperlipidemic agents, regular intake of anti-inflammatory drugs, history of severe renal failure, history of severe hepatic dysfunction, and a recent infection. Fasting blood samples were collected by antecubital venipuncture into two 9 ml serum tubes (VACUETTE TUBE 9 ml Z Serum Clot Activator). The samples were centrifuged, separated, and frozen at − 80 °C. Written informed consent of all control probands was obtained in accordance with the Declaration of Helsinki.

Follow-up

All AF patients were followed in the outpatient clinic after catheter ablation. During the follow-up period, 4-day Holter ECG recordings were performed at 3, 6, and 12 months after the ablation. Additional ECGs and Holter ECG recordings were obtained when patients' symptoms were suggestive of AF. AF recurrences were defined as any atrial arrhythmia lasting > 30 s. If electrical or pharmacologic cardioversion and/or repeat procedure were needed after 3 months blanking period, this was also considered as an AF recurrence. Blood was collected 12–18 months following the ablation procedure during a follow-up visit when restoration of sinus rhythm was confirmed.

Lipid and lipoprotein profiling by nuclear magnetic resonance (NMR) spectroscopy

The lipid and lipoprotein profiles of serum samples (total cholesterol, HDL-C, LDL-C, triglycerides and total, and small and large HDL-particle concentration) were analyzed using the AXINON® lipoFIT®-S100 test system (Numares Health, Regensburg, Germany) as described previously [25]. In brief, serum (630 µl) was gently mixed with 70 µl of an internal standard (with reference substances, NaN_3 and D_2O). From this solution, 600 µl were transferred into 5 mm NMR tubes, followed by the recording of NMR spectra at a

temperature of 310 K on a shielded 600 MHz Bruker Avance III HD spectrometer with a 5 mm triple resonance TXI probe head including a deuterium lock channel and a z-grade coil. Only samples and their spectra which met a defined set of quality criteria were analyzed, to ensure data quality. Lipoprotein particle concentrations were calibrated to an NMR-based lipoprotein profiling method [15].

Preparation of apolipoprotein B (apoB)-depleted serum

ApoB-depleted serum was prepared by addition of 40 µl polyethylene glycol (20% in 200 mmol/l glycine buffer) to 100 µl serum [32]. Serum was incubated at room temperature for 20 min and the supernatant recovered after centrifugation (10,000 rpm, 20 min, 4 °C).

HDL-cholesterol efflux capability

Cholesterol efflux capacity was assessed using an established assay [4, 13]. J774 macrophages, maintained in DMEM with 10% fetal bovine serum, were plated on 48-well plates (300,000 cells/plate). Cells were labeled for 24 h with 1 µCi/ml [^{3}H] cholesterol (Perkin Elmer, Boston, MA, USA). [^{3}H]-cholesterol-labeled J774 macrophages were incubated in the presence of 0.3 mmol/l 8-(4-chlorophenylthio)-cyclic AMP (cAMP) to stimulate ATP-binding cassette transporter A1 (ABCA1) expression. After labeling, cells were washed twice with serum-free DMEM and subsequently equilibrated in serum-free DMEM containing 0.2% BSA for 2 h. After additional two washing steps, [^{3}H]-cholesterol efflux was determined by incubating cells for 3 h with serum-free DMEM containing 2.8% apoB-depleted serum. Cholesterol efflux was expressed as the radioactivity in the medium relative to total radioactivity in medium and cells. All steps were performed in the presence of 2 µg/ml of the acyl coenzyme A cholesterol acyltransferase inhibitor Sandoz 58-035 (Sigma, Darmstadt, Germany).

Paraoxonase activity

Arylesterase activity of HDL-associated paraoxonase was determined using a photometric assay with phenylacetate as substrate, as described [32].

Serum amyloid A

Serum amyloid A (SAA) was determined using an enzyme-linked immunosorbent assay (Life Technologies, Vienna, Austria).

Lecithin–cholesterol acyltransferase activity

Serum lecithin–cholesterol acyltransferase (LCAT) activity was assessed according to the manufacturers instruction (Merck, Darmstadt, Germany).

ApoA-I

ApoA-I was determined by immunoturbidimetry on an Olympus AU640 analyzer (Olympus Diagnostika, Hamburg, Germany) as described [14].

Statistical analysis

Differences between two groups were analyzed with the Student's *t* test (unpaired, two-tailed). For non-Gaussian data, the Mann–Whitney *U* test was used. Differences between AF patients at baseline and following sinus rhythm restoration after catheter ablation were analyzed with Student's *t* test (paired, two-tailed).

Correlations were determined using the Pearson product-moment estimates. Group differences were considered statistically significant for $*p < 0.05$. Statistical analyses were performed using GraphPad Prism (Version 4.0, GraphPad Software) or IBM SPSS Statistics for Windows Version 23 (IBM Corp, Armonk, NY, USA).

Results

Characteristics of study population

The clinical characteristics of study subjects are given in Table 1. In total, 91 patients with AF and 54 age- and sex-matched healthy controls were included in the study. There were 46 patients (51%) with paroxysmal AF, 45 patients (49%) with persistent AF. The median CHA2DS2-VASc score in the AF cohort was 2 (IQR 1–4). A subgroup of 21 patients with restored sinus rhythm of the original cohort had accessible follow-up data and blood samples.

We did not observe differences in serum levels of triglycerides and LDL-C between healthy controls and AF patients ($p = 0.981$, $p = 0.164$), while levels of total cholesterol and HDL-C were reduced ($p = 0.009$, $p < 0.001$). There was no difference in body mass index between the two groups ($p = 0.968$) (Table 1).

HDL function

We observed a strikingly reduced cholesterol efflux capacity of apoB-depleted serum in patients with AF when compared with controls ($p < 0.001$) (Fig. 1a). There was no change in cholesterol efflux capacity in

Table 1 Clinical overview of study subjects

	Controls	AF	*p* value
N	54	91	
Age (year)	60 (53–66)	63 (56–71)	0.153[a]
Male/female	30/24	50/41	0.943[b]
BMI (kg/m^2)	27 (25–33)[c]	30 (26–33)	0.968[a]
Triglycerides (mg/dl)	117 (95–152)	108 (78–145)	0.981[a]
Total cholesterol (mg/dl)	194 (168–222)	180 (154–203)	0.009[a]
LDL-cholesterol (mg/dl)	108 (100–134)	103 (85–125)	0.164[a]
HDL-cholesterol (mg/dl)	58 (52–70)	50 (42–56)	< 0.001[a]
HDL-particle number (nnmol/l)	36,156 (33,180–41,601)	29,794 (27,127–32,053)	< 0.001
Type			
Paroxysmal		46 (51%)	
Persistent		45 (49%)	

Values are given as medians with the interquartile range

AF atrial fibrillation, *BMI* body mass index, *n.d.* not determined, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein

[a]*p* value based on *t* test

[b]*p* value based on Chi-square test

[c]BMI was available from 25 controls

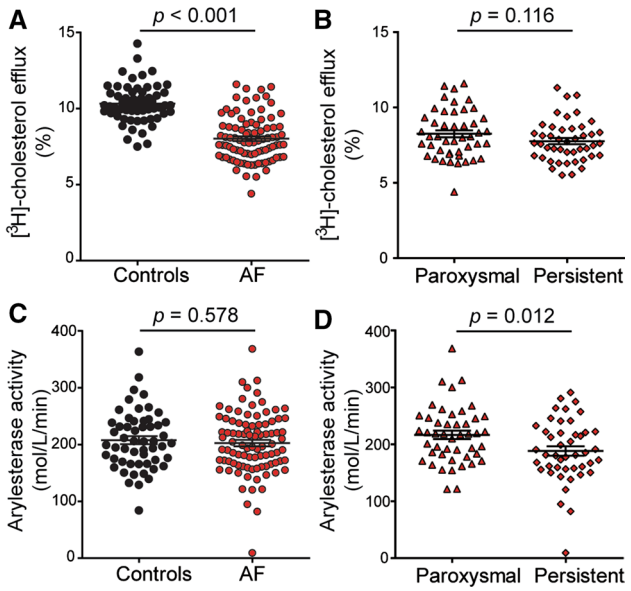

Fig. 1 Cholesterol efflux capacity and arylesterase activity of HDL-associated paraoxonase of healthy subjects and patients with AF. ApoB-depleted sera of 54 healthy subjects (control) and 91 patients with AF were examined for (**a**, **b**) their ability to promote cholesterol efflux from [^{3}H]-cholesterol-labeled J774 macrophages, (**c**, **d**) their arylesterase activity of HDL-associated paraoxonase. **b** Cholesterol efflux capacity of patients with paroxysmal ($n=46$) and persistent ($n=45$) AF. **d** Arylesterase activity of HDL-associated paraoxonase of patients with paroxysmal ($n=46$) and persistent ($n=45$) AF. **a**–**d** Values represent means of two independent experiments measured in duplicates. Levels of significance were calculated using Student's *t* test. Bars indicate mean and standard error of mean of the different test groups. *AF* atrial fibrillation, *Apo* apolipoprotein, *HDL* high-density lipoprotein, *SAA* serum amyloid A

patients with persistent AF when compared to paroxysmal AF ($p=0.116$) (Fig. 1b). Paraoxonase activity of apoB-depleted serum in AF patients was not altered, as monitored by arylesterase activity ($p=0.578$) (Fig. 1c). We observed no changes in the HDL-associated pro-inflammatory mediator SAA ($p=0.681$) (Online Fig. 1A). However, patients with persistent AF depicted reduced paraoxonase-mediated arylesterase activity of apoB-depleted serum when compared to patients with paroxysmal AF ($p=0.0122$) (Fig. 1d), while SAA levels were not different ($p=0.852$) (Online Fig. 1B).

HDL-C, HDL-P, and apoA-I levels

Prompted by the low cholesterol efflux capacity observed in AF patients, we next assessed HDL-C and apoA-I as well as HDL-P. Levels of HDL-C, apoA-I, and total HDL-P were markedly decreased in AF patients (all $p<0.001$) (Table 1, Fig. 2a, c). Notably, patients with persistent AF depicted reduced HDL-P when compared to patients with paroxysmal AF ($p=0.009$) (Fig. 2d). We observed significantly lower apoA-I values in patients with persistent AF compared to patients with paroxysmal AF ($p=0.006$). Large HDL-P between healthy subjects and patients with AF was not significantly changed ($p=0.110$) (Fig. 2e), while small HDL-P were markedly reduced ($p<0.001$) (Fig. 2g). Both, large and small HDL-P, respectively, were unchanged between patients with paroxysmal and persistent AF ($p=0.202$, $p=0.218$) (Fig. 2f, h). Both LDL-C and LDL-P did not differ between controls and AF patients ($p=0.164$, $p=0.130$)

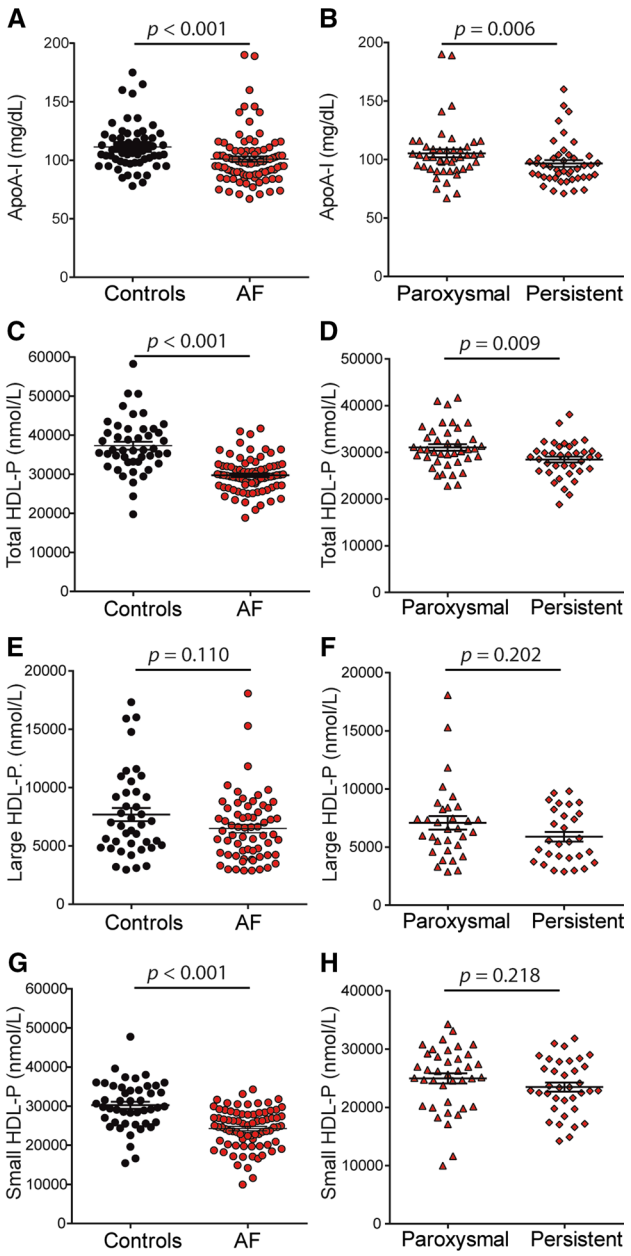

Fig. 2 ApoA-I levels and HDL-P of healthy subjects and patients with AF. Serum levels of apoA-I (**a**, **b**) and HDL-P (**c**–**h**) of 54 healthy subjects (control) and 91 patients with AF were determined by immunoturbidimetry (**a**, **b**) and using NMR spectroscopy (**c**–**h**). Levels of apoA-I (**b**), total HDL-P (**d**), large HDL-P (**f**), and small HDL-P (**h**) of AF patients with paroxysmal ($n=46$) and persistent ($n=45$) AF. Levels of significance were calculated using Student's t test or Mann–Whitney U test. Bars indicate mean and standard error of mean of the different test groups. *AF* atrial fibrillation, *apo* apolipoprotein, *HDL* high-density lipoprotein, *HDL-P* HDL-particle number, *NMR* nuclear magnetic resonance

(Table 1, Online Fig. 2). It is noteworthy that there was a strong association of HDL-P with the cholesterol efflux capacity ($r=0.494$, $p<0.001$) (Table 2).

Lecithin–cholesterol acyltransferase (LCAT) activity

During HDL maturation, free cholesterol is esterified to cholesteryl ester by LCAT. This converts discoidal HDL into spherical HDL and increases the size of HDL [12]. We hypothesized that LCAT activity in patients with AF is altered. Indeed, patients with AF displayed a vast reduction in serum LCAT activity when compared to controls ($p<0.001$) (Fig. 3a). LCAT activity between paroxysmal and persistent AF did not differ ($p=0.212$) (Fig. 3b). Of particular interest, LCAT activity was significantly associated with cholesterol efflux capacity ($r=0.409$, $p<0.001$), HDL-P ($r=0.443$, $p<0.001$) and apoA-I ($r=0.174$, $p=0.036$), but not with HDL-C ($r=0.097$, $p=0.150$) and paraoxonase-mediated arylesterase activity of apoB-depleted serum ($r=0.088$, $p=0.294$) (Table 2). Persistent AF is characteristic of AF progression; therefore, we performed a detailed analysis including gender as an important risk factor for all measurements of HDL functionality. However, there were no significant differences between sex and AF type alone or in combination that influenced the parameters analyzed (Online Fig. 3).

Restoration of sinus rhythm and association with cholesterol efflux capacity, LCAT activity, apoA-I levels, and HDL-P

Serum of 21 AF patients with restored sinus rhythm was available for measurement of HDL functionality, LCAT activity, and apoA-I levels and of 18 patients for determination of serum lipid profile and HDL-P. Of particular interest, restoration of sinus rhythm ameliorated cholesterol efflux capacity of apoB-depleted serum ($p<0.001$) (Fig. 4a) and LCAT activity of serum ($p<0.001$) (Fig. 4c) reaching levels that were still lower than of healthy controls ($p=0.034$, $p=0.009$) (Fig. 4b, d). Restoration of sinus rhythm led to increased levels of apoA-I ($p=0.001$) (Fig. 5a), total HDL-P ($p<0.001$) (Fig. 5c), large HDL-P ($p<0.001$) (Fig. 5e), and small HDL-P ($p<0.001$) (Fig. 5g) serum. Notably, restoration of sinus rhythm normalized apoA-I (Fig. 5b), total HDL-P (Fig. 5d), large HDL-P (Fig. 5f), and small HDL-P (Fig. 5h) to control values ($p=0.234$, $p=0.255$, $p=0.074$, $p=0.276$). Restoration of sinus rhythm had no impact on SAA levels ($p=0.570$), by trend increased arylesterase activity of HDL-associated paraoxonase ($p=0.096$), and was associated with increased levels of LDL-C levels ($p=0.025$), triglycerides ($p=0.001$), total cholesterol ($p<0.001$), and HDL-C ($p<0.001$) (Table 3).

Discussion

In the present study, we provide first evidence that indices of HDL function, including cholesterol efflux capacity of apoB-depleted serum, LCAT activity, and HDL-P are markedly

Table 2 Correlation of HDL functionality with HDL-particle number and LCAT activity

	HDL-P		HDL-C		ApoA-I	Arylesterase activity			LCAT activity	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
CEC	0.494	< 0.001	0.437	< 0.001	0.533	< 0.001	0.274	0.001	0.409	< 0.001
HDL-P	–	–	0.743	< 0.001	0.617	< 0.001	0.296	0.001	0.443	< 0.001
HDL-C	–	–	–	–	0.668	< 0.001	0.194	0.031	0.150	0.097
ApoA-I	–	–	–	–	–	–	0.301	< 0.001	0.174	0.036
Arylesterase activity	–	–	–	–	–	–	–	–	0.088	0.294

ApoA-I apolipoprotein A-I, *CEC* cholesterol efflux capacity, *HDL-C* high-density cholesterol, *HDL-P* HDL-particle number, *LCAT* lecithin–cholesterol acyltransferase

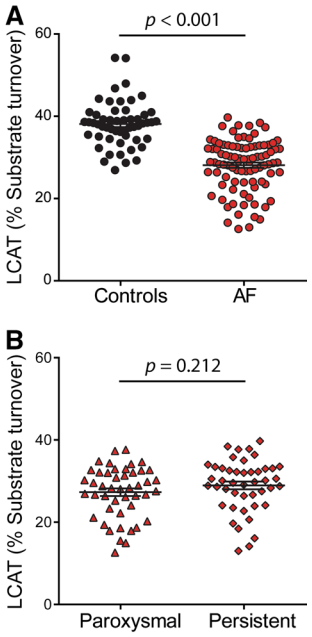

Fig. 3 LCAT activity of healthy subjects and patients with AF. **a**, **b** Serum LCAT activity of 54 healthy subjects (control) and 91 patients with AF. **b** Serum LCAT activity of patients with paroxysmal ($n=46$) and persistent ($n=45$) AF. Levels of significance were calculated using Student's *t* test. Bars indicate mean and standard error of mean of the different test groups. *AF* atrial fibrillation, *LCAT* lecithin–cholesterol acyl transferase

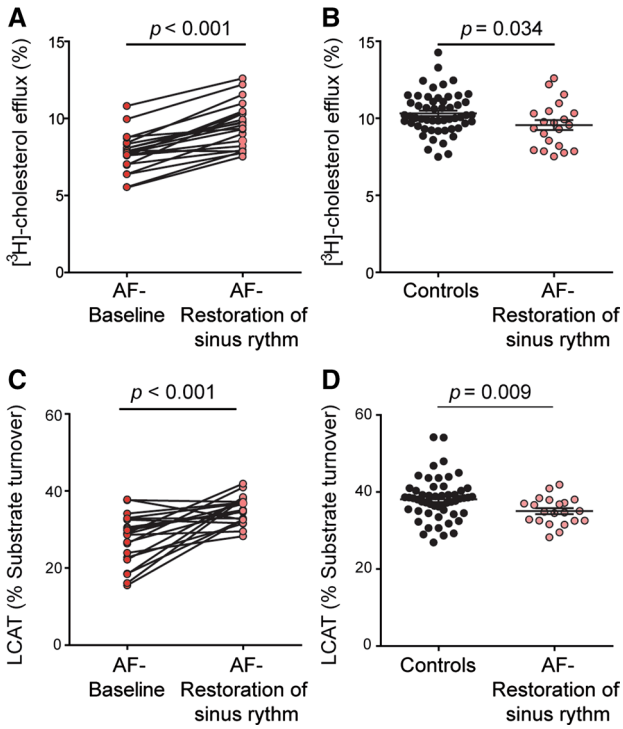

Fig. 4 Cholesterol efflux capacity and LCAT activity of 21 patients with restoration of sinus rhythm after catheter ablation. ApoB-depleted sera (**a**, **b**) and sera (**c**, **d**) were examined for (**a**, **b**) their ability to promote cholesterol efflux from [^{3}H]-cholesterol-labeled J774 macrophages and (**c**, **d**) LCAT activity. **a**, **c** Cholesterol efflux capacity and LCAT activity of AF patients before (AF baseline) and after successful catheter ablation (AF restoration of sinus rhythm). **b**, **d** Cholesterol efflux capacity and LCAT activity of healthy subjects (controls) ($n=54$) and AF patients after successful catheter ablation. **a**, **b** Values represent means of two independent experiments measured in duplicates. Levels of significance were calculated using Student's *t* test or Mann–Whitney *U* test. Bars indicate mean and standard error of mean of the different test groups. *AF* atrial fibrillation, *apo* apolipoprotein, *HDL* high-density lipoprotein, *LCAT* lecithin–cholesterol acyl transferase

reduced in AF patients. Furthermore, this reduction partially correlates with AF progression stage characterized by a switch from paroxysmal to persistent AF. Of particular interest, restoration of sinus rhythm improved HDL functionality, LCAT activity, apoA-I levels, and HDL-P.

Both, cholesterol efflux capacity [17, 26] and HDL-P, have been inversely associated with cardiovascular disease [20] and heart failure mortality [25], independently of HDL-C levels. Our data suggest that the reduced HDL-P probably explains an altered cholesterol efflux capacity in patients with AF, as cholesterol efflux capacity and HDL-P

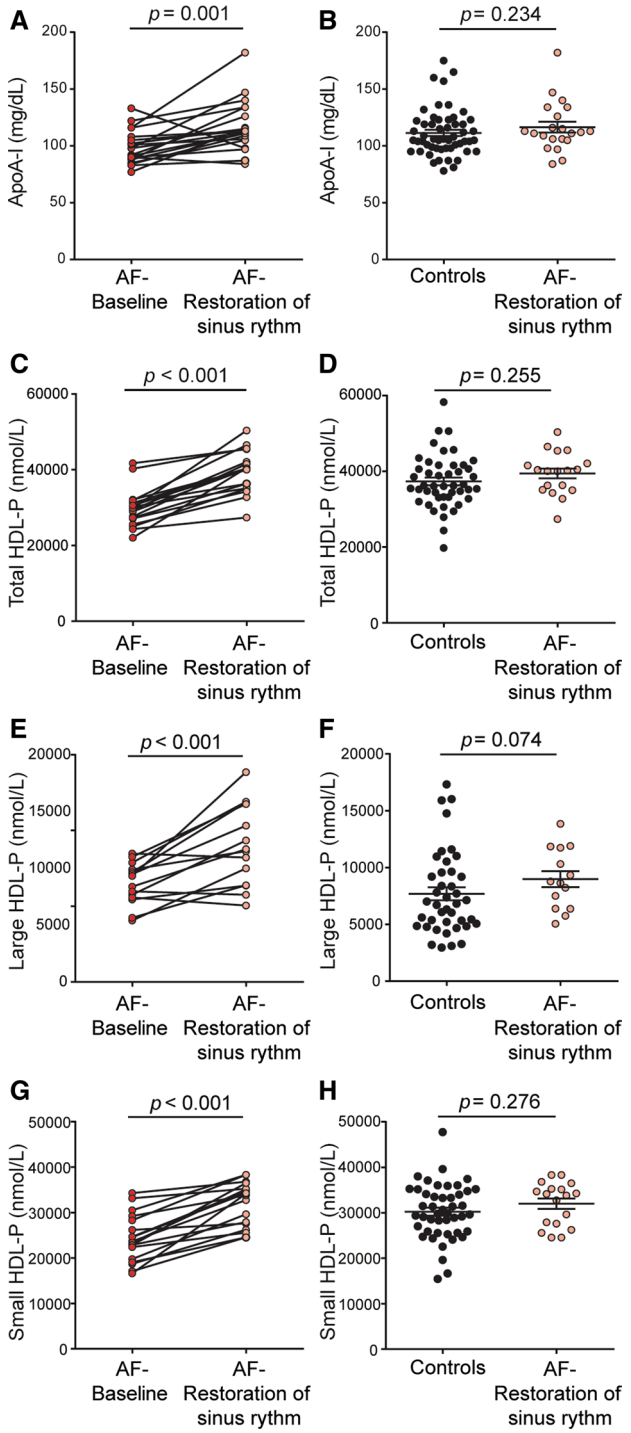

Fig. 5 Apo-AI levels and HDL-P of healthy subjects and patients with AF at baseline and with restored sinus rhythm 12–18 months after catheter ablation. Serum levels of apoA-I ($n=21$) (**a**, **b**) and HDL-P ($n=18$) **c**–**h** of AF patients and of healthy subjects (controls) ($n=54$) (**b**, **d**, **f**, **h**) were determined by immunoturbidimetry (**a**, **b**) and by NMR spectroscopy (**c**–**h**). Levels of significance were calculated using Student's *t* test or Mann–Whitney *U* test. Bars (**b**, **d**, **f**, **h**) indicate mean and standard error of mean of the different test groups. *AF* atrial fibrillation, *apo* apolipoprotein, *HDL* high-density lipoprotein, *HDL-P* HDL-particle number, *NMR* nuclear magnetic resonance

are strongly correlated. However, we cannot exclude that some other AF induced changes in the lipid or protein composition of HDL also affect cholesterol efflux capacity. Interestingly, we observed a reduced activity of LCAT in the serum of AF patients. LCAT is responsible for the synthesis of cholesteryl esters in plasma and subsequent incorporation into HDL; through this action, it plays a key role in the formation and maturation of HDL particles. We observed a strong association of LCAT activity and HDL-P, suggesting that low serum LCAT activity in AF patients leads to decreased HDL-particle formation and affects cholesterol efflux capacity.

AF is associated with oxidative stress [19] and inflammation [16]. HDL-associated paraoxonase activity reduces systemic oxidative stress and is associated with a decreased risk of major adverse cardiac events [30]. The acute-phase protein SAA impairs the anti-inflammatory properties of HDL [2] and is a clinically applicable surrogate for the vascular functionality of HDL [35]. Interestingly, AF did neither change serum SAA content nor paraoxonase-mediated arylesterase activity of apoB-depleted serum, both indices of HDL quality that are affected during inflammation [8]. Our data, therefore, argue against a significant contribution of inflammation to changing HDL function in AF. Future studies may prove fruitful in elucidating additional components of HDL that determine functionality, such as the HDL proteome [8] or HDL-associated lipids like sphingosine 1 phosphate [27, 28, 31] or lysophosphatidylcholine [10, 33].

In summary, our data support the growing evidence that HDL function and HDL-P are superior to HDL-C in predicting cardiovascular disease [23] and mortality in coronary heart disease and patients with acute heart failure [20, 25].

A very important observation of the present study is that cholesterol efflux capacity, LCAT activity, levels of apoA-I, and HDL-P improved significantly after sinus rhythm restoration. There is evidence that restoration of sinus rhythm after catheter ablation in AF patients with symptomatic heart failure results in lower rates of death of any cause [21], lower risk of AF recurrence, and improved maintenance of the sinus rhythm [11]. Our data raise the possibility that recovery of LCAT activity, levels of apoA-I and HDL-P after sinus rhythm restoration contribute to these beneficial effects.

Study limitations

Our results, albeit robust, are observational, do not prove causality, and may be subject to unmeasured confounders. Indeed, AF patients typically receive comprehensive medication including anti-arrhythmic, anti-coagulation, and anti-hypertensive drugs. Therefore, it was not possible to study the impact of all potential confounders.

Table 3 Lipid parameters, SAA levels and arylesterase activity of HDL-associated paraoxonase of AF patients before and after catheter ablation

	AF cohort		*p* value
	Baseline	Restoration of sinus rhythm	
Triglycerides (mg/dl)	102 (77–129)	145 (89–183)	0.001
Total cholesterol (mg/dl)	170 (152–194)	209 (184–234)	< 0.001
HDL-cholesterol (mg/dl)	50 (43–54)	61 (52–71)	< 0.001
LDL-cholesterol (mg/dl)	97 (85–113)	114 (97–151)	0.025
SAA (µg/ml)	22 (14–56)	28 (17–63)	0.570
Arylesterase activity (mol/l/min)	221 (175–250)	206 (171–257)	0.096

Values are given as medians with the interquartile range

AF atrial fibrillation, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *SAA* serum amyloid A

Conclusions

Our data provide first evidence of marked alterations in HDL-P, apoA-I levels, HDL-mediated cholesterol efflux capacity, and HDL maturation in AF patients. Importantly, restoration of sinus rhythm is associated with improved HDL functionality.

Funding This work was supported by the Austrian Science Fund FWF (FWF W1241, DK-MOLIN, and P22976-B18 to GM). PB and DH were supported by the Volkswagen Foundation Germany through the Lichtenberg professorship program to DH (#84901)

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Alonso A, Yin X, Roetker NS, Magnani JW, Kronmal RA, Ellinor PT, Chen LY, Lubitz SA, McClelland RL, McManus DD, Soliman EZ, Huxley RR, Nazarian S, Szklo M, Heckbert SR, Benjamin EJ (2014) Blood lipids and the incidence of atrial fibrillation: the multi-ethnic study of atherosclerosis and the Framingham Heart Study. J Am Heart Assoc. https://doi.org/10.1161/jaha.114.001211
2. Artl A, Marsche G, Lestavel S, Sattler W, Malle E (2000) Role of serum amyloid A during metabolism of acute-phase HDL by macrophages. Arterioscler Thromb Vasc Biol 20:763–772
3. Barter PJ, Nicholls S, Rye K-A, Anantharamaiah GM, Navab M, Fogelman AM (2004) Antiinflammatory properties of HDL. Circ Res. https://doi.org/10.1161/01.res.0000146094.59640.13
4. Bauer L, Kern S, Rogacev KS, Emrich IE, Zawada A, Fliser D, Heinemann A, Heine GH, Marsche G (2017) HDL cholesterol efflux capacity and cardiovascular events in patients with chronic kidney disease. J Am Coll Cardiol. https://doi.org/10.1016/j.jacc.2016.10.054
5. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D (1998) Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. Circulation 98:946–952
6. Besler C, Heinrich K, Rohrer L, Doerries C, Riwanto M, Shih DM, Chroni A, Yonekawa K, Stein S, Schaefer N, Mueller M, Akhmedov A, Daniil G, Manes C, Templin C, Wyss C, Maier W, Tanner FC, Matter CM, Corti R, Furlong C, Lusis AJ, Eckardstein A, Fogelman AM, Lüscher TF, Landmesser U (2011) Mechanisms underlying adverse effects of HDL on eNOS-activating pathways in patients with coronary artery disease. J Clin Investig. https://doi.org/10.1172/jci42946
7. Bhattacharyya T, Nicholls SJ, Topol EJ, Zhang R, Yang X, Schmitt D, Fu X, Shao M, Brennan DM, Ellis SG, Brennan M-L, Allayee H, Lusis AJ, Hazen SL (2008) Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. JAMA. https://doi.org/10.1001/jama.299.11.1265
8. Birner-Gruenberger R, Schittmayer M, Holzer M, Marsche G (2014) Understanding high-density lipoprotein function in disease: recent advances in proteomics unravel the complexity of its composition and biology. Prog Lipid Res. https://doi.org/10.1016/j.plipres.2014.07.003
9. Büttner P, Schumacher K, Dinov B, Zeynalova S, Sommer P, Bollmann A, Husser D, Hindricks G, Kornej J (2018) Role of NT-proANP and NT-proBNP in patients with atrial fibrillation. Heart Rhythm. https://doi.org/10.1016/j.hrthm.2018.03.021
10. Curcic S, Holzer M, Pasterk L, Knuplez E, Eichmann TO, Frank S, Zimmermann R, Schicho R, Heinemann A, Marsche G (2017) Secretory phospholipase A2 modified HDL rapidly and potently suppresses platelet activation. Sci Rep. https://doi.org/10.1038/s41598-017-08136-1
11. Di Biase L, Mohanty P, Mohanty S, Santangeli P, Trivedi C, Lakkireddy D, Reddy M, Jais P, Themistoclakis S, Dello Russo A, Casella M, Pelargonio G, Narducci ML, Schweikert R, Neuzil P, Sanchez J, Horton R, Beheiry S, Hongo R, Hao S, Rossillo A, Forleo G, Tondo C, Burkhardt JD, Haissaguerre M, Natale A (2016) Ablation versus amiodarone for treatment of persistent atrial fibrillation in patients with congestive heart failure and an implanted device: results from the AATAC multicenter randomized trial. Circulation. https://doi.org/10.1161/circulationaha.115.019406
12. Francone OL, Gurakar A, Fielding C (1989) Distribution and functions of lecithin: cholesterol acyltransferase and cholesteryl ester transfer protein in plasma lipoproteins. Evidence for a functional unit containing these activities together with apolipoproteins A-I and D that catalyzes the esterification and transfer of cell-derived cholesterol. J Biol Chem 264:7066–7072
13. Holzer M, Schilcher G, Curcic S, Trieb M, Ljubojevic S, Stojakovic T, Scharnagl H, Kopecky CM, Rosenkranz AR, Heinemann A, Marsche G (2015) Dialysis modalities and HDL composition and function. J Am Soc Nephrol. https://doi.org/10.1681/asn.2014030309
14. Holzer M, Trieb M, Konya V, Wadsack C, Heinemann A, Marsche G (2013) Aging affects high-density lipoprotein composition and

function. Biochim Biophys Acta. https://doi.org/10.1016/j.bbalip.2013.06.004
15. Hopkins PN, Pottala JV, Nanjee MN (2015) A comparative study of four independent methods to measure LDL particle concentration. Atherosclerosis. https://doi.org/10.1016/j.atherosclerosis.2015.08.042
16. Issac TT, Dokainish H, Lakkis NM (2007) Role of inflammation in initiation and perpetuation of atrial fibrillation: a systematic review of the published data. J Am Coll Cardiol. https://doi.org/10.1016/j.jacc.2007.06.054
17. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, Mohler ER, Rothblat GH, Rader DJ (2011) Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med. https://doi.org/10.1056/nejmoa1001689
18. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, Castella M, Diener H-C, Heidbuchel H, Hendriks J, Hindricks G, Manolis AS, Oldgren J, Popescu BA, Schotten U, van Putte B, Vardas P (2016) 2016 ESC guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur Heart J. https://doi.org/10.1093/eurheartj/ehw210
19. Korantzopoulos P, Kolettis TM, Galaris D, Goudevenos JA (2007) The role of oxidative stress in the pathogenesis and perpetuation of atrial fibrillation. Int J Cardiol. https://doi.org/10.1016/j.ijcard.2006.04.026
20. Mackey RH, Greenland P, Goff DC, Lloyd-Jones D, Sibley CT, Mora S (2012) High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis). J Am Coll Cardiol. https://doi.org/10.1016/j.jacc.2012.03.060
21. Marrouche NF, Brachmann J, Andresen D, Siebels J, Boersma L, Jordaens L, Merkely B, Pokushalov E, Sanders P, Proff J, Schunkert H, Christ H, Vogt J, Bänsch D (2018) Catheter ablation for atrial fibrillation with heart failure. N Engl J Med. https://doi.org/10.1056/nejmoa1707855
22. Mineo C, Deguchi H, Griffin JH, Shaul PW (2006) Endothelial and antithrombotic actions of HDL. Circ Res. https://doi.org/10.1161/01.res.0000225982.01988.93
23. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM (2009) Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. Circulation. https://doi.org/10.1161/circulationaha.108.816181
24. Navab M, Yu R, Gharavi N, Huang W, Ezra N, Lotfizadeh A, Anantharamaiah GM, Alipour N, van Lenten BJ, Reddy ST, Marelli D (2007) High-density lipoprotein: antioxidant and anti-inflammatory properties. Curr Atheroscler Rep 9:244–248
25. Potočnjak I, Degoricija V, Trbušić M, Pregartner G, Berghold A, Marsche G, Frank S (2017) Serum concentration of HDL particles predicts mortality in acute heart failure patients. Sci Rep. https://doi.org/10.1038/srep46642
26. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos James A, Shaul PW (2014) HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med. https://doi.org/10.1056/nejmoa1409065
27. Sattler K, Gräler M, Keul P, Weske S, Reimann C-M, Jindrová H, Kleinbongard P, Sabbadini R, Bröcker-Preuss M, Erbel R, Heusch G, Levkau B (2015) Defects of high-density lipoproteins in coronary artery disease caused by low sphingosine-1-phosphate content: correction by sphingosine-1-phosphate-loading. J Am Coll Cardiol. https://doi.org/10.1016/j.jacc.2015.07.057
28. Sattler KJE, Elbasan S, Keul P, Elter-Schulz M, Bode C, Gräler MH, Bröcker-Preuss M, Budde T, Erbel R, Heusch G, Levkau B (2010) Sphingosine 1-phosphate levels in plasma and HDL are altered in coronary artery disease. Basic Res Cardiol. https://doi.org/10.1007/s00395-010-0112-5
29. Spieker LE, Sudano I, Hürlimann D, Lerch PG, Lang MG, Binggeli C, Corti R, Ruschitzka F, Lüscher TF, Noll G (2002) High-density lipoprotein restores endothelial function in hypercholesterolemic men. Circulation 105:1399–1402
30. Tang WHW, Wu Y, Mann S, Pepoy M, Shrestha K, Borowski AG, Hazen SL (2011) Diminished antioxidant activity of high-density lipoprotein-associated proteins in systolic heart failure. Circ Heart Fail. https://doi.org/10.1161/circheartfailure.110.958348
31. Theilmeier G, Schmidt C, Herrmann J, Keul P, Schäfers M, Herrgott I, Mersmann J, Larmann J, Hermann S, Stypmann J, Schober O, Hildebrand R, Schulz R, Heusch G, Haude M, von Wnuck Lipinski K, Herzog C, Schmitz M, Erbel R, Chun J, Levkau B (2006) High-density lipoproteins and their constituent, sphingosine-1-phosphate, directly protect the heart against ischemia/reperfusion injury in vivo via the S1P3 lysophospholipid receptor. Circulation. https://doi.org/10.1161/circulationaha.105.607135
32. Trieb M, Horvath A, Birner-Gruenberger R, Spindelboeck W, Stadlbauer V, Taschler U, Curcic S, Stauber RE, Holzer M, Pasterk L, Heinemann A, Marsche G (2016) Liver disease alters high-density lipoprotein composition, metabolism and function. Biochim Biophys Acta. https://doi.org/10.1016/j.bbalip.2016.04.013
33. Trieb M, Wolf P, Knuplez E, Weger W, Schuster C, Peinhaupt M, Holzer M, Trakaki A, Eichmann T, Lass A, Wadsack C, Schuligoi R, Heinemann A, Marsche G (2019) Abnormal composition and function of high-density lipoproteins in atopic dermatitis patients. Allergy. https://doi.org/10.1111/all.13620
34. Wilson PW, Garrison RJ, Castelli WP, Feinleib M, McNamara PM, Kannel WB (1980) Prevalence of coronary heart disease in the Framingham Offspring Study: role of lipoprotein cholesterols. Am J Cardiol 46:649–654
35. Zewinger S, Drechsler C, Kleber ME, Dressel A, Riffel J, Triem S, Lehmann M, Kopecky C, Säemann MD, Lepper PM, Silbernagel G, Scharnagl H, Ritsch A, Thorand B, las de Heras Gala T, Wagenpfeil S, Koenig W, Peters A, Laufs U, Wanner C, Fliser D, Speer T, März W (2015) Serum amyloid A: high-density lipoproteins interaction and cardiovascular risk. Eur Heart J. https://doi.org/10.1093/eurheartj/ehv352

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