



3-31-2021

Fontan-Associated Dyslipidemia

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Repository Citation

Lubert, Adam M.; Alsaied, Tarek; Palermo, Joseph J.; Anwar, Nadeem; Urbina, Elaine M.; Brown, Nicole M.; Alexander, Craig; Almeneisi, Hassan; Wu, Fred; Leventhal, Andrew R.; Aldweib, Nael; Mendelson, Michael; and Opotowsky, Alexander R., "Fontan-Associated Dyslipidemia" (2021). *Gill Heart & Vascular Institute Faculty Publications*. 27.

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Digital Object Identifier (DOI)

<https://doi.org/10.1161/JAHA.120.019578>

Notes/Citation Information

Published in *Journal of the American Heart Association*, v. 10, no. 7, 019578.

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ORIGINAL RESEARCH

Fontan-Associated Dyslipidemia

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BACKGROUND: Hypocholesterolemia is a marker of liver disease, and patients with a Fontan circulation may have hypocholesterolemia secondary to Fontan-associated liver disease or inflammation. We investigated circulating lipids in adults with a Fontan circulation and assessed the associations with clinical characteristics and adverse events.

METHODS AND RESULTS: We enrolled 164 outpatients with a Fontan circulation, aged ≥ 18 years, in the Boston Adult Congenital Heart Disease Biobank and compared them with 81 healthy controls. The outcome was a combined outcome of nonelective cardiovascular hospitalization or death. Participants with a Fontan (median age, 30.3 [interquartile range, 22.8–34.3 years], 42% women) had lower total cholesterol (149.0 \pm 30.1 mg/dL versus 190.8 \pm 41.4 mg/dL, $P < 0.0001$), low-density lipoprotein cholesterol (82.5 \pm 25.4 mg/dL versus 102.0 \pm 34.7 mg/dL, $P < 0.0001$), and high-density lipoprotein cholesterol (42.8 \pm 12.2 mg/dL versus 64.1 \pm 16.9 mg/dL, $P < 0.0001$) than controls. In those with a Fontan, high-density lipoprotein cholesterol was inversely correlated with body mass index ($r = -0.30$, $P < 0.0001$), high-sensitivity C-reactive protein ($r = -0.27$, $P = 0.0006$), and alanine aminotransferase ($r = -0.18$, $P = 0.02$) but not with other liver disease markers. Lower high-density lipoprotein cholesterol was independently associated with greater hazard for the combined outcome adjusting for age, sex, body mass index, and functional class (hazard ratio [HR] per decrease of 10 mg/dL, 1.37; 95% CI, 1.04–1.81 [$P = 0.03$]). This relationship was attenuated when log high-sensitivity C-reactive protein was added to the model (HR, 1.26; 95% CI, 0.95–1.67 [$P = 0.10$]). Total cholesterol, low-density lipoprotein cholesterol, and triglycerides were not associated with the combined outcome.

CONCLUSIONS: The Fontan circulation is associated with decreased cholesterol levels, and lower high-density lipoprotein cholesterol is associated with adverse outcomes. This association may be driven by inflammation. Further studies are needed to understand the relationship between the severity of Fontan-associated liver disease and lipid metabolism.

Key Words: adult congenital heart disease ■ Fontan ■ high-density lipoprotein cholesterol ■ lipids ■ single ventricle

Patients born with a functional single ventricle typically undergo a series of operations that culminate in a Fontan procedure, which creates a circulation where systemic venous blood flows to the pulmonary arteries without the benefit of a subpulmonary ventricular pump. This leads to elevated venous pressure, constrained systemic ventricular preload, and relatively low cardiac output. Long-term sequelae include heart failure, arrhythmia, liver fibrosis/cirrhosis, and premature death. However, predicting which patients are at highest risk of adverse outcomes remains difficult.

Circulating biomarkers that can aid in risk stratification would be useful to guide care in this challenging population.

Several studies have evaluated circulating biomarkers in patients with a Fontan circulation.^{1–4} Further biomarker research may aid in better understanding the Fontan circulation and, more directly, a set of biomarkers representing orthogonal pathophysiologies may be able to identify patients at risk for adverse outcomes. Serum lipid levels are an inexpensive, potentially useful biomarker in patients with

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Supplementary Material for this article is available at <https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.019578>

For Sources of Funding and Disclosures, see page 9.

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CLINICAL PERSPECTIVE

What is New?

- Adults with a Fontan circulation had lower total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol (HDL-C) than healthy controls.
- HDL-C in patients with a Fontan was associated with higher alanine aminotransferase and lower albumin but not with other markers of liver disease severity, including Model for End-stage Liver Disease Excluding INR or VAST score (features of portal hypertension: 1 point each for varices, ascites, splenomegaly, or thrombocytopenia).
- Lower HDL-C was independently associated with the composite outcome of nonelective cardiovascular hospitalization or death; the relationship between HDL-C and the composite outcome was attenuated when adjusting for high-sensitivity C-reactive protein.

What are the Clinical Implications?

- Low HDL-C in adults with a Fontan circulation is associated with adverse outcomes and this may be driven by chronic inflammation.
- Further studies are needed to understand the relationship between the severity of Fontan-associated liver disease and lipid metabolism.

Nonstandard Abbreviations and Acronyms

FALD	Fontan-associated liver disease
MELD	Model for End-stage Liver Disease
MELD-XI	Model for End-stage Liver Disease Excluding INR
VAST	Imaging features of portal hypertension: 1 point each for varices, ascites, splenomegaly, or thrombocytopenia

a Fontan. A prior study including 88 patients, mostly in the pediatric age range, reported lower cholesterol in those with a Fontan circulation compared with healthy controls.⁵ Since cholesterol is synthesized in the liver, decreased total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) may be caused by liver dysfunction from Fontan-associated liver disease (FALD).⁶ In other chronic liver diseases, hypocholesterolemia indicates more severe liver dysfunction, and HDL-C may be the best indicator of the degree of liver disease and prognosis.^{7,8}

This study aims to increase our understanding of lipid levels, focusing on HDL-C, in patients with a Fontan circulation. We compared lipid levels between a relatively large cohort of prospectively enrolled adults with a Fontan circulation and a control group. Associations between lipid levels, clinical characteristics, and clinical outcomes were analyzed. We hypothesized that lipid levels would be lower in patients with a Fontan and that lower cholesterol levels would be associated with an increased risk for adverse cardiac outcomes in patients with a Fontan circulation.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Sample

We evaluated patients aged ≥ 18 years with a Fontan circulation who were enrolled prospectively in the Boston Adult Congenital Heart Disease Biobank between March 15, 2012, and May 1, 2019. We excluded patients with a history of Fontan circulation who had subsequently undergone 2-ventricle repair or cardiac transplantation before enrollment. We also included controls enrolled in the Biobank who were aged < 61 years, with the age cutoff based on the age of the oldest participant with Fontan. Controls were nonsmokers without diabetes mellitus or known cardiovascular disease who were family/friends of patients with congenital heart disease or who were recruited using postings on the Boston Children's Hospital website. The study was approved by the Boston Children's Hospital institutional review board, and written informed consent was obtained from all participants; there was a formal reliance agreement between the institutional review boards of Partners HealthCare/Brigham and Women's Hospital and Boston Children's Hospital.

Lipid and Other Laboratory Assessment

Laboratory testing was performed on nonfasting samples. Blood was collected in a serum separator tube and allowed to clot for 30 to 60 minutes at room temperature, followed by centrifugation at 1300 g for 10 minutes at 4°C. Triglycerides, total cholesterol, and HDL-C were measured using an enzymatic, colorimetric method (Cholesterol Gen.2, Roche Diagnostics) meeting standard goals of $\leq 3\%$ precision and bias by a Clinical Laboratory Improvement Amendments–approved laboratory (LabCorp, Laboratory Corporation of America). LDL-C was calculated using the Friedewald equation. Patients with

markedly elevated triglycerides (>400 mg/dL; n=3 Fontan, n=1 control) were excluded from analyses of LDL-C but were included in other analyses. Non-HDL-C was calculated as total cholesterol minus HDL-C. hs-CRP (high-sensitivity C-reactive protein) was measured from these fresh serum samples using a latex particle-enhanced immunoturbidimetric assay (Roche Diagnostics) with a lower limit of detection of 0.1 mg/L and between-run coefficients of variation of 3.1% and 2.3% at mean values of 1.5 mg/L and 11.4 mg/L, respectively. A subset of the blood was collected in an EDTA tube and processed to plasma by centrifugation at 1300 g for 10 minutes at 4°C, and then aliquoted and frozen at -80°C. All measurements were performed on specimens with no prior freeze-thaw cycles. NT-proBNP (N-terminal pro-B-type natriuretic peptide) was measured in a research laboratory using an electrochemiluminescence immunoassay (Roche Diagnostics). Other assays were performed as previously outlined.⁹

Clinical and Outcomes Assessment

Demographic, anthropometric, and other clinical data, including age at Fontan, type of Fontan, primary congenital heart disease diagnosis, and medications were extracted from medical records. Imaging and laboratory data were used to calculate Model for End-stage Liver Disease Excluding INR (MELD-XI)¹⁰ and VAST (features of portal hypertension: 1 point each for varices, ascites, splenomegaly, or thrombocytopenia) scores.¹¹ If the presence of a variable for the VAST score was not assessed, then it was assumed that the patient was negative for that variable. The primary outcomes of interest were all-cause mortality and a combined outcome of nonelective cardiovascular hospitalization or death. Nonelective cardiovascular hospitalization was defined as an overnight admission for heart failure, arrhythmia or symptoms of arrhythmia, thrombotic or embolic events, cerebral hemorrhage, or a specific Fontan-related complication (ie, ascites, protein-losing enteropathy, plastic bronchitis).

Statistical Analysis

Continuous variables are presented as mean±SD for normally distributed variables and median (interquartile range) for non-normally distributed variables. We also categorized HDL-C according to tertile to assess for associations with clinical variables. Unadjusted linear regression was used to compare continuous variables between 2 groups, while either the chi-square or Fisher exact test was used to analyze categorical variables between groups. Variables with heavily skewed distributions (eg, NT-proBNP, hs-CRP, and triglycerides) were natural log-transformed.

Associations between continuous variables were assessed using Pearson product-moment correlation. The log-rank test and Cox regression were used to perform univariate and multivariable survival analysis, respectively. Time-to-event was defined from a time origin of the date of biospecimen collection until the date of the first clinical event, with censoring of event-free individuals at the most recent clinical follow-up date when event status was known. Multivariable models adjusted for age, sex, body mass index (BMI), and New York Heart Association class I versus II and higher. The assumption of proportional hazards was assessed for each model, and there was no apparent violation.

SAS version 9.4 (SAS Institute Inc.) and GraphPad Prism (GraphPad Software) were used for analyses. A 2-sided *P* value <0.05 was considered statistically significant.

RESULTS

Baseline demographic and clinical data for patients with a Fontan (n=164) and for controls (n=81) are shown in Table 1. Additional baseline characteristics of the Fontan cohort are shown in Table 2. Compared with controls, patients with a Fontan circulation had 22% lower total cholesterol (149.0±30.1 mg/dL versus 190.8±41.4 mg/dL, *P*<0.0001; adjusted for age, sex, and BMI), 33% lower HDL-C (42.8±12.2 mg/dL versus 64.1±16.9 mg/dL, *P*<0.0001), 19% lower LDL-C (82.5±25.4 mg/dL versus 102.0±34.7 mg/dL, *P*<0.0001), and 16% lower non-HDL-C (106.1±32.6 mg/dL versus 126.7±42.5 mg/dL, *P*<0.0001) (Figure 1). There was no difference in triglyceride level (119.3±85.2 mg/dL versus 124.4±84.8 mg/dL, Wilcoxon rank sum test *P*=0.82). Only 4 patients (2.4%) with a Fontan circulation were taking a statin, and those 4 patients had similar HDL (37.5±3.9 mg/dL) and slightly higher LDL (100±36.7 mg/dL) levels compared with patients with a Fontan not taking a statin. One of the 4 patients taking a statin had heterozygous familial hypercholesterolemia (HDL=33, LDL=142); excluding this patient did not affect the findings substantially. No patient was taking a nonstatin lipid medication, and no control participant was taking a lipid-lowering medication.

Among the 164 patients with a Fontan circulation, HDL-C inversely correlated with BMI (*r*=-0.30, *P*<0.0001), LDL-C (*r*=-0.25, *P*=0.002), triglycerides (*r*=-0.40, *P*<0.0001), and hs-CRP (*r*=-0.27, *P*=0.0006) (Figure 2). HDL-C positively correlated with percent predicted peak oxygen consumption on cardiopulmonary exercise testing (*r*=0.28, *P*=0.001). Correlations between lipid levels, clinical factors, and laboratory values are presented in Table 3. HDL-C was not associated

with age at the time of measurement or the duration of time since the Fontan procedure; however, female sex was significantly associated with higher HDL-C (Table 4). HDL-C correlated modestly with alanine aminotransferase ($r=-0.18$, $P=0.02$) and albumin ($r=0.18$, $P=0.02$). However, there were no other statistically significant associations between HDL-C and laboratory tests associated with liver disease including aspartate aminotransferase, alkaline phosphatase, platelet count, VAST score, or MELD-XI score (Tables 3 and 4).

Outcomes Analysis

The median follow-up duration was 2.8 years (interquartile range, 1.2–5.5 years). The composite outcome of death or nonelective cardiovascular hospitalization occurred in 56 patients (34%), including 15 deaths (9%). Causes of death included heart failure ($n=3$, 20%), arrhythmia or sudden death ($n=3$, 20%), infection ($n=2$, 13%), procedural complication ($n=1$, 7%), hemoptysis ($n=1$, 7%), hepatocellular carcinoma ($n=1$, 7%), and unknown ($n=4$, 27%). Factors associated with the composite outcome are shown in Table S1.

Lower HDL-C was associated with a greater risk of the combined outcome of death or nonelective hospitalization (unadjusted hazard ratio [HR] per decrease of 10 mg/dL, 1.30; 95% CI, 1.01–1.67 [$P=0.045$], C statistic=0.575). In a multivariable model adjusting for age, sex, BMI, and New York Heart Association functional

class I versus II and higher, HDL-C remained associated with a greater hazard for the combined outcome (HR per decrease of 10 mg/dL, 1.37; 95% CI, 1.04–1.81 [$P=0.0264$], model C statistic=0.690). Adding log hs-CRP to the multivariable model attenuated the relationship of HDL-C with the combined outcome (HR per decrease of 10 mg/dL, 1.26; 95% CI, 0.95–1.67 [$P=0.10$], model C statistic=0.708). The addition of other variables associated with the outcome (Table S1) did not attenuate the relationship between HDL-C and the composite outcome to the same extent (eg, HR, 1.35 [95% CI, 1.02–1.80]; HR, 1.30 [95% CI, 0.98–1.72]; HR, 1.31 [95% CI, 0.99–1.72]; and HR, 1.38 [95% CI, 1.03–1.85] adjusting the model for hemoglobin, albumin, VAST score, and log NT-BNP, respectively, rather than for log hs-CRP).

In contrast to HDL-C, no other lipid level was associated with the combined outcome: total cholesterol (HR per decrease of 10 mg/dL, 0.95; 95% CI, 0.86–1.05 [$P=0.88$], model C statistic=0.549); LDL-C (HR per decrease of 10 mg/dL, 0.91; 95% CI, 0.81–1.02 [$P=0.10$], model C statistic=0.570); and log triglycerides (HR, 0.84; 95% CI, 0.50–1.42 [$P=0.52$], model C statistic=0.518). No lipid variable was associated with all-cause mortality, although the point estimate of the effect size was similar to that observed for the composite outcome (eg, for HDL-C: HR per decrease of 10 mg/dL, 1.26; 95% CI, 0.78–2.05 [$P=0.339$], C statistic=0.583).

Table 1. Baseline Demographic and Clinical Data for Patients With a Fontan Circulation and Controls

	Fontan (n=164)	Controls (n=81)	Unadjusted P Value	Adjusted P Value
Age, y	30.3 (22.8–34.4)	34.8 (23.9–44.4)	0.003	0.002
Female	69 (42)	61 (75)	<0.0001	<0.0001
Body mass index, kg/m ²	25.2±4.6	24.9±5.3	0.65	0.64
Creatinine, mg/dL	0.87±0.2	0.78±0.1	0.0002	<0.0001
AST, U/L	26.6±9.1	20.6±6.1	<0.0001	<0.0001
ALT, U/L	27.7±12.3	18.5±13.5	<0.0001	<0.0001
Alkaline phosphatase, mg/dL	88.1±30.5	62.5±17.8	<0.0001	<0.0001
Bilirubin, total, mg/dL	1.0±0.9	0.5±0.3	<0.0001	<0.0001
Albumin, gm/dL	4.7±0.5	4.6±0.3	0.31	0.34
Platelet count, K/ μ L	180±55.1	272±55.4	<0.0001	<0.0001
MELD-XI score	10.6 (9.4–10.8)	9.4 (9.4–9.4)	<0.0001	<0.0001
hs-CRP, mg/dL	1.3 (0.7–3.2)	0.6 (0.3–1.2)	0.09	0.09
Total cholesterol, mg/dL	149.0±30.1	190.8±41.4	<0.0001	<0.0001
LDL-C, mg/dL	82.5±25.4	102.0±34.7	<0.0001	<0.0001
HDL-C, mg/dL	42.8±12.2	64.1±16.9	<0.0001	<0.0001
Non-HDL-C, mg/dL	106.1±32.6	126.7±42.5	<0.0001	<0.0001
Triglycerides, mg/dL	95.5 (71.5–147.0)	102 (70–148)	0.66	0.66

Results are presented as mean±SD, median (interquartile range), or frequency (percentage). Data on low-density lipoprotein cholesterol (LDL-C) were missing for 4 participants ($n=3$ Fontan and $n=1$ control) because of triglyceride level >400 mg/dL. Adjusted P values reflect linear regression adjusted for age, sex, and body mass index (analyses for these 3 variables adjust for the other 2).

ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; MELD-XI, Model for End-stage Liver Disease score excluding INR; and non-HDL-C, non-high-density lipoprotein cholesterol.

DISCUSSION

These data demonstrate that: (1) patients with a Fontan circulation have lower total cholesterol, HDL-C, LDL-C, and non-HDL-C compared with controls; (2) HDL-C is affected by the same key drivers in patients with a Fontan circulation as is seen in the general population (lower HDL-C is associated with male sex, higher BMI, and higher hs-CRP); and (3) lower HDL-C is independently

Table 2. Baseline Characteristics of the Fontan Cohort

	Patients With Fontan (n=164)
Age, y	27.2 (22.8–34.4)
Age at Fontan, y	3.8 (2.5–8.3)
Fontan duration, y	23.1±5.6
Cardiac diagnosis, n (%)	
Tricuspid atresia	43 (26)
Double inlet left ventricle	37 (22)
Hypoplastic left heart	25 (15)
Double-outlet right ventricle	19 (12)
Unbalanced AVSD	6 (4)
Pulmonary atresia intact septum	3 (2)
Other	29 (18)
Unknown	2 (1)
Systemic ventricular morphology	
Left ventricle	54 (33)
Right ventricle	108 (66)
Unknown/indeterminate	2 (1)
Fontan type	
Lateral tunnel	100 (61)
Atriopulmonary	33 (20)
Extracardiac conduit	24 (15)
Atrioventricular/Bjork	5 (3)
Unknown	2 (1)
Medications	
ACEI/ARB	91 (55)
β-Blocker	37 (23)
Digoxin	47 (29)
Loop diuretic	52 (32)
MRA	31 (19)
Statin	4 (2)
Warfarin	53 (32)
Current tobacco smoker	5 (3)
Diabetes mellitus	5 (3)
Hepatitis B infection (63/164 tested)	0
Hepatitis C infection (65/164 tested)	2 (3)
History of PLE	8 (5)

Results are presented as mean±SD, median (interquartile range), or frequency (percentage).

ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; AVSD, atrioventricular septal defect; MRA, mineralocorticoid receptor antagonist; and PLE, protein-losing enteropathy.

associated with adverse outcomes in patients with a Fontan circulation. Prior studies have suggested an association between hypocholesterolemia and Fontan circulation, which we have confirmed with a prospectively enrolled cohort of participants with Fontan and controls.^{5,6} In addition, we describe the relationship between lipid levels and clinical outcomes in patients with a Fontan circulation. While the effect size of low HDL-C is too small to be used alone to predict which patients are at increased risk of morbidity or mortality, HDL-C has the potential to be used in combination with other biomarkers and clinical factors to aid in risk stratifying this high-risk population.

The Fontan procedure has been successful in allowing patients with single ventricles to live to adulthood. However, after a "honeymoon period" during early childhood, there is an increasing incidence of complications in adolescence and adulthood, with 60% of patients experiencing serious morbidities by age 40.^{12,13} While overt signs of worsening cardiac function occasionally occur, many adult patients with a Fontan have preserved ventricular systolic function with stable valvular function, making predictions about who will experience cardiac complications challenging. Many studies have attempted to identify circulating biomarkers that may aid in determining which patients are at increased risk of adverse outcomes.^{2-4,14} The current study has identified HDL-C as an additional biomarker that is independently associated with adverse Fontan outcomes. HDL-C has the benefit of being inexpensive and frequently obtained in clinical care allowing for easy adaptation into the routine screening of adults with a Fontan.

The potential causes of hypocholesterolemia in patients with Fontan and the association of HDL-C with worse outcomes deserves further discussion. Atherosclerosis is unlikely to play a role in these findings considering the young age and the low prevalence of coronary artery disease in patients with cyanotic heart disease even after repair resulting in resolution of hypoxemia.¹⁵ Some possible explanations for these findings could include low levels of physical activity, chronic inflammation, or liver dysfunction. The associations between low HDL-C and a sedentary lifestyle are well established,^{16,17} and clinical trials have demonstrated that increased physical activity can lead to increased HDL-C.¹⁸ However, low HDL-C in the sedentary general population is usually associated with elevated triglycerides.¹⁹ In distinction, triglyceride levels in the Fontan cohort did not differ from those in controls. Additionally, lower physical activity is unlikely to be the sole driver of the observed pattern of lower total cholesterol, LDL-C, and HDL-C with no difference in triglycerides compared with controls.

Fontan hypocholesterolemia could also be related to chronic low-level inflammation. Chronic

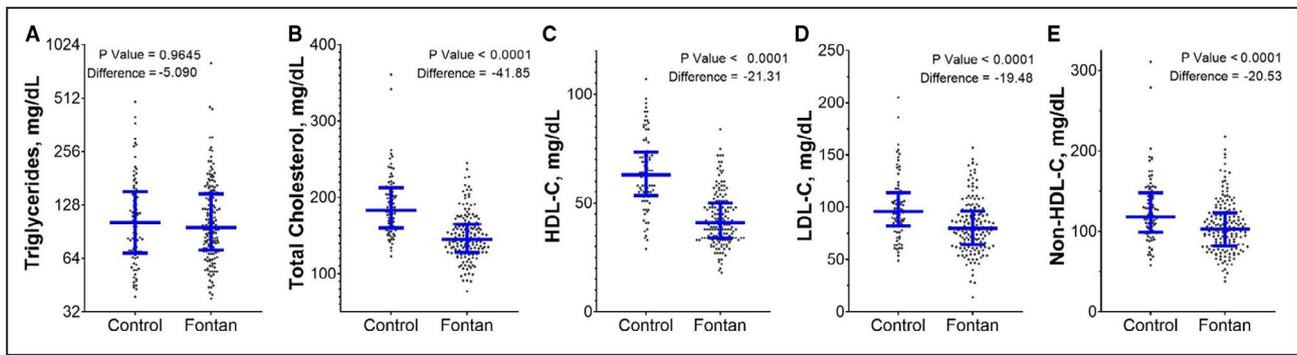


Figure 1. Distribution of lipids for patients with a Fontan circulation and control patients.

Values for each participant are plotted for (A) triglycerides, (B) total cholesterol, (C) high-density lipoprotein cholesterol (HDL-C), (D) low-density lipoprotein cholesterol (LDL-C), and (E) non-high-density lipoprotein cholesterol (non-HDL-C). The thick middle lines indicate median values, and error bars represent the 25th to 75th percentiles.

inflammatory conditions are known to be associated with low HDL-C,^{20,21} and the degree of elevation of inflammatory markers correlates inversely with HDL-C.²² At least a third of patients with a Fontan have elevated markers of systemic inflammation.^{23–25} Our present study found an inverse relationship between hs-CRP and HDL-C levels. The relationship between low HDL-C and adverse outcomes was attenuated after accounting for the contribution of hs-CRP. Therefore, HDL-C may be a marker of chronic low-level inflammation.

Liver dysfunction secondary to FALD could also underlie the observed findings. The Fontan circulation combines chronic elevation of venous pressure and low cardiac output. These hemodynamic derangements likely cause FALD, a poorly understood form of liver disease characterized by heterogeneous fibrosis, portal hypertension, and eventual cirrhosis.²⁶ Liver fibrosis is ubiquitous in adult patients with a Fontan circulation; however, methods to discern different degrees of FALD have not been identified.²⁷ The liver plays a

key role in lipid metabolism, and lipoprotein deficiencies are common among patients with chronic liver disease.²⁸ In diverse causes of chronic liver disease, hypocholesterolemia is inversely related to the severity of liver disease and declining lipid levels are indicative of liver disease progression.^{29–32} One study of patients with decompensated cirrhosis reported that a combination of elevated MELD (Model for End-stage Liver Disease) score and low cholesterol was able to more accurately discriminate 1-year mortality than the MELD score alone.³³ Another study of cirrhotic patients listed for liver transplant showed that HDL-C levels represent the best predictor of survival, and HDL-C <30 mg/dL was associated with a 3.4-fold higher hazard for death.⁸

Furthermore, some data suggest that hypocholesterolemia may directly contribute to complications seen in cirrhosis. Lipoproteins play a crucial role in the inactivation of circulating endotoxin; therefore, hypocholesterolemia may contribute to the increased risk of infections seen with cirrhosis.⁷ This study was not

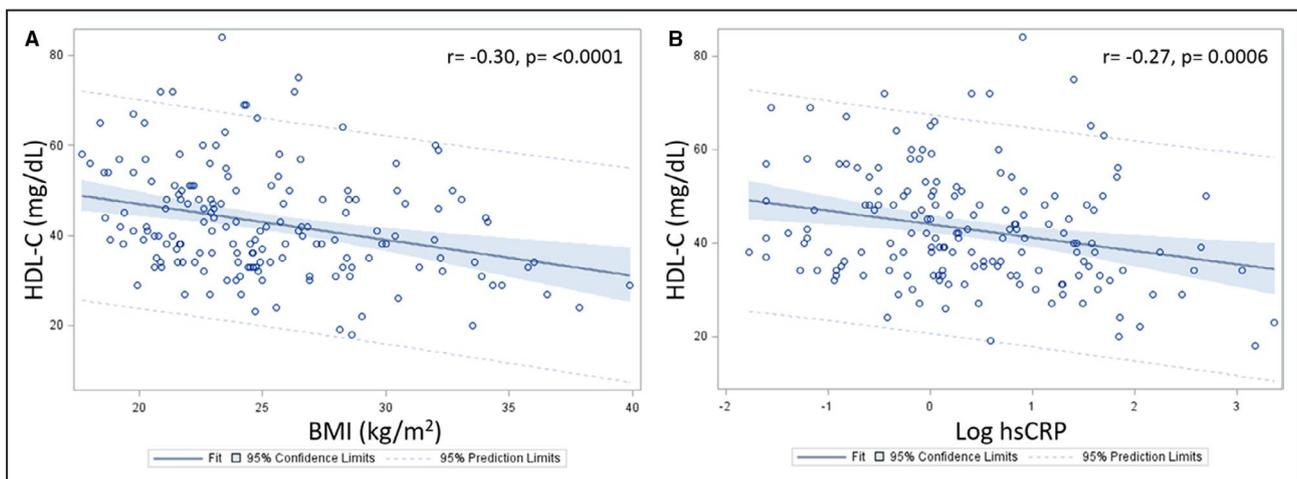


Figure 2. Associations between high-density lipoprotein cholesterol (HDL-C) and body mass index (BMI; A) and HDL-C and log high-sensitivity C-reactive protein (hs-CRP; B) among patients with a Fontan circulation.

Table 3. Correlation Between Lipid Measures and Various Clinical and Laboratory Variables of the Fontan Cohort

	LDL-C	Log Triglycerides	TC	Non-HDL-C	Age	Time Since Fontan, y	BMI	O ₂ Saturation	% Predicted VO ₂	Platelets	ALT	Albumin	Log NT-proBNP	Log hs-CRP
HDL-C	-0.25**	-0.44**	-0.01	-0.38**	0.09	0.05	-0.30**	0.008	0.28**	-0.009	-0.18*	0.18*	-0.03	-0.25*
LDL-C	1	0.35**	0.90**	0.94**	0.17*	0.17*	0.25**	-0.05	0.03	0.18*	0.10	0.04	0.09	0.33**
Log triglycerides		1	0.57**	0.69**	0.22**	0.24**	0.25**	-0.03	-0.03	0.16*	0.14	-0.15	0.07	0.24**
TC			1	0.93**	0.28**	0.26**	0.21**	-0.06	0.12	0.22**	0.04	-0.04	0.12	0.30**
Non-HDL-C				1	0.22**	0.23**	0.30**	-0.06	-0.01	0.21**	0.14	-0.10	0.12	0.37**
Age					1	0.63**	-0.07	-0.07	0.02	-0.19*	-0.12	-0.11	0.50**	0.33**
Time since Fontan, y						1	0.09	0.06	0.07	-0.06	0.06	-0.06	-0.03	0.24**
BMI							1	-0.02	-0.07	0.08	0.27**	0.03	-0.03	0.34**
O ₂ saturation								1	0.27**	0.10	0.04	0.16	-0.05	-0.13
% Predicted VO ₂									1	0.14	-0.05	0.13	-0.14	-0.25**
Platelets										1	-0.03	-0.001	-0.12	0.02
ALT											1	0.05	-0.30**	-0.05
Albumin												1	-0.13	-0.08
Log NT-proBNP													1	0.25**
Log hs-CRP														1

BMI indicates body mass index; HDL-C, high-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; O₂ saturation, resting oxygen saturation; and TC, total cholesterol. Data were missing for alanine aminotransferase (ALT)=1, aspartate aminotransferase (AST)=1, high-sensitivity C-reactive protein (hs-CRP)=1, low-density lipoprotein cholesterol (LDL-C)=3, NT-proBNP (N-terminal pro-B-type natriuretic peptide)=33, oxygen saturation=18, percent predicted peak oxygen consumption (% predicted VO₂)=33, platelets=7, and years since Fontan=2.

*Denotes P<0.05, **Denotes P<0.01.

Table 4. Clinical and Laboratory Variables of the Fontan Cohort by HDL-C Tertile

	HDL-C Tertile			P Value
	Lowest (<36 mg/dL)	Middle (36–47 mg/dL)	Highest (>47 mg/dL)	
Age, y	28.7 (23.5–32.6)	27.6 (22.2–35.5)	27.3 (22.6–38.3)	0.13
Time since Fontan, y	23.1 (19.6–26.9)	22.2 (18.8–25.7)	23.1 (19.9–26.8)	0.79
Female	13 (25)	25 (42)	31 (58)	0.002*
Body mass index, kg/m ²	27.4±5.1	24.5±3.9	23.9±4.0	<0.0001*
Creatinine, mg/dL	0.9±0.2	0.9±0.2	0.9±0.2	0.73
AST, U/L (n=163)	28.2±10.2	25.9±9.8	25.8±6.6	0.18
ALT, U/L (n=163)	32.4±14.9	26.2±11.5	24.9±8.5	0.001*
Alkaline phosphatase, U/L (n=153)	86.5±28.7	86.1±25.2	91.5±36.7	0.41
Total bilirubin, mg/dL (n=163)	1.0±1.0	1.2±1.1	0.9±0.5	0.47
Albumin, mg/dL	4.6±0.7	4.6±0.4	4.7±0.3	0.11
MELD-XI score (n=163)	9.4 (9.4–10.8)	9.4 (9.4–11.5)	9.4 (9.4–10.8)	0.59
Total cholesterol, mg/dL	153±33	143±29	152±27	0.87
Triglycerides, mg/dL	124 (85–184)	96 (71–140)	81 (62–103)	<0.0001*
Log triglycerides	4.9±0.6	4.6±0.5	4.4±0.4	<0.0001*
LDL-C, mg/dL	91.4±24.3	80.2±25.1	76.5±25.0	0.003*
Non-HDL-C, mg/dL	122.3±34.1	102.2±29.7	94.7±28.2	<0.0001*
Peak VO ₂ , % predicted (n=131)	56.9±15.6	59.5±13.5	63±14.1	0.049*
hs-CRP, mg/dL (n=163)	1.9 (0.9–5.2)	1.3 (0.8–2.5)	1.0 (0.6–2.0)	0.002*
Log hs-CRP	0.8±1.2	0.3±1	0.17±0.96	0.002*
Oxygen saturation, % (n=146)	95 (92–96)	93 (91–95)	95 (92–96)	0.36
Hemoglobin, g/dL	15.7±1.7	15.3±1.9	15.0±1.9	0.055
Platelets, K/μL (n=157)	183±59	172±55	188±51	0.68
NT-proBNP, pg/mL (n=131)	134 (64–261)	120 (66–323)	173 (82–331)	0.37
VAST score (n=160)	1 (0–2)	1 (0–2)	0 (0–2)	0.16

Results are presented as mean±SD, median (interquartile range), or frequency (percentage). ALT indicates alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MELD-XI, model for End-stage Liver Disease score excluding INR; non-HDL-C, non-high-density lipoprotein cholesterol; NT-proBNP, N-terminal pro-B-type natriuretic peptide; VO₂, peak oxygen consumption (as measured during exercise testing); and VAST, imaging features of portal hypertension: 1 point each for varices, ascites, splenomegaly, or thrombocytopenia.

*P<0.05.

designed to ascertain whether hypocholesterolemia is secondary to liver dysfunction. We identified a correlation between hypocholesterolemia and both alanine aminotransferase and albumin, however, other assessments of liver disease severity including MELD-XI and VAST scores did not correlate with lipid levels. The lack of a gold standard for determining stages of FALD serves as an obstacle to understanding the relationship between lipid levels and FALD. As FALD becomes better understood, further investigations may be able to identify whether HDL-C could be useful in discriminating degrees of FALD.

There are several limitations to our current study. Patients with a Fontan may be taking several cardiac medications that may affect lipid levels. However, there were only 4 patients taking statin medications, which have modest effects on HDL-C, and no patients taking nonstatin lipid-lowering medications. Many were also taking β-blockers, which may also alter serum lipid

levels. It is, however, doubtful that medication use accounts for the marked differences observed in HDL-C. Another limitation is that low HDL-C is known to be associated with lower physical activity. Adequately capturing the type, duration, and frequency of physical activity is not possible with medical record review, and lower physical activity is likely causally associated with worse Fontan outcomes. Last, patients were not fasting at the time of the blood draws, which may limit interpretation of the triglyceride levels, and HDL-C level does not necessarily indicate HDL function.

CONCLUSIONS

Patients with a Fontan circulation have lower total cholesterol, HDL-C, and LDL-C compared with healthy volunteer controls, and lower HDL-C is independently associated with adverse outcomes. Further

investigation is needed to determine the cause of decreased lipid levels and whether HDL-C could be a marker of more advanced liver disease in patients with a Fontan circulation.

ARTICLE INFORMATION

Received September 29, 2020; accepted February 11, 2021.

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Sources of Funding

This work was conducted with support from Harvard Catalyst/The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award UL1 TR001102, Harvard University, and its affiliated academic healthcare centers). A.R.O. and F.W. were supported by the Dunlevie Family Fund. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the article, and its final contents.

Disclosures

None.

Supplementary Material

Table S1

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Supplemental Material

Table S1. Clinical and Laboratory Variables of the Fontan cohort, by composite outcome.

	Non-elective hospitalization or death		P value	Adjusted P value*
	Yes (n=56)	No (n=108)		
Age (years)	32.6 (23.9-42.6)	25.2 (22.6-30.6)	0.001	0.13
Female	26 (46%)	43 (40%)	0.50	0.42
Body mass index (kg/m ²)	25.3 ± 4.5	25.1 ± 4.6	0.79	0.13
Creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.2	0.91	0.07
AST (U/L)	25.9 ± 8.8	27.2 ± 9.2	0.48	0.63
ALT (U/L)	26.7 ± 13.2	28.3 ± 11.7	0.41	0.76
Alkaline phosphatase (U/L)	92.0 ± 30.62	86.2 ± 30.5	0.27	0.67
Bilirubin, total (mg/dL)	0.9 ± 1.0	1.1 ± 0.9	0.34	0.75
Albumin (mg/dL)	4.5 ± 0.7	4.7 ± 0.3	0.002	0.07
MELD-XI score (n=163)	9.4 (9.4-10.8)	9.4 (9.4-11.2)	0.60	0.17
Total cholesterol (mg/dL)	151 ± 30	148 ± 30	0.62	0.68
LDL cholesterol (mg/dL)	86.1 ± 25.5	80.6 ± 25.4	0.19	0.07
HDL cholesterol (mg/dL)	40.2 ± 10.7	44.2 ± 12.7	0.04	0.02
Triglycerides (mg/dL)	124 (77-133)	116 (71-149)	0.61	0.83
Log Triglycerides	4.7 ± 0.5	4.6 ± 0.5	0.72	0.94
Log hsCRP	0.8 ± 1.1	0.2 ± 1.0	<0.001	<0.001
Hemoglobin (gm/dL)	14.8 ± 1.6	15.7 ± 1.9	0.004	0.42
Platelets (K/mcl)	190 ± 63	176 ± 50	0.13	0.053
Log NT-proBNP	5.4 ± 1.1	4.7 ± 1.2	0.002	0.04
VAST score	1 (0-2)	0 (0-1)	<0.001	0.004
Peak VO ₂ (% predicted) (n=131)	56.1 ± 16.1	61.6 ± 13.3	0.004	0.07
NYHA	1 (1-2)	1 (1-2)	0.04	0.004

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, hsCRP: high-sensitivity C-reactive protein, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, MELD-XI: Model for End-stage Liver Disease (MELD) score excluding INR, NT-proBNP: N-terminal pro-B-type natriuretic peptide, VO₂: peak oxygen consumption as measured during exercise testing, VAST: features of portal hypertension (1 point each for Varices, Ascites, Splenomegaly, or Thrombocytopenia). Results are presented as mean ± standard deviation, median (IQR), or frequency (%).

*Adjusted P values reflect Cox proportional hazards model, with the variable listed in the table adjusted for age, sex, VAST score, and NYHA functional class 1 vs 2+. Platelet count was not adjusted for VAST score as thrombocytopenia is included in the VAST score.