Lipoprotein Particle Concentrations in Children and Adults following Kawasaki Disease

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ABSTRACT

Background: The long-term sequelae of Kawasaki disease (KD) are largely unknown and concern has been voiced that the damaged arterial wall may be more susceptible to development of premature atherosclerosis. We sought to determine whether children and adults with a history of KD are more likely to have abnormal lipoprotein particle profiles that could place them at increased risk of atherosclerosis later in life.

Methods and Results: Fasting serum samples were obtained from 190 children and 68 adults with a history of KD and 90 age-similar healthy controls. Lipoprotein particle (P) concentrations and sizes were measured by Nuclear Magnetic Resonance (NMR) spectroscopy (Liposcience Diagnostics, Raleigh, NC) and serum was assayed for total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL)-C. Low-density lipoprotein cholesterol (LDL)-C was estimated using the Friedewald formula. Data were analyzed in a least square means model adjusting for age and sex and using Holm's correction for multiple comparisons. Compared to their respective control groups, both adult and pediatric KD subjects had significantly lower mean very-low-density lipoprotein-chylomicron particle concentrations (VLDLC-P), intermediate-density lipoproteins (IDL), TG, and TC concentrations. Pediatric KD subjects also had significantly lower total LDL-P and LDL-C concentrations and a TC/HDL-C ratio. In contrast, the adult KD subjects had significantly lower HDL-P and HDL-C concentrations, but HDL-C was within the normal range.

Conclusions: NMR lipoprotein particle analysis suggests that pediatric and adult KD subjects are no more likely than age-similar, healthy controls to have lipid patterns associated with an increased risk of atherosclerosis.

Keywords: Kawasaki disease, atherosclerosis, lipoproteins

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DISCLOSURES:

None

Since the first published report in 1967, Kawasaki disease (KD) has become the leading cause of acquired pediatric heart disease in developed countries [1]. KD is an acute, self-limited vasculitis that typically presents as a febrile illness with mucocutaneous changes in young children. [2] Coronary artery aneurysms will develop in 25% of untreated patients, putting them at increased risk for developing long-term cardiovascular complications including myocardial ischemia and infarction. [3-6] The current American Heart Association (AHA) guidelines recommend lipid profile screening for those who have recovered from KD, due to concerns that these patients are at increased risk of accelerated atherosclerosis. [7] However, data to support this concern are lacking. Studies to date have yielded conflicting results on many measures used to assess atherosclerotic risk, including traditional lipid panels, carotid intima-media thickness, endothelial cell function testing, and arterial stiffness [8-12]. Concern has also been raised that KD children who develop coronary artery aneurysms will develop accelerated atherosclerosis compared to children with normal coronary arteries [13, 14]. In a murine model of KD using intraperitoneal injection of *Lactobacillus casei* cell wall extract, mice fed an atherogenic diet developed accelerated atherosclerosis with aortic lesions rich in collagen, lipid, and foam cells [15]. However, autopsy and explanted heart studies report that KD coronary artery lesions did not contain the characteristic features of atherosclerosis, such as cholesterol clefts and foam cells.[16] Determination of lipoprotein profiles is one component of risk stratification for the development of atherosclerosis, a process that may be superimposed upon existing arterial wall damage, termed KD vasculopathy.

The protective role of high-density lipoprotein (HDL), particularly large HDL, and pathogenic role of low-density lipoprotein (LDL), especially the small-dense LDL, in atherosclerosis and coronary artery disease (CAD) are well-established. However, the traditional lipid panel only provides concentrations of TC, TG, and cholesterol in LDL and all HDL particles, and may not provide the most robust measurement of lipoprotein-attributable risk[17]. Nuclear magnetic resonance (NMR) spectroscopy directly quantifies the number of LDL and HDL particles (LDL-P and HDL-P) and their size distribution, and may yield a more accurate assessment of atherosclerotic risk [18-22]. Results of multivariable analyses from several studies in adults have supported the hypothesis that it is the number of lipoprotein particles, not lipoprotein size or concentration of cholesterol, that is most strongly associated with atherosclerotic risk[23-25]. The goal of our study was to assess whether pediatric and young adult KD patients are at increased risk of accelerated atherosclerosis compared to healthy controls using NMR lipoprotein particle counts.

Methods:

Study Population: Pediatric subjects included 190 children and adolescents with a history of KD diagnosed and treated at Rady Children's Hospital San Diego, between November 2005 and June 2011. Inclusion criteria were initial diagnosis of KD according to AHA criteria and phlebotomy performed at least 11 months after KD onset.[26] Serum samples were also obtained from 45 age-similar, healthy control (HC) children who were fasting prior to undergoing minor orthopedic surgical procedures. Adult KD subjects included 68 young adults enrolled in the San Diego Adult KD Collaborative study. Fasting serum samples were obtained at least 14 years following acute KD. Adult healthy controls included 45 age-similar healthy volunteers with no history of KD or heart disease. None of the study subjects were on any lipid-

lowering medication. Written informed consent, and assent when appropriate, were obtained from the parents of subjects or the subjects themselves. The protocol was approved by the Institutional Review Board at the University of California San Diego.

Lipoprotein Analysis: Fasting serum samples (stored at -80°C prior to testing) were assayed for total cholesterol (TC), triglycerides and high-density lipoprotein cholesterol (HDL-C) using standard automated methods on a Vitros 5,1 FS Chemistry System instrument. Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald formula. Lipoprotein particle profiles were measured by NMR spectroscopy with the LipoProfile-3 algorithm at LipoScience Inc.(Raleigh, NC). Very-Low-Density Lipoprotein (VLDL) + Chylomicron particle (VLDLC-P), LDL-P and HDL-P subclasses were quantified by the amplitudes of their spectroscopically distinct lipid methyl group NMR signals. Weighted-average VLDL, LDL and HDL particle sizes were derived from the sum of the diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal.

Clinical Data: Body mass index (BMI) was calculated from hospital records (pediatric subjects) or by measurements obtained for this study at the time of phlebotomy (adult subjects). Coronary artery status was determined by echocardiography for the pediatric KD subjects. Subjects were classified as dilated if the internal diameter of the coronary artery normalized for body surface area and expressed as standard deviation units from the mean (Z score) exceeded 2.5 for the left anterior descending or right coronary arteries assessed by echocardiography during the first 6 weeks after disease onset. Aneurysms were defined as a segment $\geq$ 1.5 times the diameter of the adjacent segment. Adult KD subjects were evaluated by a combination of invasive, computed tomography, and magnetic resonance angiography and classified as having normal or aneurysmal coronary arteries.

Statistical Analysis:

Patient characteristics were summarized by group. Medians and interquartile ranges (IQRs) were reported for continuous variables and frequency counts and percentages were reported for categorical variables. For each of the lipoprotein outcomes, linear regression models were used to compare the differences between KD subjects and controls, after adjusting for age and sex. Least-square means from the models were reported with 95% confidence intervals (CIs), and two-sided p-values <0.05 were considered statistically significant. Holm's multiple testing adjustment procedure was applied. Statistical analyses were performed in R (http://cran.r-project.org), version 2.14.0.

Results:

There were no significant differences in the demographic or clinical features of the pediatric and adult KD groups and their respective controls except for an excess of females in the adult HC group (Table 1). These differences were taken into account in the analysis model adjusting for age and sex.

The analysis of serum using the *NMR LipoProfile*® test provided lipoprotein particle concentrations for all groups (Table 2) whereas the lipid panel provided cholesterol and TG concentrations. Table 3 separates lipoprotein particles and cholesterol concentrations that are known to be atherogenic and atheroprotective. High concentrations of VLDL, IDL, LDL, and tryglyercide concentrations are all known to be associated with atherosclerosis. Both pediatric and adult KD subjects had significantly lower mean VLDLC-P, IDL-P, and TG concentrations compared to their respective control groups. Pediatric KD subjects also had significantly lower mean total LDL-P and LDL-C concentrations ($p=0.001$ and $p<0.001$, respectively), and a lower mean TC/HDL ratio compared to the pediatric HC ($p<0.001$).

Higher concentrations of total and large HDL-P are thought to be atheroprotective while small HDL-P is known to be associated with coronary artery disease[27]. Pediatric KD subjects had a significantly higher large HDL-P concentration ($p<0.001$) and small HDL-P ($p=0.003$). The adult KD subjects had significantly lower mean HDL-P and HDL-C concentrations compared to the adult HC ($p<0.001$) (Table 2). In contrast to the pediatric subjects, adult KD subjects displayed significantly lower small HDL-P.

Neither the pediatric nor the adult KD cohort had the combination of higher concentrations of small LDL-P and lower concentrations of large HDL-P, which would be considered the canonical risk profile for atherosclerosis. Compared to controls, the pediatric KD cohort had significantly higher levels of both atherogenic and atheroprotective particles, specifically small LDL-P and large HDL-P ($p=0.002$ and $p<0.001$, respectively). In contrast, the adult KD cohort was similar to controls for these particle concentrations.

When both adult KD and their control cohorts were compared to subjects in the Framingham and MESA studies, their LDL-C and LDL-P values were below the 30th percentile for both studies. Within the adult KD cohort, the mean LDL-C and LDL-P values fell within the 10-20 percentile range of both the Framingham and MESA population comparisons (Table 4).

Discussion:

This is the first report of NMR lipoprotein particle analysis in KD subjects. Pediatric and adult KD subjects displayed a mix of both atherogenic and atheroprotective lipoprotein particle profiles compared to healthy controls, after controlling for age and sex. The most robust predictors of atherosclerotic risk are thought to be the concentrations of very low density lipoprotein/chylomicron particle (VLDLC-P), triglycerides (TG), and LDL-P as well as the ratio of TC to HDL-C[25, 28, 29]. Compared to controls, the pediatric KD group had lower mean

concentrations of all of these lipids and lipoproteins consistent with a lower atherosclerotic risk profile. In contrast, the adult KD group presented a mixed profile with lower VLDLC-P and TG but similar LDL-P concentrations and a similar ratio of TC to HDL-C.

The acute inflammatory vasculitis of KD produces a spectrum of damage to the coronary arteries and other medium-sized, extra-parenchymal muscular arteries throughout the body.[16] Concerns have been raised over the potential for KD patients to develop accelerated atherosclerosis in these vascular beds.[8, 11, 30-33] Evidence cited to support this concern includes greater carotid intima-media thickness (IMT), abnormal brachial artery reactivity (BAR), and abnormal ankle-brachial indices (ABI) in some studies. However, a more recent study using finger plethysmography (Endo PAT Index) as a more accurate tool to assess endothelial cell function found no difference between KD subjects and controls.[34, 35] In addition, the pathologic record of atherosclerosis complicating regions of KD vasculopathy is scant and does not suggest an increased risk of focal atherosclerotic changes.[36-43] In fact, autopsy reports of sudden death in young adults with a history of KD in childhood have remarked on the relative absence of atherosclerosis.[37] Similarly, the assumption that medial necrosis and calcification of the coronary arteries as documented by IVUS represents early atherosclerosis in KD patients may not be appropriate.[44]

Whether or not KD vasculopathy alone predisposes individuals to an increased risk of atherosclerosis remains unanswered. Individual KD patients with documented hyperlipidemia, such as elevated LDL-C, should be managed aggressively. However, based on the data presented here, as a group, neither pediatric nor adult KD patients have lipoprotein particle counts or lipid profiles associated with increased atherosclerotic risk.

Characteristics	Pediatric KD (n=190)	Pediatric HC (n=45)	Adult KD (n=68)	Adult HC (n=45)
Median age, years (IQR; range)	5.3 (3.5-7.8;1.1-15.3)	4.7 (3.3-6.5;0.7-15.9)	21.2 (18.4-28.8;16.0-47.0)	23.3 (22.0-25.8;16.4-49.0)
Male, n (%)	123 (64.7)	23 (51.1)	39 (57.4)	13 (28.9)
Interval between KD onset and phlebotomy, years (IQR; range)	1.4 (1.1-4.6;0.9-12.6)*	N/A	18.4 (15.1-27.2;1.1-41.8)‡	N/A
Coronary artery status of subjects: n (%)				
Normal	133 (70.0)	N/A	51 (75.0)	N/A
Dilated	36 (19.0)		3 (4.4)	
Aneurysm	21 (11.0)		12 (17.6)	
Unknown	0 (0.0)		2 (3.0)	
BMI, median (IQR; range)	16.5 (15.2-18.6)	16.4 (15.7-18.0) †	22.5 (20.2-25.8)§	22.3 (20.8-23.9)
Ethnicity: n (%)				
Asian	29 (15.3)	2(4.5)	15 (22.1)	21 (46.7)
Black/African American	8 (4.2)	4 (8.9)	2 (2.9)	0 (0.0)
Caucasian	46 (24.2)	29 (64.4)	35 (51.5)	20 (44.4)
Hispanic	61(32.1)	8 (17.8)	8 (11.8)	3 (6.7)
More than one race	38 (20.0)	2 (4.4)	7 (10.3)	1 (2.2)
Native Hawaiian or Other Pacific Islander	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	6 (3.2)	0 (0.0)	1 (1.4)	0 (0.0)

Table 1. Demographic and clinical characteristics of study cohorts.

HC= healthy controls; BMI = Body Mass Index calculated, kg/m^2; IQR = Interquartile range

*n=186†n=27‡n=65§ n=67

	Pediatric KD (n=190)	Pediatric HC (n=45)	P-value	Adult KD (n=68)	Adult HC (n=45)	P-value
Total VLDL/Chylomicron particles (VLDLC-P) (nmol/L)	43.4 (39.8-46.9)	51.6 (44.4-58.9)	0.047	52.0 (45.2-58.9)	73.5 (65.1-81.9)	<0.001
Large VLDL/Chylomicron particles (VLC-P) (nmol/L)	1.4 (0.8-1.9)	3.1 (1.9-4.2)	0.01	2.3 (1.7-3.0)	2.2 (1.5-3.0)	0.79
Medium VLDL particles (VM-P) (nmol/L)	18.2 (15.9-20.5)	14.2 (9.5-18.9)	0.13	18.1 (14.4-21.8)	24.3 (19.7-28.8)	0.054
Small VLDL particles (VS-P) (nmol/L)	23.8 (21.9-25.7)	34.4 (30.4-38.3)	< 0.001	31.6 (26.9-36.3)	47.0 (41.2-52.8)	< 0.001
Total LDL particles (LDL-P) (nmol/L)	934 (893-975)	1098 (1014-1182)	0.001	953 (869-1036)	1082 (977-1187)	0.078
IDL particles (IDL-P) (nmol/L)	14 (9-18)	113 (104-123)	< 0.001	67 (54-80)	89 (73-105)	0.035
Large LDL particles (LL-P) (nmol/L)	382 (358-407)	610 (560-660)	< 0.001	479 (433-525)	537 (479-595)	0.12
Small LDL particles (total) (LS-P) (nmol/L)	538 (493-583)	375 (282-467)	0.002	407 (324-489)	455 (350-560)	0.54
Total HDL particles (HDL-P) (µmol/L)	30.4 (29.8-30.9)	30.5 (29.3-31.8)	0.79	34.0 (32.7-35.4)	40.5 (38.8-42.2)	< 0.001
Large HDL particles (HL-P) (µmol/L)	9.0 (8.6-9.5)	4.9 (4.0-5.8)	< 0.001	6.6 (5.8-7.4)	7.5 (6.5-8.5)	0.15
Medium HDL particles (HM-P) (µmol/L)	3.1 (2.6-3.7)	9.6 (8.5-10.7)	< 0.001	11.5 (9.9-13.0)	12.3 (10.4-14.2)	0.53
Small HDL particles (HS-P) (µmol/L)	18.2 (17.6-18.8)	16.0 (14.7-17.3)	0.003	16.0 (14.6-17.3)	20.7 (19.1-22.4)	< 0.001
VLDL particle size (VZ) (nm)	53.4 (52.0-54.8)*	44.2 (41.2-47.3)†	< 0.001	47.6 (46.3-48.8)‡	43.5 (42.0-45.0)§	< 0.001
LDL particle size (LZ) (nm)	21.2 (21.1-21.3)	21.1 (20.9-21.3)	0.47	21.1 (20.9-21.2)	20.9 (20.8-21.1)	0.21
HDL particle size (HZ) (nm)	9.1 (9.0-9.2)	9.2 (9.1-9.3)	0.06	9.2 (9.1-9.3)	9.2 (9.1-9.3)	0.52
Total Triglyceride (TG) (mg/dL)	82 (75-89)	105 (90-119)	0.007	98 (88-108)	118 (106-131)	0.02
Total VLDL/Chylomicron Triglyceride (NVCTG) (mg/dL)	53 (46-60)	70 (55-84)	0.05	67 (58-76)	83 (71-95)	0.048
Total HDL cholesterol (HDL-C) (mg/dL)	50 (49-52)	48 (45-51)	0.12	53 (51-56)	62 (59-65)	< 0.001
Total LDL cholesterol (LDL-C) (mg/dL)	85 (82-88)	106 (100-113)	< 0.001	87 (81-94)	97 (89-105)	0.08
Total Cholesterol (TC) (mg/dL)	148 (145-152)	169 (161-176)	< 0.001	154 (148-161)	178 (169-187)	< 0.001
Ratio of Total cholesterol/HDL cholesterol	3.0 (2.9-3.2)	3.6 (3.4-3.8)	<0.001	3.0 (2.8-3.2)	3.0 (2.8-3.2)	0.83

Table 2: NMR lipoprotein particle concentrations and sizes. Values are model-estimated means (95% CI). P values are after Holm's correction for multiple testing. HC=healthy controls

* n=192 † n=39 ‡ n=66 § n=43

Lipoprotein Subclass	KD Cohorts	
Atherogenic Subclasses	**Pediatric KD compared to PHC**	**Adult KD compared to AHC**
Small LDL-P	↑	NS
Small HDL-P	↑	↓
Total LDL-P	↓	NS
IDL-P	↓	↓
Total VLDL/Chylomicrons	↓	↓
LDL-C	↓	NS
Total Triglycerides	↓	↓
Atheroprotective Subclasses	**Pediatric KD**	**Adult KD**
Large HDL-P	↑	NS
Total HDL-P	NS	↓
HDL-C	NS	↓

Table 3: Atherogenic and atheroprotective lipoprotein subclasses and cholesterol concentrations adapted from Ref.[45, 46]

	Framingham Offspring* n=3367 (1367 men; 1732 women)		MESA† n=6697 (3154 men; 3543 women)	
Percentile	**LDL-C** (mg/dL)	**LDL-P** (nmol/L)	**LDL-C** (mg/dL)	**LDL-P** (nmol/L)
2	70	720	58	670
5	78	850	69	770
10	88	940	79	870
20	100	1100	91	990
30	111	1220	100	1090
40	120	1330	108	1170
50	130	1440	115	1260
60	139	1540	123	1350
70	149	1670	131	1440
80	160	1820	141	1560
90	176	2020	157	1740
95	191	2210	170	1900

Table 4: Population Comparisons of Lipid and Lipoprotein Particle Concentrations
*Specimens collected in 1988-1991 (exam cycle 4). Analysis restricted to subjects with TG <400 mg/dL. Ethnic make-up 99% Caucasian.[47]
†Specimens collected in 2000-2002. Analysis restricted to subjects with TG <400 mg/dL. Ethnic make-up 27.4% African-American, 38.0% Caucasian, 12.3% Chinese, 22.3% Hispanic.[48]

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