Lipoprotein Particle Concentrations in Children and Adults following Kawasaki Disease

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Objective To test the hypothesis that children and adults with a history of Kawasaki disease (KD) are more likely to have abnormal lipoprotein particle profiles that could place them at increased risk for developing atherosclerosis later in life.

Study design Fasting serum samples were obtained from 192 children and 63 adults with history of KD and 90 age-similar healthy controls. Lipoprotein particle concentrations and sizes were measured by nuclear magnetic resonance spectroscopy (LipoScience Inc, Raleigh, North Carolina), and serum was assayed for total cholesterol (TC), triglycerides, and high-density lipoprotein (HDL) cholesterol (HDL-C). Low-density lipoprotein (LDL) cholesterol was estimated using the Friedewald formula. Data were analyzed in a least-square means model, with adjustment for age and sex and with the use of Holm correction for multiple comparisons.

Results Compared with respective control groups, both adult and pediatric subjects with KD had significantly lower mean very low-density lipoprotein-chylomicron particles, intermediate-density lipoproteins, triglycerides, and TC concentrations. Pediatric subjects with KD had significantly lower LDL particle and LDL cholesterol concentrations and lower mean TC/HDL-C ratio ($P < .001$). In contrast, the adult subjects with KD had significantly lower HDL particle, small HDL particle, and HDL-C concentrations ($P < .001$), but HDL-C was within normal range.

Conclusions Nuclear magnetic resonance lipoprotein particle analysis suggests that pediatric and adult subjects with KD, regardless of their aneurysm status, are no more likely than age-similar, healthy controls to have lipid patterns associated with increased risk of atherosclerosis. *(J Pediatr 2014;165:727-31)*.

Since the first published report in 1967, Kawasaki disease (KD) has become the leading cause of acquired pediatric heart disease in developed countries.[1,2] Coronary artery aneurysms develop in 25% of untreated patients, placing them at increased risk for cardiovascular complications, including myocardial ischemia and infarction.[3-6] The current guidelines of the American Heart Association recommend lipid profile screening for those who have recovered from KD because of concerns that these patients may be at increased risk of accelerated atherosclerosis.[7] 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) and pathogenic role of low-density lipoprotein (LDL), especially the small-dense LDL, in atherosclerosis and coronary artery disease are well-established. However, the traditional lipid panel may not provide the most robust measurement of lipoprotein-attributable risk.[8] Nuclear magnetic resonance (NMR) spectroscopy directly quantifies the number of LDL and HDL particles (LDL-Ps and HDL-Ps) and their size distribution and may yield a more accurate assessment of atherosclerotic risk.[9-13] Results of multivariable analyses from several studies in adults have supported the hypothesis that it is the number of lipoprotein particles, not lipoprotein particle size or concentration of cholesterol, that is most strongly associated with atherosclerotic risk.[14-16] Studies of lipid profiles in small cohorts of acute and convalescent patients with KD have yielded conflicting results.[17-21] Using NMR lipoprotein particle counts, we sought to assess whether pediatric and young adults with KD are more likely to have atherogenic lipid profiles compared with healthy control subjects.

CAA	Coronary artery abnormality	LDL-P	Low-density lipoprotein particle
HDL	High-density lipoprotein	NMR	Nuclear magnetic resonance
HDL-C	High-density lipoprotein cholesterol	TC	Total cholesterol
		TG	Triglyceride
HDL-P	High-density lipoprotein particle	VLDL	Very low-density lipoprotein
KD	Kawasaki disease	VLDLC-P	Very low-density lipoprotein-chylomicron particle
LDL	Low-density lipoprotein		
LDL-C	Low-density lipoprotein cholesterol		

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Methods

Pediatric subjects included 192 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 criteria from the American Heart Association and phlebotomy performed at least 11 months after the onset of KD.[7] Serum samples also were obtained from 45 age-similar, healthy control children who were fasting before undergoing minor orthopedic surgical procedures. Adults with KD included 63 young adults enrolled in the San Diego Adult KD Collaborative study. Fasting serum samples were obtained at study enrollment. Adult healthy controls included 45 age-similar healthy volunteers with no history of KD or heart disease. One pediatric subject and 11 adult subjects who were on lipid-lowering medications were excluded. Only 2 subjects with mild mixed hyperlipidemia were on statin therapy for lipid-lowering effects. The remaining 10 subjects were on statin therapy either as standard practice postmyocardial infarction or for the potential anti-inflammatory benefits of statins in the setting of coronary artery abnormalities (CAAs) after acute KD. None of the control subjects were on any lipid-lowering medication. Written informed consent, and assent when appropriate, was 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.

Fasting serum samples (stored at −80°C before testing) were assayed for total cholesterol (TC), triglycerides (TGs), and HDL cholesterol (HDL-C) via the use of standard automated methods on a Vitros 5.1 FS Chemistry System instrument (Ortho Clinical Diagnostics, Rochester, New York). LDL cholesterol (LDL-C) was estimated with the Friedewald formula. Lipoprotein particle profiles were measured by NMR spectroscopy with the LipoProfile-3 algorithm from LipoScience Inc (Raleigh, North Carolina). 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.

Body mass index 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 subjects with KD. Subjects were classified as dilated if the internal diameter of the coronary artery normalized for body surface area and expressed as SD 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 ≥1.5 times the diameter of the adjacent segment. Adult subjects with KD were evaluated by a combination of invasive, computed tomography, and magnetic resonance angiography and classified as having normal or aneurysmal coronary arteries.

Statistical Analyses

Patient characteristics were summarized by group. Medians and 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 subjects with KD and control subjects, as well as between subjects with KD with and without CAAs ($Z \leq 2.5$), after we adjusted for age and sex. Least-square means from the models were reported with 95% CIs, and 2-sided *P*-values <.05 were considered statistically significant. Holm 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 groups with KD and their respective controls except for an excess of females in the adult healthy control group (**Table I**). These differences were taken into account in the analysis model, adjusting for age and sex.

The analysis of serum using the NMR LipoProfile test (LipoScience Inc) provided lipoprotein particle concentrations for all groups (**Table II**), whereas the lipid panel provided cholesterol and TG concentrations. **Table III**[22,23] separates lipoprotein particles and cholesterol concentrations that are known to be atherogenic and atheroprotective. High concentrations of VLDL, intermediate-density lipoprotein, LDL, and TG concentrations are all known to be associated with atherosclerosis. Both pediatric and adult subjects with KD had significantly lower mean VLDLC-P, intermediate-density lipoprotein particles, and TG concentrations compared with their respective control groups. Pediatric subjects with KD also had significantly lower mean total LDL-P and LDL-C concentrations ($P = .001$ and $P < .001$, respectively), and a lower mean TC/HDL compared with the healthy pediatric control subjects ($P < .001$). For the pediatric cohort with KD, we compared lipoprotein particle counts with the maximum Z score of the right and left anterior descending coronary arteries measured by echocardiography during the first 6 weeks after illness onset. For the adult cohort, we compared lipoprotein particle counts between subjects with and without CAA. When we compared the pediatric or adult cohorts via linear regression analysis, we found no significant relationship between lipoprotein particle counts and coronary artery status. Similarly, both pediatric and adult subjects with CAA had similar lipoprotein particle counts that did not differ significantly from the respective healthy control cohort (data not shown).