Atherosclerosis Plus 44 (2021) 18–24

Contents lists available at ScienceDirect

Atherosclerosis Plus

journal homepage: www.elsevier.com/locate/atherosclerosis

Cholesterol efflux promoting function of high-density lipoproteins in calcific aortic valve stenosis

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ARTICLE INFO

Article history:
Received 26 May 2021
Received in revised form
5 August 2021
Accepted 6 August 2021
Available online 11 August 2021

Keywords:
Aortic stenosis
Aortic valve calcium score
Lipids
Matrix metalloproteinase
Choline

ABSTRACT

Background and aims: Cholesterol efflux capacity is a functional property of high-density lipoproteins (HDL) reflecting the efficiency of the atheroprotective reverse cholesterol transport process in humans. Its relationship with calcific aortic valve stenosis (CAVS) has not been fully assessed yet.
Methods: We evaluated HDL-CEC in a patient population with varying degrees of aortic valvular calcific disease, assessed using echocardiography and cardiac computed tomography. Measurement of biomarkers that reflect osteogenic and tissue remodeling, along with dietary and gut microbiota-derived metabolites were performed.
Results: Patients with moderate-severe CAVS had significantly lower HDL-CEC compared to both control and aortic sclerosis subjects (mean: 6.09%, 7.32% and 7.26%, respectively). HDL-CEC displayed negative correlations with peak aortic jet velocity and aortic valve calcium score, indexes of CAVS severity ($\rho = -0.298$, $p = 0.002$ and $\rho = -0.358$, $p = 0.005$, respectively). In multivariable regression model, HDL-CEC had independent association with aortic valve calcium score (B: -0.053, SE: 0.014, $p < 0.001$), GFR (B: -0.034, SE: 0.012, $p = 0.007$), as well as with levels of total cholesterol (B: 0.018, SE: 0.005, $p = 0.002$).
Conclusion: These results indicate an impairment of HDL-CEC in moderate-severe CAVS and may contribute to identify potential novel targets for CAVS management.

Introduction

Calcific aortic valve stenosis (CAVS) is a multifactorial phenomenon that courses with aortic valve calcification and hemodynamic obstruction. So far, the only available approach to treat patients with CAVS is surgical or transcatheter aortic valve replacement, rendering the search of novel pharmacological targets an important and still unmet need [1].

Initiation of CAVS shares common cardiovascular risk factors with atherosclerosis including age, male gender, active smoking, hypertension and dyslipidemia [1,2]. Concerning the latter, the role of high-density lipoprotein (HDL) in CAVS was investigated in a few studies although the main focus was plasma levels of this lipoprotein rather than its function. In particular, reduced amount of plasma HDL-cholesterol (HDL-C) [3] and aortic valve-localized apolipoprotein A-I (apoA-I) [4], the main protein component of HDL, have been reported in subjects with aortic stenosis. Conversely, in another study HDL-C levels did not differ between subjects with and without aortic stenosis [5]. *In vitro* studies have suggested HDL to act against aortic valvular calcification through regulation of several proteins involved in maintenance of homeostasis for valvular calcification (i.e. osteoprotegerin and tumor

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https://doi.org/10.1016/j.athplu.2021.08.002

necrosis-alpha) [4]. Furthermore, apolipoprotein A-I mimetic peptide infusions were shown to improve aortic valve area in mice [6] and rabbit [7] models of aortic stenosis.

Notably, in recent human studies, HDL cholesterol efflux capacity (HDL-CEC), a functional property reflecting the efficiency of the atheroprotective reverse cholesterol transport process in humans [8], has emerged as a more robust parameter compared to plasma HDL-C levels to assess the likelihood of angiographically confirmed coronary artery disease [9] and incident cardiovascular disease events [10–12], independently of plasma HDL-C levels. Nonetheless, not all reports univocally indicated CEC as a biomarker of cardiovascular risk [13–15]. Up to now, scarce evidence exists on the potential relationship between HDL cholesterol efflux promoting function and CAVS in humans [5].

In recent years, dietary-derived choline and its metabolism by the gut microbiota and liver into betaine and trimethylamine-N-oxide (TMAO) have drawn interest following the demonstration that dietary supplementation with choline promotes atherogenesis in mice [16]. An association between coronary artery disease and choline metabolites has been successively reported in clinical studies [17,18], whereas the relation of choline levels with CAVS presence and severity has been recently highlighted for the first time [19].

The aim of the study was to evaluate HDL-CEC in a patient population with varying degrees of aortic valvular calcific disease (specifically, i) control, ii) aortic sclerosis (ASc), iii) moderate-severe CAVS). It was also aimed to assess the association of HDL-CEC with baseline demographics and laboratory characteristics (including plasma levels of osteogenic and tissue remodeling biomarkers as well as dietary and gut microbiota-derived metabolites). Thereby, results of the present study sought to suggest new mechanisms underlying CAVS pathogenesis, particularly with regards to HDL functionality.

Materials and methods

Patient population

This study is a subgroup analysis of a case-control study conducted in (1) Department of Cardiology, Hacettepe University Faculty of Medicine (from May 2016 to July 2016) and (2) Department of Cardiovascular Surgery, Medicana International Ankara Hospital (from May 2016 to December 2016). The design and primary study results have been reported previously [19].

In brief, 'cases' were consecutive subjects who were diagnosed with moderate-severe CAVS or ASc, and 'controls' were age and gender-matched subjects without functional or morphological abnormalities in aortic valves [19]. Diagnosis was made on transthoracic echocardiography according to the 2014 American Heart Association/American College of Cardiology guideline on the management of patients with valvular heart disease [20] and recommendations on the echocardiographic assessment of aortic valve stenosis [21,22]. Severity of CAVS was determined based on peak aortic jet velocity, mean pressure gradient and aortic valve area [20–22]. Echocardiographic criteria used to categorize patients are provided in ***Supplementary Materials***.

Subjects who had prior history of cardiovascular disease (myocardial infarction, revascularization, peripheral arterial disease, stroke or transient ischemic attack), chronic liver disease, chronic kidney disease or other systemic diseases that interfere with bone metabolism were not included. Other exclusion criteria are noted in ***Supplementary Materials***. Peripheral venous blood sampling was carried out at the time of echocardiographic evaluation, and after a 12-h fast and 2-h avoidance of smoking and physical exercise. Reference values for the laboratory test results are provided in ***Supplementary Materials***. Of the final study sample, 107 patients had serum samples available for HDL-CEC analysis.

The study was carried in compliance with 1975 Helsinki Declaration. Informed consent was obtained from all participants. The original study protocol was approved by the local ethics committee [2016/04-34 (KA-16031) and an addendum for the subgroup analysis was approved in 2016/09-21 (KA-16031)].

Measurement of HDL-CEC

HDL-CEC was assessed on J774 murine macrophages with a radioisotopic technique, by exposing cells to the apolipoprotein B-depleted serum fraction as previously described [23]. Further details are described in ***Supplementary Materials***.

Measurement of aortic valve calcium score

Aortic valve calcium scores were measured according to the guidelines of the Society of Cardiovascular Computed Tomography [24], and recorded in 60 patients with available prospectively electrocardiogram-triggered non-contrast cardiac computed tomography data (Siemens SOMATOM Definition, Siemens Healthcare, Erlangen, Germany) within 12 months, using syngo. CT CaScoring, Siemens software.

Measurement of osteogenic and tissue remodeling biomarkers

Serum levels of markers related with osteogenesis (namely osteopontin) and tissue remodeling (namely tissue inhibitor of matrix metalloproteinase- 1 [TIMP-1] and matrix metalloproteinase-9 [MMP-9]) were measured by enzyme-linked immunosorbent analysis [25]. Osteopontin (Human Osteopontin PicoKine™ ELISA Kit, Boster, CA, USA), TIMP-1 (Human TIMP-1 PicoKine™ ELISA Kit, Boster, CA, USA) and MMP-9 (LEGEND MAX™ Human MMP-9 ELISA Kit, BioLegend, CA, USA) ELISA kits were used according to the manufacturer's instructions.

Measurement of dietary and gut microbiota-derived metabolites

Measurement of plasma levels of choline and its oxidative metabolites, betaine and TMAO, was undertaken using ultra-performance liquid chromatography-tandem mass spectroscopy method [26].

Statistical analysis

The Kolmogorov-Smirnov test was used to determine the normality of the distribution. Continuous variables were reported as mean ± standard deviation (SD) (for normal distribution) or median (interquartile range [IQR] defined as 25th percentile- 75th percentile) (for skewed distribution). Independent samples *t*-test or Mann-Whitney *U* test was utilized to compare continuous variables with and without normal distribution, respectively, between two groups. To compare three groups of continuous variables with normal distribution, Levene's test for homogeneity of variances was performed. One-way analysis of variance (ANOVA) was done if this assumption was met and Bonferroni post-hoc test was preferred for making multiple comparisons. Skewed variables were compared between three groups using Kruskal-Wallis test. The Chi-square test was used to compare categorical data presented as percentages. Spearman's test was used to determine the correlation between two variables. Strength of the correlation was graded based on the absolute correlation coefficients; values between 0 and 0.3, 0.3–0.7 and 0.7–1.0 indicated weak, moderate and strong association, respectively [27]. To find independent associates of HDL-CEC,

linear regression analysis was done, where variables with p values < 0.2 when compared between groups of < and ≥ median HDL-CEC were included in the univariable model and those that yielded p values < 0.2 in the univariable linear regression analysis were incorporated to the multivariable model (namely, glomerular filtration rate [GFR], HDL-C, low-density lipoprotein-cholesterol [LDL-C], total cholesterol, MMP-9, choline, peak aortic jet velocity, aortic valve calcium score). SPSS software was utilized for statistical analysis (Version 20.0. Armonk, NY: IBM Corp.). Statistical significance was defined as a p value < 0.05.

RESULTS

Among 107 patients included in the analysis, number of subjects categorized into control, ASc and moderate-severe CAVS groups were 30, 34 and 43, respectively. Baseline characteristics of the study population is shown in Table 1.

Patients with moderate-severe CAVS, reflected by marked increased peak aortic jet velocity and aortic valve calcium score, were older than the controls (median: 74.00 vs. 61.50 years, p = 0.007) but had similar age distribution to that of patients with aortic sclerosis (vs. median: 70.00 years, p = 0.881). No significant difference regarding gender distribution existed within controls (43.3% male) and cases of ASc (47.1% male) and moderate-severe CAVS (44.2% male) (between groups p value = 0.950) (Table 1).

Concerning lipids, HDL-C was significantly lower in patients with moderate-severe CAVS compared to controls (median: 45.00 vs. 50.00 mg/dL, p = 0.040) (Table 1). In regards of HDL function, HDL-CEC was lower in patients with moderate-severe CAVS compared to both controls and patients with ASc (mean: 6.09 ± 1.98%, 7.32 ± 1.45% and 7.26 ± 1.99%, respectively) (Fig. 1).

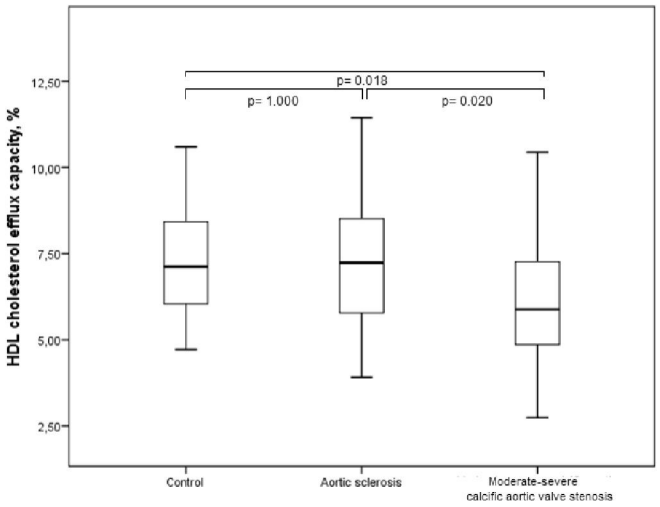

Fig. 1. Cholesterol efflux capacity within controls and patients with aortic sclerosis and moderate-severe calcific aortic valve stenosis The box displays median values with the 25th and 75th percentiles; the whiskers represent minimum and maximum values. One-way ANOVA with Bonferroni post-hoc test was utilized to compare cholesterol efflux capacity between groups.

Mean HDL-CEC of the study population was 6.80 ± 1.93% and the median was 6.71 (IQR: 2.69%). By stratifying subjects according to

Table 1
Baseline characteristics of the study population.

	Control (G1) (n = 30)	Aortic sclerosis (G2) (n = 34)	Moderate- severe CAVS (G3) (n = 43)	p value
Clinical characteristics				
Age, years	61.50 (17.25)	70.00 (16.50)	74.00 (13.00)	0.009[b]
Gender: male, n (%)	13 (43.3)	16 (47.1)	19 (44.2)	0.950
Hypertension, n (%)	22 (73.3)	30 (88.2)	37 (86.0)	0.229
Diabetes mellitus, n (%)	10 (33.3)	13 (38.2)	23 (53.5)	0.184
Current smoking, n (%)	4 (13.3)	6 (17.6)	6 (14.0)	0.865
Statin therapy, n (%)	7 (23.3)	8 (23.5)	13 (30.2)	0.735
Laboratory test results				
ALT, U/L	20.50 (11.75)	16.50 (8.00)	19.00 (17.00)	0.205
GFR (CKD-EPI), mL/min/1.73 m^2	96.50 (13.15)	84.00 (23.40)	91.50 (34.00)	0.319
Fasting blood glucose, mg/dL	100.00 (16.75)	95.50 (19.75)	105.00 (30.25)	0.250
HDL-C, mg/dL	50.00 (16.50)	46.00 (11.00)	45.00 (11.00)	0.043[b]
LDL-C, mg/dL	134.50 ± 27.61	135.47 ± 38.68	138.23 ± 25.89	0.865
Triglyceride, mg/dL	120.50 (111.50)	115.00 (73.25)	133.50 (89.75)	0.112
Total cholesterol, mg/dL	218.50 (48.15)	202.40 (48.18)	214.40 (40.20)	0.235
HDL-CEC				
HDL-CEC, %	7.32 ± 1.45	7.26 ± 1.99	6.09 ± 1.98	0.006[b,c]
Osteogenic and tissue remodeling biomarkers				
OPN, pg/mL	2780.00 (2127.25)	3450.00 (1434.00)	3210.00 (2650.00)	0.439
MMP-9, pg/mL	2898.50 (470.50)	3123.00 (684.00)	3231.00 (436.00)	0.003[a,b]
TIMP-1, pg/mL	605.00(405.50)	460.00 (424.00)	396.00 (216.00)	<0.001[b]
Dietary and gut microbiota-derived metabolites				
Choline, μM	12.14 (2.40)	12.41 (3.31)	14.63 (4.30)	<0.001[b,c]
Betaine, μM	52.57 (19.06)	48.59 (24.47)	45.62 (20.80)	0.370
TMAO, μM	2.95 (3.73)	3.17 (2.33)	3.81 (4.19)	0.654
CAVS severity imaging assessment				
AVmax, m/s	1.24 (0.30)	1.73 (0.31)	4.32 (0.85)	NA[d]
AVC score[a], AU	0 (0)	0 (184.25)	1910 (1342.25)	NA[d]

ALT alanine transaminase, AU Agatston unit, AVmax peak aortic jet velocity, AVC aortic valve calcium, CAVS calcific aortic valve stenosis, CEC cholesterol efflux capacity, GFR glomerular filtration rate, HDL-C high-density lipoprotein-cholesterol, LDL-C low-density lipoprotein-cholesterol, MMP matrix metalloproteinase, NA non-available, OPN osteopontin, TC total cholesterol, TIMP tissue inhibitor of matrix metalloproteinase, TMAO trimethylamine N-oxide.

[a]Performed in subjects with prior CT scans (n = 60).

[a] Denotes statistical significance (p < 0.05) when compared between G1 and G2.

[b] Denotes statistical significance (p < 0.05) when compared between G1 and G3.

[c] Denotes statistical significance (p < 0.05) when compared between G2 and G3.

[d] p values are not reported for the two variables that were employed to define G1-3.

Table 2
Baseline characteristics of the study population stratified for < and ≥ median HDL-CEC (6.71%).

	HDL-CEC <6.71% (n = 53)	HDL-CEC ≥6.71% (n = 54)	p value
Age, years	72 (15)	72 (18.75)	0.988
Gender: male, n (%)	23 (43.4)	25 (46.3)	0.763
Hypertension, n (%)	45 (84.9)	44 (81.5)	0.636
Diabetes mellitus, n (%)	23 (43.4)	23 (42.6)	0.933
Current smoking, n (%)	8 (15.1)	8 (14.8)	0.968
Statin therapy, n (%)	16 (30.2)	12 (22.2)	0.349
Alanine transaminase, U/L	16.50 (12.00)	21.00 (16.50)	0.101
GFR (CKD-EPI), mL/min/1.73 m^2	95.00 (19.00)	86.00 (23.40)	0.121
Fasting blood glucose, mg/dL	98.50 (20.25)	100.00 (28.50)	0.501
HDL-cholesterol, mg/dL	45.00 (9.80)	49.00 (17.00)	0.023*
LDL-cholesterol, mg/dL	128.75 ± 27.81	143.17 ± 31.41	0.016*
Triglyceride, mg/dL	123.00 (101.50)	133.00 (83.50)	0.768
Total cholesterol, mg/dL	207.21 (46.20)	222.60 (43.80)	0.011*
Osteopontin, pg/mL	3210.00 (1969.25)	3450.00 (2166.50)	0.957
MMP-9, pg/mL	3234 (467.75)	3041.50 (404.75)	0.021*
TIMP-1, pg/mL	487.00 (333.50)	499.00 (374.00)	0.685
Choline, μM	13.10 (4.48)	12.62 (3.32)	0.191
Betaine, μM	47.00 (24.31)	49.41 (22.99)	0.430
TMAO, μM	3.32 (3.14)	3.34 (15.90)	0.847
AVmax, m/s	2.62 (2.82)	1.71 (2.07)	0.019*
AVC score, AU (n = 60)	131 (1900)	0 (1200)	0.193

AVC aortic valve calcium, AVmax peak aortic jet velocity, GFR glomerular filtration rate, HDL high-density lipoprotein, LDL low-density lipoprotein, MMP matrix metalloproteinase, TIMP tissue inhibitor of matrix metalloproteinase, TMAO trimethylamine N-oxide.
* p value < 0.05 demonstrates statistical significance.

Table 3
Bivariate correlation analysis for HDL cholesterol efflux capacity with other baseline characteristics.

Age, years	Spearman correlation coefficient	-0.099
	P value	0.312
Alanine transaminase, U/L	Spearman correlation coefficient	0.101
	P value	0.305
Glomerular filtration rate (CKD-EPI), mL/min/1.73 m^2	Spearman correlation coefficient	-0.207
	P value	0.033*
Fasting blood glucose, mg/dL	Spearman correlation coefficient	0.024
	P value	0.813
HDL-cholesterol, mg/dL	Spearman correlation coefficient	0.209
	P value	0.041*
Low-density lipoprotein cholesterol, mg/dL	Spearman correlation coefficient	0.199
	P value	0.044*
Triglyceride, mg/dL	Spearman correlation coefficient	0.036
	P value	0.717
Total cholesterol, mg/dL	Spearman correlation coefficient	0.256
	P value	0.011*
Osteopontin, pg/mL	Spearman correlation coefficient	-0.065
	P value	0.524
Matrix metalloproteinase-9, pg/mL	Spearman correlation coefficient	-0.251
	P value	0.013*
Tissue inhibitor of matrix metalloproteinase-1, pg/mL	Spearman correlation coefficient	0.040
	P value	0.698
Peak aortic jet velocity, m/s	Spearman correlation coefficient	-0.298
	P value	0.002*
Aortic valve calcium score, AU (n = 60)	Spearman correlation coefficient	-0.358
	P value	0.005*
Choline, μM	Spearman correlation coefficient	-0.216
	P value	0.026*
Betaine, μM	Spearman correlation coefficient	-0.137
	P value	0.162
Trimethylamine N-oxide, μM	Spearman correlation coefficient	0.010
	P value	0.920

HDL high-density lipoprotein.
* p value < 0.05 demonstrates statistical significance.

this median value, we observed that patients with lower HDL-CEC also had significantly lower HDL-C (median: 45.00 vs. 49.00 mg/dL, p = 0.023), LDL-C (mean: 128.75 vs. 143.17 mg/dL, p = 0.016), total cholesterol (mean: 202.61 vs. 221.57 mg/dL, p = 0.012), higher MMP-9 (median: 3234 vs. 3041.50 pg/mL, p = 0.021) levels and higher peak aortic jet velocity (median: 2.62 vs 1.71 m/s, p = 0.019) (Table 2).

Correlations between HDL-CEC and baseline demographics and laboratory characteristics are given in Table 3. Weak positive correlations existed between HDL-CEC and HDL-C (ρ = 0.209, p = 0.041), LDL-C (ρ = 0.199, p = 0.044) and total cholesterol (ρ = 0.256, p = 0.011). Among other laboratory tests, HDL-CEC had weak negative correlations with GFR (ρ = -0.207, p = 0.033); levels of MMP-9 (ρ = -0.251, p = 0.013) and choline (ρ = -0.216,

p = 0.026). No significant correlations were observed between HDL-CEC and other biomarkers of osteogenesis and tissue remodeling (namely osteopontin and TIMP-1) or levels of the choline oxidative-metabolites betaine and TMAO (all p > 0.05). With regards to parameters that define the severity of CAVS, HDL-CEC displayed a weak negative correlation with peak aortic jet velocity (ρ = -0.298, p = 0.002) and a moderate negative correlation with aortic valve calcium score (ρ = -0.358, p = 0.005) (Table 3). Within each group of subjects, the relationship between aortic valve calcium score and HDL-CEC% were not significant (r = 0.030, p = 0.879; r = -0.157, p = 0.666 and r = -0.204, p = 0.363 in controls, patients with ASc and moderate-severe CAVS, respectively).

Linear regression analysis was performed to identify independent associates of HDL-CEC, including laboratory test variables that had p values < 0.2 in Table 2 (alanine transaminase, GFR, HDL-C, LDL-cholesterol, total cholesterol, MMP-9, choline, peak aortic jet velocity, aortic valve calcium score). Results of the multivariable linear regression analysis, where the model included variables with p values < 0.2 in the univariable linear regression test (GFR, HDL-C, LDL-cholesterol, total cholesterol, MMP-9, choline, peak aortic jet velocity, aortic valve calcium score), showed that HDL-CEC was independently associated with aortic valve calcium score (B: -0.053, SE: 0.014, p < 0.001), GFR (B: -0.034, SE: 0.012, p = 0.007), as well as with levels of total cholesterol (B: 0.018, SE: 0.005, p = 0.002) (Table 4).

Discussion

The main findings of our study may be summarized as follows: 1) HDL-CEC is impaired with a pattern depending on severity of CAVS, 2) HDL-CEC has negative weak and moderate correlations with peak aortic jet velocity and aortic valve calcium score, respectively, which are established diagnostic and prognostic imaging parameters in CAVS, 3) HDL-CEC is independently associated with aortic valve calcium score, GFR and total cholesterol.

In details, an impairment in HDL-CEC was noted in moderate-severe CAVS compared to both control and ASc subjects. HDL-CEC not only had a negative correlation with the hemodynamic severity of the disease identified on echocardiography (peak aortic jet velocity) but also with the valvular calcium load identified on computed tomography (aortic valvular calcium score). In this regard, the only other clinical study that investigated HDL functionality in patients with (n = 86) and without (n = 86) aortic stenosis of similar distributions of age, gender and presence of coronary artery disease, reported no impairment of total HDL-CEC in aortic stenosis [5]. The reasons of the discrepancy compared to our results are far from being completely clear and may be related to several factors. First, the inclusion criteria were different, since we excluded subjects with prior diagnosis of cardiovascular disease, while patients with history of coronary artery disease were included in the previously published work [5]. Another possible explanation may be related to the slightly different assay to evaluate HDL-CEC. We have measured total HDL-CEC from cyclic adenosine monophosphate (cAMP)-stimulated J774 macrophages, in which the major contributions are from aqueous diffusion and the ATP-binding cassette A1 (ABCA1)-mediated efflux [28], whereas Arsenault and colleagues used cholesterol-loaded and cAMP-stimulated J774 [5]. Cholesterol loading may induce overexpression of other cholesterol transporters besides ABCA1 [29], and the contribution of additional cholesterol efflux pathways may have blunted the differences that we instead observed. This hypothesis fits with higher absolute efflux percentages measured in the previous work compared to ours [5]. However, since cAMP-incubated macrophages are the most widely used cell model to investigate association with CV risk [30,31], we feel confident in drawing reliable conclusions on the relationship between CEC and CAVS. Finally, mild aortic stenosis patients were included in the case group in the prior study [5], which may have also contributed to the lack of statistically significant difference with regards to HDL-CEC between two groups.

Beside the aforementioned study, other works have examined the association between HDL-CEC and coronary calcium score (CAC). Using the same cell model, murine macrophages J774 incubated with cAMP, we previously demonstrated no significant relationship between HDL-CEC and CAC in a cohort of very old, healthy individuals [15]. Differently, in obese subjects a paradoxical positive association was observed between the HDL-CEC specifically mediated by ABCA1 and CAC [32]. Considering the limited number of available data, further evidence is needed to elucidate the relationship between the capacity of HDL to remove cholesterol from macrophages and the calcium deposition processes, either in the arterial wall or specifically at the valve district.

Levels of total cholesterol were independently associated with HDL-CEC in our study. The positive relation between total cholesterol and HDL-CEC is consistent with results of a previous study in pre-clinical models [33] and of a substudy of the PREVEND (Prevention of Renal and Vascular End-stage Disease) cohort that assessed the role of HDL-CEC in predicting incident cardiovascular disease events at follow-up of 12 years (n = 8267) [11]. In 351 cases with incident CVD events and 354 matched controls, a significant positive weak correlation existed between total cholesterol and HDL-CEC when adjusted for age, sex and HDL-C [11].

Our findings suggest the existence of an independent and negative association between HDL-CEC and the GFR, index of kidney functionality. Indeed, this result is not surprising, since this inverse relationship has been previously observed in patients at

Table 4
Linear regression analysis to identify associates of HDL cholesterol efflux capacity.

Variable	UNIVARIATE LINEAR REGRESSION MODEL		MULTIVARIABLE LINEAR REGRESSION MODEL	
	Unstandardized B coefficient, standard error	p value	Unstandardized B coefficient, standard error	p value
ALT, U/L	0.010, 0.015	0.513	–	–
GFR (CKD-EPI), mL/min/1.73 m^2	-0.024, 0.011	0.038*	-0.034, 0.012	0.007*
HDL-cholesterol, mg/dL	0.025, 0.018	0.169	-0.010, 0.016	0.539
LDL-cholesterol, mg/dL	0.013, 0.006	0.035*	-0.012, 0.019	0.536
Total cholesterol, mg/dL	0.012, 0.005	0.017*	0.018, 0.005	0.002*
MMP-9, pg/mL	-0.001, 0.000	0.032*	-0.001, 0.001	0.242
Choline, μM	-0.140, 0.058	0.018*	-0.108, 0.079	0.177
Peak aortic jet velocity, m/s	-0.360, 0.125	0.005*	0.263, 0.199	0.194
Aortic valve calcium score, 100 AU[a] (n = 60)	-0.045, 0.015	0.005*	-0.053, 0.014	<0.001*

ALT alanine transaminase, GFR glomerular filtration rate, HDL high-density lipoprotein, LDL low-density lipoprotein, MMP-9 matrix metalloproteinase-9.
* p value < 0.05 demonstrates statistical significance.
[a] Reflects each 100 AU increase in aortic valve calcium score.

different degrees of renal impairment [34]. Although the mechanistic link explaining this association is still far from being understood, an increased production of nascent pre-β1 HDL resulting from reduced activity of LCAT, the enzyme responsible for plasma HDL maturation [35] may be suggested. In fact, a role of LCAT deficiency in predicting chronic kidney disease has been recently observed not only in renal impaired patients [35,36], but also in subjects with normal GFR values, comparable to these of our study [37]. It will be interesting in a future study to evaluate LCAT activity and the serum pre-β1 content in our cohort of subjects, investigating their relationship with CAVS severity.

In situ hybridization and immunohistochemical studies performed in excised human aortic valves with CAVS have demonstrated that MMP-9 was exclusively located in proximity to the calcific nodules [38,39]. We found a negative association between HDL-CEC from ABCA1-stimulated macrophages and MMP-9, but the significance was lost after multivariable regression analysis. In any case, the relationship once again points to the involvement of cholesterol efflux promoting function of HDL in mechanisms underlighting atheroprotection and the absence of an independent association may be the result of the small sample size. For instance, a previous study suggested that proteases released from inflammatory cells lead to reduction of the apoA-I-containing preβ-migrating HDL and other lipid-deprived HDL particles resulting in reduced ABCA1-mediated cholesterol removal from the foam cells [40,41]. Similarly, incubation of apolipoprotein A-I with MMP-8 in MMP-8 deficient mice was shown to reduce its capacity of facilitate cholesterol efflux [42].

As demonstrated in a study previously published by our group, serum choline levels displayed moderate positive correlations with determinants of CAVS, specifically peak aortic jet velocity and aortic valvular calcium score [19], and are independently associated with peak aortic jet velocity [19]. Previous work indicated that high plasma choline levels are associated to low levels of HDL-C in various populations [43,44]. Findings of the current study indicate an association between HDL-CEC and plasma choline, although the significance was lost after adjustment. Further investigation conducted on a wider number of subjects with a strict diet control, in order to estimate choline intake and quantify metabolite plasma levels, will be necessary to explore the robustness of this association.

Study limitations

The major limitation of this study is its small study sample, and additional work is required to conclude on the relationship between HDL-CEC and CAVS. Another limitation of this study is its observational nature that does not allow establishing a causal relationship and the mechanistic explanation of this link. Third, the younger age of controls compared to moderate-severe CAVS patients in our study may be an additional limitation. However the inclusion of patients with age-matched ASc as a separate group and the observed statistically significant difference in HDL-CEC extent between them and those with moderate-severe CAVS suggests that impairment in HDL-CEC was independent from age.

Conclusions

This study shows that HDL-CEC is impaired in moderate-severe CAVS when compared to patients with normal aortic valves and ASc. In addition, HDL-CEC is independently associated with aortic valve calcium score, GFR and total cholesterol. Whether this complex interplay between different factors presents a common denominator in the context of CAVS remains to be established and deserves further investigations.

Financial support

This work was supported by Turkish Society of Cardiology (Project number: 2016/03) and Hacettepe University Scientific Research Coordination Unit (Project number: 2017/11-17).

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by DK, FZ and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Competing interest

Prof. Lale Tokgozoglu (LT) has served as a company consultant to Abbott, Amgen, Bayer, MSD, Mylan, Novartis, Sanofi. LT has received company speaker honorarium from Abbott, Actelion, Amgen, Bayer, Daiichi Sankyo, MSD, Mylan, Novartis, Novo Nordisk, Sanofi, Servier, Pfizer, Recordati, Abdi-İbrahim. LT has participated in trials of Amgen.

Other authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.athplu.2021.08.002.

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