Elevated Remnant-Like Particle Cholesterol and Triglyceride Levels in Diabetic Men and Women in the Framingham Offspring Study

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OBJECTIVE — Remnants of triglyceride-rich lipoproteins are thought to be atherogenic. A new antibody-based assay allows for the isolation of remnant-like particles (RLPs) from plasma or serum, and the subsequent measurement of RLP cholesterol (RLPC) and triglycerides (RLPTGs). We hypothesized that diabetic patients would have higher remnant levels than nondiabetic patients.

DESIGN AND METHODS — We compared RLPC and RLPTG levels of diabetic subjects (68 women, 121 men) participating in the Framingham Heart Study with those of nondiabetic subjects (1,499 women, 1,357 men).

RESULTS — Mean RLPC values for diabetic women were 106% higher than those for nondiabetic women (0.367 ± 0.546 mmol/l [14.2 ± 21.1 mg/dl] vs. 0.179 ± 0.109 mmol/l [6.9 ± 4.2 mg/dl]; $P < 0.0001$), and RLPTG values for diabetic women were 385% higher than those for nondiabetic women (1.089 ± 2.775 mmol/l [93.1 ± 245.6 mg/dl] vs. 0.217 ± 0.235 mmol/l [19.2 ± 20.8 mg/dl]; $P < 0.0001$). Similar but less striking differences were observed in diabetic men, who had mean RLPC values 28% higher than those seen in nondiabetic men (0.285 ± 0.261 mmol/l [11.0 ± 10.1 mg/dl] vs. 0.223 ± 0.163 mmol/l [8.6 ± 6.3 mg/dl]; $P < 0.001$) and mean RLPTG values 70% higher than those seen in nondiabetic men (0.606 ± 1.019 mmol/l [53.6 ± 90.2 mg/dl] vs. 0.357 ± 0.546 mmol/l [31.6 ± 48.3 mg/dl]; $P < 0.001$). Moreover, diabetic men and women had significantly higher total triglycerides and lower HDL cholesterol levels than nondiabetic subjects.

CONCLUSIONS — The data indicate that RLP particles are elevated in diabetic subjects. To achieve optimal reduction of risk for cardiovascular disease, treatment of elevated RLP values, along with the control of LDL cholesterol levels, should be considered.

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Abbreviations: apo, apolipoprotein; CHD, coronary heart disease; CVD, cardiovascular disease; DBP, diastolic blood pressure; FHS, Framingham Heart Study; HDLC, HDL cholesterol; HMG, hydroxymethylglutaryl; LDLC, LDL cholesterol; NCEP, National Cholesterol Education Program; RLP, remnant-like lipoprotein particle; RLPC, RLP cholesterol; RLPTG, RLP triglyceride; SBP, systolic blood pressure; VA-HIT, Veterans Affairs HDL Intervention Trial.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Elevated serum triglyceride concentrations and decreased levels of HDL cholesterol (HDLC) are frequently observed in patients with premature cardiovascular disease (CVD) and diabetes. In Framingham Heart Study (FHS) participants, diabetes is an independent risk factor for coronary heart disease (CHD); its presence results in a 1.5-fold multivariate adjusted risk for CHD in men and a 1.8-fold adjusted risk for CHD in women (1). In addition, HDLC concentrations <0.90 mmol/l (35 mg/dl) result in a 1.5-fold increased risk of CHD in men and a 2.1-fold increased risk in women. Increased age, cigarette smoking, hypertension, elevated LDL cholesterol (LDLC), and low HDLC levels have been identified by the National Cholesterol Education Program's (NCEP) Adult Treatment Panel III as significant independent risk factors, and the presence of diabetes has been defined as a CHD risk equivalent (2).

It is difficult to fit triglycerides into this risk pattern, although elevated triglyceride levels are often associated with decreased HDLC levels. Families with premature CHD often have familial dyslipidemia (i.e., elevated triglycerides and low HDLC levels) as well as familial combined hyperlipidemia and isolated decreased HDLC levels (3). In a meta-analysis by Hokanson and Austin (4), based primarily on FHS prospective analyses, the presence of elevated triglyceride levels was a significantly more important risk factor in women than men. However, it has never been documented in intervention trials that lowering triglyceride levels is of significant benefit in reducing CHD risk. It should be noted that patients with dysbetalipoproteinemia who have elevated levels of partially metabolized triglyceride-rich lipoproteins (i.e., remnants), do appear to be at increased risk for CHD (3,5). This rare lipoprotein disorder, also called type III hyperlipoproteinemia, is associated with elevated remnant levels and defective clearance

stemming from abnormal forms of apolipoprotein (apo) E or apoE deficiency (5,6). However, it is now clear that elevated remnant levels are often observed in other more common disorders, such as renal insufficiency and diabetes, thereby potentially predisposing these patients to premature CHD (7–9).

In the past, it has been difficult to isolate remnants, and most techniques have been suitable for use in animal models only (10). However, a new assay has been developed that allows for the immunoseparation of remnant-like lipoprotein particles (RLPs) of both intestinal and liver origin (11), thereby possibly providing more sensitive information about CHD risk than does the measurement of triglycerides alone. Furthermore, this new assay is suitable for large-scale studies (12,13). The purpose of the present study was to compare RLP cholesterol (RLPC) and triglyceride (RLPTG) levels in diabetic participants from cycle 4 of the FHS Offspring Study with those from nondiabetic participants, as previous reports have indicated that diabetic subjects have significantly increased elevations of RLPC and RLPTG, which may contribute to their increased risk of CHD (7–9).

RESEARCH DESIGN AND METHODS

Study subjects

We compared RLPC and RLPTG levels of diabetic subjects (68 women, 121 men) participating in the Framingham Heart Study with those of nondiabetic subjects (1,499 women, 1,357 men). The diabetic subjects were participants in exam cycle 4 (1987–1990) of the FHS Offspring Study, under an ongoing, approved protocol, and ranged in age from 22 to 81 years (mean age 52 years). Information concerning smoking status, height, weight, and systolic and diastolic blood pressure (SBP and DBP, respectively) was collected at the time of examination (1). Subjects who reported cigarette use within the year before the exam were classified as current smokers; those who reported smoking, but not within the previous year, were classified as former smokers; the remainder were classified as never having smoked. BMI was calculated as weight in kilograms divided by the square of height in meters (k/m^2). Hypertension was defined as SBP >140 mmHg or DBP >90 mmHg, or by the intake of antihypertensive medication. Diabetes was defined as fasting blood glucose >6.85 mmol/l (125 mg/dl) or by the intake of hypoglycemic medication. The types and amounts of medications in this population were extremely variable and precluded adjustment for their effects on lipid parameters. Diagnosis of CVD included a history of angina pectoris, myocardial infarction, stroke, or transient ischemic attack, using previously published methods (1). Information was reviewed by a panel of physicians to adjudicate the diagnosis.

Lipoprotein analyses

Blood was drawn from each subject after a 12-h, overnight fast into tubes containing EDTA (0.15%). Plasma was separated by centrifugation (2,500 rpm, 4°C, 20 min). Plasma lipid and lipoprotein (total cholesterol, triglycerides, and HDLC) and glucose concentrations were measured fresh, using standard enzymatic methods, essentially as previously described (14,15). LDLC was calculated according to the formula of Friedewald et al. (16), except when triglycerides were >4.5 mmol/l (400 mg/dl). In those cases, which included 62 of the 3,045 participants (41 men [2.8%] and 21 women [1.3%]), LDLC values were omitted.

RLP analyses

RLP isolation was based on the removal of apoA-I–containing particles (HDL) and most apoB-containing particles (LDL, nascent VLDL, and nascent chylomicrons), using a previously described immunoseparation technique (Japan Immunoresearch Laboratories, Takasaki, Japan) (11,17) that has been shown to leave particles characteristic of previously described VLDL remnants and chylomicron remnants in the unbound fraction (10). Briefly, monoclonal antibodies to apoA-I and specific monoclonal antibodies to apoB that do not recognize partially hydrolyzed, apoE-enriched lipoprotein remnants were immobilized on agarose gel.

RLPC and RLPTG concentrations were measured in FHS plasma aliquots, which were then stored at −80°C until the time of analysis. Thawed plasma was incubated with the gel for 2 h, after which the gel, containing the bound (non-RLP) lipoproteins, was precipitated with low-speed centrifugation (5 min, 135*g*). RLPC and RLPTG levels were then measured in supernates on an Abbott Spectrum CCx chemistry analyzer (Abbott Diagnostics, Irving, TX) using two-reagent enzymatic, colorimetric assays containing a sensitive chromophore (Kyowa Medex, Tokyo). Precision studies have yielded among-run RLPC imprecision for two levels of RLP control over 20 runs of 9.1% at 0.18 mmol/l (7 mg/dl) and 7.3% at 0.62 mmol/l (24 mg/dl). Among-run RLPTG imprecision for the same controls was 8.3% at 0.25 mmol/l (22 mg/dl) and 5.0% at 1.23 mmol/l (109 mg/dl) (17).

Statistical analysis

Statistical analyses were performed on 3,045 individuals (1,567 women and 1,478 men). Subjects with prevalent diabetes (68 women and 121 men) were compared with those with no evidence of diabetes (1,499 women and 1,357 men) for a variety of risk factors, including RLPC and RLPTG. Student's *t* test was used to compare the mean levels of continuous measures, and a χ^2 statistic was calculated for categorical factors. For continuous measures that were highly skewed, we formally compared diabetic and nondiabetic subjects using log-transformed values, although the untransformed means and standard deviations are reported. To further evaluate the relationship between diabetes and RLPC and RLPTG, we established sex-specific quartiles for each measure and computed age-adjusted prevalence for each quartile, using direct standardization and the following age groups: ages 20–39, 40–49, 50–59, and 60+ years. The age distribution for each sex provided the standard distribution for these comparisons, and the Mantel extension test was used to calculate a test of linear trend in the prevalence across quartiles. To adjust for known diabetic risk factors, we used logistic regression, with the presence or absence of prevalent diabetes as the outcome. The covariates considered were age, hypertension (SBP >140 mmHg or DBP >90 mmHg or intake of hypertensive treatment), use of beta blockers, smoking status (current, former, or never), LDLC and HDLC concentrations, and hormonal replacement therapy in women. Analyses were conducted separately by sex. Because the age-adjusted prevalence rates indicated a sharp increase in the fourth quartile, we used dichotomous variables in these analyses for RLPC and RLPTG, which compared the odds of diabetes for those in the fourth quartile compared with all the rest.

Table 1—*Lipid and lipoprotein concentrations for nondiabetic and diabetic participants in the Framingham Heart Study (Offspring Exam 4)*

Variable	Nondiabetic	Diabetic	Difference (%)	*P*
Women				
n	1,499	68		
Age (years)	51 ± 10	57 ± 9	+12	<0.0001
BMI (kg/m^2)	26 ± 5	31 ± 7	+19	<0.0001
TC (mmol/l)	5.3 ± 1.0	5.8 ± 1.2	+10	<0.0004
TG (mmol/l)	1.2 ± 0.8	2.9 ± 3.7	+146	<0.0001*
LDLC (mmol/l)	3.3 ± 0.9	3.6 ± 1.0	+9	<0.03
HDLC (mmol/l)	1.5 ± 0.4	1.1 ± 0.4	−26	<0.0001
RLPC (mmol/l)	0.18 ± 0.11	0.37 ± 0.55	+106	<0.0001*
RLPTG (mmol/l)	0.22 ± 0.36	1.05 ± 2.78	+385	<0.0001*
Men				
n	1,357	121		
Age (years)	52 ± 10	59 ± 9	+13	<0.0001
BMI (kg/m^2)	28 ± 4	29 ± 5	+4	<0.0001
TC (mmol/l)	5.3 ± 0.9	5.4 ± 1.2	+1	NS
TG (mmol/l)	1.5 ± 1.1	2.2 ± 1.6	+41	<0.0001*
LDLC (mmol/l)	3.5 ± 0.9	3.4 ± 1.1	−2	NS
HDLC (mmol/l)	1.1 ± 0.3	1.0 ± 0.3	−11	<0.0002
RLPC (mmol/l)	0.22 ± 0.16	0.28 ± 0.26	+28	<0.0005*
RLPTG (mmol/l)	0.36 ± 0.55	0.61 ± 1.02	+70	<0.0001*

Data are means ± SD, unless otherwise indicated. *Measures are not normally distributed and were log-transformed for statistical analysis. TC, total cholesterol; TG, triglyceride.

RESULTS — As shown in Table 1, diabetic women were significantly older (+12%) and had a significantly higher BMI (+19%), modest elevation in total cholesterol (+10%), very striking elevation in triglycerides (+146%), modest increase in LDLC (+9%), and significant decrease in HDLC (−26%), in comparison with nondiabetic women. All of these differences were statistically significant. The overall prevalence of diabetes in FHS women was 4.3%. Diabetic women had a mean RLPC value that was 106% higher than that of nondiabetic women; the RLPTG elevation was even greater, with a marked 385% increase relative to nondiabetic women, so that values were more than fourfold higher.

Results for nondiabetic and diabetic men are also shown in Table 1. The diabetic men were older (+13%) and had a greater BMI (+4%), more elevated triglycerides (+41%), and lower HDLC (−11%) than nondiabetic men. These differences were all statistically significant; however, in contrast to the diabetic women, total cholesterol and LDLC levels were not significantly different in diabetic versus nondiabetic men. At 8.2%, the prevalence of diabetes in FHS men was somewhat higher than the 4.3% observed in the FHS women, but the magnitude of the differences for BMI, triglycerides, and HDLC were substantially less than what was observed in the women. Diabetic men had significantly higher RLPC (+28%) and RLPTG (+70%) concentrations than did nondiabetic men. These differences, however, were also considerably less than those observed for women.

The relation between diabetic and nondiabetic individuals with regard to NCEP cut points are shown in Table 2. Cut points reflecting 75th percentile values were tabulated for RLPC and RLPTG. These data indicate that there was a much higher prevalence of values above the cut points for triglycerides, RLPC, RLPTG, and even LDLC, as well as a significantly higher prevalence of low HDLC levels in diabetic subjects than in nondiabetic subjects. In addition, the data clearly indicate that the presence of diabetes is associated with much more profound lipid alterations in diabetic women than in diabetic men.

Prevalence rates for diabetes increased dramatically in the upper quartile of RLPC and RLPTG levels (Table 3), with age-adjusted estimates of 90 per 1,000 for women and 140 per 1,000 for men in the upper quartile of RLPC and RLPTG values ($P < 0.001$) compared with estimates of 20–40 per 1,000 for women and 60–90 for men in the lower three quartiles. These results suggest that the most striking association between remnant lipoproteins and diabetes lies in the upper quartile of RLPC and RLPTG.

Univariate correlations for RLPC and RLPTG with other CVD risk factors were performed. Risk factors included age (years), BMI (kg/m^2), SBP and DBP (mmHg), and levels of glucose, total cholesterol, triglycerides (log transformed) and LDLC and HDLC concentrations (mmol/l). Associations were highly significant ($P < 0.0001$) for all comparisons except for age in men, which showed a minimally significant association with RLPC ($P < 0.04$) and no significant association with RLPTG. RLPC values in both men and women with diabetes were strongly associated with all parameters, especially triglycerides and total cholesterol. RLPTG values in diabetic men and

Table 2—***Nondiabetic and diabetic subjects in the Framingham Heart Study (Offspring Exam 4) who exceeded NCEP cut points***

	Women			Men		
Variable	Nondiabetic	Diabetic	*P*	Nondiabetic	Diabetic	*P*
n	1,499	68		1,357	121	
Age >55/45 years	36	53	<0.004	69	93	<0.001
BMI >30 kg/m^2	17	50	<0.001	23	33	<0.02
TC >6.2 mmol/l	18	35	<0.001	16	16	NS
TG >2.25 mmol/l	6	41	<0.001	16	36	<0.001
LDLC >3.4 mmol/l	45	60	<0.02	55	55	NS
HDLC <1.0 mmol/l	9	49	<0.001	36	55	<0.001
RLPC (>75th percentile)	26	59	<0.001	27	42	<0.001
RLPTG (>75th percentile)	26	59	<0.001	27	44	<0.001

Data are %. TC, total cholesterol; TG, triglycerides.

Table 3—*Association of diabetes prevalence with RLPC and RLPTG concentrations based on quartiles*

	RLPC					RLPTG				
	Mean plasma concentration (mmol/l)	Diabetic subjects (*n*)	Total subjects (*n*)	Diabetes rate per 1,000	Age-adjusted diabetes rate per 1,000	Mean plasma concentration (mmol/l)	Diabetic subjects (*n*)	Total subjects (*n*)	Diabetes rate per 1,000	Age-adjusted diabetes rate per 1,000
Women										
Totals	—	68	1,567	—	—	—	66	1,492	—	—
Quartile										
1st	0.13 ± 0.01	5	391	12.8	19.8	0.09 ± 0.02	4	373	10.7	13.9
2nd	0.15 ± 0.01	9	392	23.0	23.6	0.14 ± 0.01	8	373	21.4	20.9
3rd	0.17 ± 0.01	14	392	35.7	29.7	0.19 ± 0.02	15	373	40.2	39.4
4th	0.29 ± 0.29	40	392	102.0	89.7*	0.59 ± 1.35	39	373	104.6	94.4*
Men										
Totals		121	1,478				118	1,456		
Quartile										
1st	0.14 ± 0.01	20	369	54.2	58.1	0.11 ± 0.03	22	364	60.4	59.9
2nd	0.16 ± 0.01	22	370	59.5	59.2	0.18 ± 0.02	12	364	33.0	33.1
3rd	0.20 ± 0.02	28	370	75.7	73.6	0.28 ± 0.05	32	364	88.2	88.6
4th	0.41 ± 0.27	51	370	138.2	140.6*	0.94 ± 1.00	52	364	142.9	141.3*

Data are means ± SD, unless otherwise indicated. *$P < 0.001$, test of linear trend across quartiles.

women were also associated with all parameters (except age in men) and were especially associated with triglycerides, total cholesterol, and HDLC (inversely).

When a logistic regression analysis was applied to the relation between RLPC and diabetes and RLPTG and diabetes, independent significance was not seen for the most part, probably because of the strong interrelation among the risk factors (Table 4). The odds ratio for RLPC at >75th percentile was 1.41 in women ($P = 0.26$) but was not quite significant in men ($P = 0.051$); the odds ratio for RLPTG at >75th percentile was independent only in men ($P = 0.048$). At the same time, a triglyceride level at >75th percentile was an independent factor in women ($P = 0.014$), with a similar trend in men ($P = 0.063$). These data indicate a strong interrelation among diabetes, hypertension, BMI, and dyslipidemia.

Table 4—*Logistic regression analysis for association of RLPC and RLPTG with diabetes*

Variable	Women	Men
RLPC >75th percentile		
Odds ratio (per year)	1.41	1.58
95% CI	0.77–2.57	0.998–2.49
P	0.26	0.051
RLPTG >75th percentile		
Odds ratio (per year)	1.28	1.59
95% CI	0.69–2.30	1.004–2.52
P	0.43	0.048
TG >2.25 mmol/l (200 mg/dl)		
Odds ratio (per year)	2.41	1.59
95% CI	1.19–4.86	0.98–2.61
P	0.014	0.063

Results given were adjusted for age, BMI, hypertension, diabetes, smoking, beta blocker use, LDLC, and HDLC.

CONCLUSIONS — Elevated triglyceride levels have been defined by the NCEP Adult Treatment Panel as being ≥4.5 mmol/l (400 mg/dl), with borderline values at 2.25–4.5 mmol/l (200–400 mg/dl). Many investigators have recommended more stringent criteria for the definition of hypertriglyceridemia (18); the difficulty in following this recommendation, of course, is the lack of clear-cut data showing benefit from lowering triglyceride levels in intervention studies. It should be noted that in the Helsinki Heart Study, in men selected for having non-HDLC >5.2 mmol/l (200 mg/dl), treatment with gemfibrozil resulted in significant reductions in triglyceride and LDLC levels, significant increases in HDLC levels, and significant reduction of CHD risk (19,20). However, benefit could be ascribed only to decreases in LDLC and increases in HDLC levels. In a subsequent analysis, it was determined that the men who received the most benefit from gemfibrozil therapy and CHD risk reduction were those with an LDLC-to-HDLC ratio >5.0 and triglyceride levels >2.25 mmol/l (200 mg/dl) at baseline.

In the Veterans Affairs HDL Intervention Trial (VA-HIT), men with CHD who had HDLC concentrations <1.0 mmol/l (40 mg/dl), triglyceride levels <3.4 mmol/l (300 mg/dl), and LDLC levels <3.6 mmol/l (140 mg/dl) were randomized to gemfibrozil versus placebo (21). In that study, the gemfibrozil group had a 22% reduction in CHD risk, substantial reductions in triglycerides, and small but significant increases in HDLC, compared with the placebo group.

It is known that patients with diabetes frequently do not have elevated levels of LDLC. VA-HIT participants, in fact, had a mean LDLC concentration at baseline of 2.90 mmol/l (112 mg/dl), but appeared to receive significant benefit from gemfibrozil therapy. This raises questions regard-

ing what the optimal drug therapy is in diabetic patients who have dyslipidemias. It is well known that diabetic patients received significant benefit in the Scandinavian Simvastatin Survival Study (4S), as well as in the VA-HIT, with lipid-lowering therapies (21,22). In fact, in some cases, diabetic patients have received even greater benefit than nondiabetic subjects with regard to risk reduction. A recent analysis of the VA-HIT data has indicated that the subjects receiving the greatest benefit from gemfibrozil treatment with regard to CHD risk reduction were those with diabetes and/or elevated insulin levels (23).

Preliminary data from our laboratory have shown that gemfibrozil can result in significant reductions in RLPC and RLPTG of >30% (unpublished observations). We have seen similar significant reductions with the use of the hydroxymethylglutaryl (HMG)-CoA reductase inhibitors atorvastatin and simvastatin in another small study (unpublished observations). It is known that elevated RLP levels are associated with CVD (6,11,12). In patients with type III hyperlipoproteinemia, there is a significant increase in these particles (6), and such patients have long been known to be prone to premature CHD as well as peripheral vascular disease (24). Moreover, these patients frequently are homozygous for apoE2, a form of apoE resulting in delayed clearance of triglyceride-rich lipoprotein remnants by the liver (5).

The choice of therapy at the present time in diabetic patients may depend on their baseline LDLC level. In general, diabetic patients have hypertriglyceridemia along with low HDLC levels. They also appear to have elevated RLPC and RLPTG levels. These particles have been clearly shown to be atherogenic and more recently have been shown to affect endothelial function (25). Moreover, in a Japanese study of > 500 subjects, elevated levels of these particles were observed in those who had either impaired glucose tolerance or frank diabetes (7). If the LDLC level in these subjects is <3.2 mmol/l (125 mg/dl), an appropriate primary therapy would probably be a fibric acid derivative such as gemfibrozil or fenofibrate; if, on the other hand, the LDLC level is ≥3.2 mmol/l (125 mg/dl) and the patient is being treated with monotherapy, the recommended drug of choice would be an HMG-CoA reductase inhibitor. The safety and efficacy of the combination of a statin and fibric-acid derivative in long-term intervention studies has not been evaluated, but recently some deaths have been associated with cerivastatin and gemfibrozil in combined treatment.

In our view, patients with diabetes are more likely to have not only elevated triglyceride and decreased HDLC levels, but also elevations in RLPC and RLPTG. To determine the risk profiles associated with RLP elevations that may predispose individuals to accelerated atherosclerotic risk, more research needs to be done. It also needs to be determined what target goals might be appropriate in diabetic patients with regard to these types of particles.

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