

# Are Remnant-Like Particles Independent Predictors of Coronary Heart Disease Incidence?

## The Honolulu Heart Study

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**Background**—Remnant-like particles have been proposed as a new risk factor for coronary heart disease (CHD). This is the first long-term prospective investigation of the relationship between remnant-like particles and a cardiovascular disease outcome in healthy men.

**Methods and Results**—A cohort of 1156 Japanese-American men aged 60 to 82 from the Honolulu Heart Program was followed for 17 years. During that period 164 incident cases of CHD were identified. In multivariate Cox regression analyses, baseline remnant-like particle cholesterol (RLP-C) and triglyceride (RLP-TG) levels were significantly related to CHD incidence independently of nonlipid cardiovascular risk factors and of total cholesterol or high-density and low-density lipoprotein cholesterol levels. Total triglyceride levels were an independent predictor of CHD incidence. However, in models including RLP and triglyceride level simultaneously, neither variable was significant when adjusted for the other. This finding can be attributed to the strong correlation between RLP-C and RLP-TG levels and total triglycerides. When individuals with normal triglyceride levels (n=894) were separated from those with elevated triglycerides (n=260), the association between RLPs and CHD relative risk was only significant for the group with elevated triglyceride levels.

**Conclusions**—RLP levels predicted CHD incidence independently of nonlipid risk factors and of total cholesterol or high-density and low-density lipoprotein cholesterol levels. However, RLP levels did not provide additional information about CHD incidence over and above total triglyceride levels. Therefore, this study does not support the need for testing of remnants in men if measures of fasting triglycerides are available. (*Arterioscler Thromb Vasc Biol.* 2005; 25:1718-1722.)

**Key Words:** coronary heart disease ■ lipoproteins ■ triglycerides ■ remnant ■ Asian Americans

Recent prospective epidemiologic studies and a metaanalysis indicate that triglyceride levels in plasma or serum predict risk of coronary heart disease (CHD) independently of other cardiovascular risk factors.<sup>1-7</sup> Triglycerides are a measure of triglyceride-rich lipoproteins (TRLs). TRLs are heterogeneous and only certain TRLs were found to be atherogenic. Measurement of total plasma triglycerides does not distinguish the various subspecies of TRLs. Isolation of remnant-like particles (RLPs) allows the measurement of particles within TRLs, which are thought to be atherogenic.<sup>8</sup> Therefore, RLP particles have been proposed as a new risk factor for CHD, but there is debate as to whether they offer any predictive ability beyond triglycerides alone.

Chylomicrons produced in the intestine go through lipolysis when they reach the bloodstream. During this process, TRLs lose much of the triglyceride and C apolipoproteins and gain cholesteryl ester and apolipoprotein E (apoE) through

the action of cholesteryl ester transfer protein. These particles become chylomicron remnants and contain apoB-48 and apoE as their principal protein components. The last step in the metabolism of chylomicron remnants is uptake by the liver.<sup>9</sup>

The liver also produces TRLs, known as very-low-density lipoproteins (VLDLs), which, like chylomicrons, have a density of <1.006 kg/L. Newly formed VLDLs also go through lipolysis, losing much of their apoC and triglyceride and gaining a cholesteryl ester and apoE. These are then known as VLDL remnants and contain apoB-100 and apoE as their major protein components. VLDL remnants can then be metabolized to form intermediate-density lipoproteins (density 1.006 to 1.019 kg/L) and low-density lipoproteins (LDLs) (density 1.019 to 1.063 kg/L). These can also be taken up by the liver.<sup>9</sup>

Epidemiologic studies have shown associations between RLP concentrations and atherosclerosis, cardiovascular dis-

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ease (CVD), and CHD.<sup>10–21</sup> In a study conducted in Japan, Kugiyama et al found that higher levels of remnants in fasting serum were an independent predictor of developing coronary events in 135 patients with coronary artery disease.<sup>20</sup> RLP cholesterol (RLP-C) in fasting plasma was associated with prevalent CVD in 1567 white women from the Framingham Heart Study after adjustment for other major cardiovascular risk factors.<sup>16</sup> In addition, plasma concentration of RLP-C was associated with carotid artery intima-media thickness, especially for RLP-C levels 3 hours after receiving a rich fat meal.<sup>17</sup>

Because the Honolulu Heart Program (HHP) is a large prospective study of CHD with one of the longest follow-up periods in the world and its participants have a wider range of baseline triglyceride levels compared with other American white populations,<sup>22</sup> the HHP is an ideal population for defining the independent relationship between RLP and CHD. The objectives of this study were the following: (1) to examine the association of fasting RLP-C and RLP triglyceride (RLP-TG) levels with 17-year incidence of CHD in a sample of elderly Japanese-American men after adjustment for nonlipid cardiovascular risk factors; and (2) to evaluate how this relationship is affected by other lipids and lipoproteins such as total cholesterol, LDL and high-density lipoprotein (HDL) cholesterol or total triglycerides.

## Methods

### Study Population

The HHP cohort of Japanese-American men aged 45 to 68 and living on Oahu, Hawaii, was identified and recruited from selective service records. A total of 8006 subjects completed a baseline examination between 1965 and 1968.<sup>23–25</sup>

A 30% random sample of the participants was selected for the Cooperative Lipoprotein Phenotyping study from 1970 to 1972.<sup>26</sup> Survivors of this cohort were subsequently examined from 1980 to 1982. Data and samples from this latter examination, Lipoprotein Examination 3, were used as the baseline data for the present analysis.<sup>22</sup>

Fasting plasma samples were obtained for lipid determinations. At that time the study participants were aged 60 to 82. Of the 1379 men, 223 had prevalent CHD or stroke or were taking lipid-lowering medication and, therefore, were excluded from these analyses, leaving 1156 subjects who constituted the study population.

### Data Collection

Follow-up for incident CHD was conducted through 1997 using standardized clinical criteria.<sup>24,25</sup> Definite CHD included nonfatal myocardial infarction confirmed by electrocardiogram and/or enzyme changes. It also included any angina diagnosis that went on to surgical intervention after confirmation of coronary artery stenosis by angiography, fatal CHD, and sudden death within 1 hour caused by an unknown cause. These CHD outcomes were assessed from hospital records, death certificates, and periodic HHP examinations by the Honolulu Heart Program Surveillance Conferencing Committee following strict classification criteria of clinical evidence.<sup>27,28</sup>

Measurements of total cholesterol, triglycerides, and HDL cholesterol were performed on plasma collected after the subjects fasted overnight for at least 12 hours, using standard methods of the Lipid Research Clinics Program. The LDL cholesterol was estimated.<sup>22,29,30</sup>

Standard risk factor data, including physical activity, current smoking and drinking habits, and systolic blood pressure, were collected under standardized procedures during the Lipoprotein Examination 3; body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m<sup>2</sup>). Measurements of fasting glucose were obtained using frozen specimens from this examination. Diabetes was considered present if

subjects were taking oral hypoglycemic medication or insulin or were on a diabetic diet.

Further details on risk factor definitions and measurements are provided elsewhere.<sup>22,31–33</sup>

### RLP Analyses

RLP-C and RLP-TG measurements were carried out on frozen fasting EDTA-containing plasma samples obtained from 1980 to 1982 and stored at  $-70^{\circ}\text{C}$  under stable conditions until measurements were performed. RLP isolation was based on removal of apoA-I-containing particles (HDL) and most apoB-containing particles (LDL, nascent VLDL, and nascent chylomicrons) using an immunoseparation technique (Japan Immunoresearch Laboratories, Takasaki, Japan), as previously described,<sup>34–36</sup> which has been shown to leave remnants of both intestinal and hepatic origin in the unbound fraction. Briefly, monoclonal antibodies to apoA-I and specific monoclonal antibodies to apoB (JI-H), which do not recognize partially hydrolyzed, apoE-enriched lipoprotein remnants, were immobilized on agarose gel. EDTA-treated plasma was incubated with the gel for 2 hours on a Japan Immunoresearch Laboratories incubator in Hitachi sample cups (Boehringer Mannheim Diagnostics, Indianapolis, Ind), after which, the gel containing the bound (non-RLP) lipoproteins was precipitated by low-speed centrifugation (5 minutes, 135g). Cholesterol and triglyceride concentrations (RLP-C and RLP-TG, respectively) were then measured in the unbound supernatants, using 2-reagent enzymatic measurements in an Abbott Spectrum analyzer (Abbott Diagnostics). The sensitive chromophore was necessary because remnant concentrations are very low in most individuals, particularly in the fasting state. Calibrators were provided by Pacific Biometrics Research Foundation (Seattle, Wash). Because small differences were observed between fresh and frozen plasma, concentrations measured in previously frozen samples were subjected to correction factors to provide fresh-equivalent concentrations; the respective fit equations were  $y=1.22x-0.032$  (1.22) for RLP-C and  $y=0.95x-0.013$  (1.14) for RLP-TG. Coefficients of variation were typically within 10% for RLP-C and 15% for RLP-TG.<sup>37</sup>

### Statistical Analysis

Multivariate Cox proportional hazard models<sup>38</sup> were used to estimate the 17-year relative risk of CHD for RLP-C and RLP-TG levels associated with an increase of 2 SD. Adjustments were made for age, BMI, smoking, alcohol intake, physical activity, systolic blood pressure, fasting glucose, history of diabetes and intake of antihypertensive medication. Total cholesterol, HDL and LDL cholesterol, or total triglycerides were included as additional covariates in some of the models. These analyses were repeated using log-transformed RLP variables.

Fit of the Cox model was evaluated by permitting the effect of RLP-C and RLP-TG levels to vary with time and by entering squared terms for RLP-variables into the models. All reported probability values are based on 2-sided tests of significance.

## Results

Among the 1156 observations included in the analyses, 164 incident cases of CHD were identified. Table 1 displays descriptive statistics for RLP-C and RLP-TG levels and all covariates that were used in the models.

The effect of RLP-C and RLP-TG levels on CHD relative risk after adjustment for potential confounding variables are presented in Table 2. Each model included the basic set of nonlipid variables: age, BMI, smoking, alcohol intake, physical activity, systolic blood pressure, fasting glucose, history of diabetes and intake of antihypertensive medication, and 1 additional set of covariates.<sup>1–4</sup> Adding squared terms of the RLP variables did not improve the fit of the models (results not shown). CHD relative

**TABLE 1. Descriptive Statistics for RLP Levels and Covariates**

	Mean	SD
RLP-C, mg/dL	10.46 [6.9]*	11.93
RLP-TG, mg/dL	35.55 [18.6]*	56.41
Age, years	67.70	4.98
BMI, kg/m <sup>2</sup>	23.55	2.95
Smoking, cigarettes/day	3.93	9.15
Alcohol intake, oz/week	3.41	3.41
Physical activity index	30.81	3.12
Systolic blood pressure, mm Hg	138.90	17.87
Fasting glucose, mg/dL	113.40	28.35
Total cholesterol, mg/dL	209.62	34.96
HDL cholesterol, mg/dL	47.82	13.18
LDL cholesterol, mg/dL	135.71	33.71
Total triglycerides, mg/dL	164.23 [131.0]*	122.75

\*Measures are skewed to the right; therefore, medians are provided in brackets.

risk estimates for RLP levels are reported for an increase of 2 SD (23.85 mg/dL for RLP-C and 112.81 mg/dL for RLP-TG).

Models that included RLP as the only lipid variable showed a significant effect for RLP-C and RLP-TG levels on CHD relative risk ( $P=0.0022$  and  $P=0.0045$ , respectively).

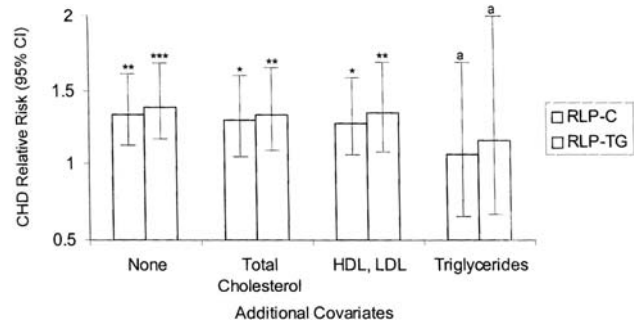
For the models that included total cholesterol or HDL and LDL cholesterol as additional covariates, RLP-C and RLP-TG were the only lipid variables that were significant.

Fasting triglyceride level was an independent predictor of CHD relative risk after adjustment for age, BMI, smoking, alcohol intake, physical activity, systolic blood pressure, fasting glucose, history of diabetes, intake of antihypertensive medication, and LDL and HDL cholesterol (relative risk estimates for triglyceride levels associated with an increase of 2 SD (245.49 mg/dL): 1.58; 95% CI: 1.15 to 2.17). In models including triglyceride level and an RLP variable simultaneously, neither variable was significant when adjusted for the other. See the Figure for the model estimates.

**TABLE 2. Age-Adjusted Correlations of Log-Transformed RLP-C and RLP-TG With CHD Risk Factors**

Variable	(Log)RLP-C, mg/dL	(Log)RLP-TG, mg/dL
BMI, kg/m <sup>2</sup>	0.22†	0.27†
Smoking, cigarettes/day	-0.048	-0.044
Alcohol intake, oz/week	0.079*	0.13†
Physical activity index	-0.090*	-0.093*
Systolic blood pressure, mm Hg	0.12†	0.16†
Antihypertensive drugs, yes/no	0.16†	0.19†
Fasting glucose, mg/dL	0.096*	0.15†
History of diabetes, yes/no	-0.0073	0.024
Total cholesterol, mg/dL	0.37†	0.19†
HDL cholesterol, mg/dL	-0.43†	-0.48†
LDL cholesterol, mg/dL	-0.022	-0.21†
(Log)total triglycerides, mg/dL	0.88†	0.93†

\* $P<0.01$ ; † $P<0.0001$ .



CHD relative risk estimates for RLP levels associated with an increase of 2 SD, after adjustment for potential confounding variables. Each model includes the basic set of nonlipid variables and one additional set of lipid covariates (None; Total Cholesterol; HDL, LDL; Triglycerides). The basic set of nonlipid variables includes age, BMI, smoking, alcohol intake, physical activity, systolic blood pressure, fasting glucose, history of diabetes, and intake of antihypertensive medication. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P=0.001$ , <sup>a</sup> $P>0.50$ .

When the previous analyses were repeated using log-transformed RLP, the results were similar, but the probability values were somewhat larger for the first 3 models; RLP-C and RLP-TG were only of borderline significance in model 3 ( $P=0.066$  and  $0.082$ , respectively). For model 4, the probability values were smaller when both RLPs and triglycerides were log-transformed ( $P=0.21$  for (log)RLP-C and  $P=0.43$  for (log)RLP-TG). The probability values for (log)RLP-C were generally smaller than the probability values for (log)RLP-TG in the corresponding models.

The previous analysis (Model 1) was also conducted separately for people with normal triglyceride levels ( $n=894$ , including 115 CHD cases) and those with elevated triglycerides ( $n=260$ , including 49 CHD cases). After adjustment for age, BMI, smoking, alcohol intake, physical activity, systolic blood pressure, fasting glucose, history of diabetes and intake of antihypertensive medication, only the group with elevated triglyceride levels showed an association between RLP-C and RLP-TG levels and CHD relative risk ( $P=0.026$  and  $P=0.014$ , respectively). However, regression-coefficient estimates (log relative risk per mg/dL) for RLP were similar for both groups: for RLP-C 0.0094 in the group with normal triglycerides and 0.011 in the group with elevated triglycerides; for RLP-TG 0.0023 in the group with normal triglycerides and 0.0028 in the group with elevated triglycerides. Standard errors for the RLP-C and RLP-TG variables were much higher in the group with normal triglyceride levels (0.037 and 0.013, respectively) than in the group with elevated triglycerides (0.0051 and 0.0011, respectively). A test for an interaction effect between RLP and triglyceride level was not significant. Similar to the result using the total sample, in models including an RLP variable and triglyceride level simultaneously, neither variable was significant when adjusted for the other in the group with elevated triglycerides.

After including the cross-product of the RLP variables with time or log-transformed time variables in the models, no evidence was found for time dependency of the relationship between RLPs and CHD relative risk.

Because of the unusual results when both RLP and triglyceride levels were included in the same model, the correlations



between the 2 log-transformed RLP variables and other CHD risk factors were computed. As shown in Table 2, (log)triglyceride level was strongly correlated with (log)RLP-C and (log)RLP-TG level ( $r=0.88$  and  $r=0.93$ , respectively).

In addition, inclusion of people taking lipid-lowering medication ( $n=20$ ) did not significantly affect the results (results not shown).

### Discussion

Since the development of the current immunoseparation method, many investigators have reported an association between RLP-C and RLP-TG levels and atherosclerosis, CVD, or CHD.<sup>10–21</sup> However, this is the first long-term prospective investigation of the relationship between remnant-like particles and a cardiovascular disease outcome in healthy men. This study adds strong evidence for such a relationship independent of other nonlipid cardiovascular risk factors for CHD. Furthermore, in models including total cholesterol or HDL and LDL cholesterol levels, only RLPs were significant. Therefore, RLP levels provided information regarding CHD risk in addition to that provided by total cholesterol or LDL and HDL cholesterol.

In this study population, fasting triglyceride level was also an independent predictor of CHD relative risk after adjustment for age, BMI, smoking, alcohol intake, physical activity, systolic blood pressure, fasting glucose, history of diabetes, intake of antihypertensive medication, and LDL and HDL cholesterol. However, in models including an RLP variable and triglyceride level simultaneously, neither variable was predictive of CHD risk when adjusted for the other. Therefore, RLP levels did not provide additional information about risk of CHD over and above total triglyceride level. This finding can be attributed to the strong correlation between RLP-C and RLP-TG levels and total triglycerides.

In a healthy middle-aged male population, Karpe et al tested log-transformed RLP variables and triglycerides as continuous variables in multiple stepwise linear regression models and found that, independently of plasma triglycerides and LDL cholesterol, the plasma concentration of fasting RLP-C is related to intima-media thickness of the carotid artery, a marker of the early development of atherosclerosis.<sup>17</sup> However, when we repeated our analyses with log-transformed RLP variables and triglycerides, no evidence was found that RLP levels provide additional information about risk of CHD over and above total triglyceride level in this population.

The Adult Treatment Panel III report of the National Cholesterol Education Program recognizes elevated triglyceride level as an independent CHD risk factor.<sup>39</sup> It identifies VLDL cholesterol as the most readily available measure of atherogenic remnant lipoproteins and recommends VLDL cholesterol as a target for cholesterol-lowering therapy. The Adult Treatment Panel III limits this recommendation to individuals with elevated triglycerides; it identifies the sum of LDL+VLDL cholesterol (termed “non-HDL cholesterol” [total cholesterol–HDL cholesterol]) as a secondary target of therapy in persons with elevated triglycerides (triglycerides  $>200$  mg/dL). When subjects in our study with normal triglyceride levels were separated from those with elevated triglycerides, a significant association between RLP levels and relative risk of CHD was found only for the

group with elevated triglycerides. However, the regression coefficients estimates were very similar. The standard errors of the regression coefficients for the group with normal triglycerides were much larger than those for the group with elevated triglyceride levels, probably attributable to the smaller variability among individual RLP levels in this group. This contributed to the nonsignificant outcome for the normal triglyceride group. Thus, it is unlikely that there is a difference in the character of the relationship between RLP levels and CHD relative risk. RLP and triglyceride levels are strongly correlated and the distributions of RLP-C and RLP-TG are highly skewed to the right. Therefore, people with elevated triglycerides are more likely to have disproportionately high levels of RLP than people with normal triglyceride levels.

In our study in models that included an RLP variable and triglyceride level simultaneously, neither variable was significant when adjusted for the other. Therefore, even when attention was restricted to men with elevated triglyceride levels, no evidence was found that RLP levels provide additional information about risk of CHD over and above total triglyceride level.

Full RLP compositional data are lacking. ApoC-III plays a major role in atherosclerosis<sup>40</sup> and is a known component of remnants in the 1.006 to 1.019 kg/L density range. Apo E is thought to be protective because it enhances receptor-mediated uptake.<sup>40</sup> The metabolism of human VLDL particles is influenced by their content of apoE and is modulated by apoC-III.<sup>41</sup> The findings of this study point to the important role of triglyceride-rich lipoprotein particles in atherosclerosis. These triglyceride-rich particles contain apoB.<sup>42</sup> Therefore, apoB-containing and probably apoC-III-containing triglyceride rich particles may be responsible, in part, for the strong relationship of triglyceride with CHD. It should be emphasized that the triglyceride relationship with CHD suggests a wider involvement of triglyceride-rich particles in atherosclerosis than RLP-TG and that other remnant particles should also be considered in risk factor analysis.

In summary, RLP levels predicted risk of CHD independently of other nonlipid cardiovascular risk factors for CHD. Furthermore, RLP levels predicted CHD relative risk independently of total cholesterol or HDL and LDL cholesterol levels. Importantly, however, RLP levels did not provide additional information about risk of CHD over and above total triglyceride levels in this cohort. This finding can be attributed to the strong correlation between RLP-C and RLP-TG levels and total triglycerides. Therefore, this study does not support the need for testing of remnants if measures of fasting triglycerides are available and provides further evidence that triglycerides are an independent risk factor for CHD in men. However, further investigation of particular component RLPs and their relationship to cardiovascular disease appears warranted.

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