Elevated Remnant-Like Particle Cholesterol Concentration
A Characteristic Feature of the Atherogenic Lipoprotein Phenotype

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Patients at increased risk of coronary artery disease (CAD) frequently exhibit an atherogenic lipoprotein phenotype characterized by elevated plasma levels of both triglyceride-rich lipoproteins (TRL) and small, dense LDL and low concentrations of HDL cholesterol. Recently, in a large observational study, the calculated non-HDL plasma cholesterol concentration (the sum of the cholesterol contents of LDL, intermediate-density lipoprotein [IDL], and very-low-density lipoprotein [VLDL]) was a stronger predictor of cardiovascular events than plasma cholesterol alone. Improvement in the predictability of CAD on inclusion of VLDL and IDL cholesterol emphasizes the proatherogenic nature of TRL and their remnant particles.

The atherogenic lipoprotein phenotype has been defined by Austin et al19 as the presence of a predominance of small, dense LDL particles, elevated plasma triglyceride (TG) levels, and low plasma HDL cholesterol levels in the lipoprotein profile, which is associated with an approximately 3-fold increased risk of atherosclerotic disease.5–8 It is now commonly accepted that small, dense LDL particles are the products of the intravascular remodeling of TG-rich VLDL particles after interaction primarily with lipoprotein lipase, hepatic lipase, and cholesterol ester transfer protein9,10 (Figure). The atherogenic lipoprotein phenotype is strongly linked to obesity, insulin resistance, familial combined hyperlipidemia (FCHL), hypertension, and abnormalities in postprandial lipid metabolism.7,11,12 Epidemiological data from the Framingham study have already revealed that plasma TG concentration is an important independent risk indicator of CAD in women13; additional evidence supporting this observation was obtained by Yarnell et al14 in a 10-year follow-up study and confirmed by others.15,16 In the PROCAM (Prospective Cardiovascular Münster) study,17 this relationship was dependent on plasma HDL cholesterol concentration. Criqui et al18 however, could not demonstrate an independent relationship between plasma TG and cardiovascular mortality in a North American population participating in the Lipid Research Clinics Follow-up. The importance of plasma TG as an independent risk factor for CAD was recognized after the publication of a meta-analysis by Austin et al19,20 in 2000, which provided support for the earlier observation that plasma TG levels predict relative risk in relatives of FCHL patients.21

The assessment of plasma TG concentration as an independent predictor for CAD is complicated by the fact that (1) there is considerable interindividual and intraindividual variation in plasma TG levels and (2) circulating TRL are highly heterogeneous in size, density, and composition and may consist of intestinally derived apolipoprotein (apo) B-48–containing chylomicrons and chylomicron remnants, in addition to liver-derived apoB-100–containing VLDL and their remnants.22,23 Partially catabolized TRL are generally considered to be highly atherogenic24–26 and have been found to be present in the atherosclerotic lesion.27–29 The concentrations of these particles have been evaluated with a number of different methods based on their density, size, charge, specific lipid components, apolipoprotein composition, or immunoaffinity.22 Quantification of apoB-100 and apoB-48 has been achieved by SDS-PAGE after isolation of TRL by density-gradient ultracentrifugation.30–32 A significant association between increased levels of apoB-48 and apoB-100 in small VLDL (Sf 20 to 60) and IDL (Sf 12 to 20) and increased risk for cardiovascular disease has been found in a number of studies.33–38 The fate of the fatty acid moiety of intestinally derived chylomicron particles can be analyzed by in vivo labeling of the particles with retinyl esters (REs).39 Several studies have shown an association between abnormalities in the postprandial response of REs and premature atherosclerosis.34,36,40–44 Although these latter techniques have provided important information on TRL remnants, they are costly and time consuming and are not ideal either for large clinical studies or for use in routine analyses.

Accurate quantification of TRL remnants is problematic22 because (1) remnants are difficult to differentiate from their larger and more TG-rich precursors, (2) their plasma concentration is typically low compared with other lipoproteins, and (3) they are biochemically difficult to isolate or detect, because they represent a heterogeneous group of lipoproteins...
of variable size and composition. An immunoaffinity separation method for isolation of remnant-like particles (RLP) has been used recently in a number of clinical and laboratory studies to gain further insight into the metabolism and atherogenicity of remnant lipoproteins. The present article summarizes our current knowledge of the biochemical and pathophysiological characteristics of such RLP and provides evidence for the potential use of this assay as a biomarker for the atherogenic lipoprotein phenotype in patients with increased risk for atherosclerosis.

**RLP: Properties and Composition**

A novel method for the isolation and quantification of plasma remnants was developed by Nakajima and Nakamura in 1993. Specific monoclonal antibodies directed against epitopes of apoA-I and apoB-100 are bound to Sepharose 4B gel. This immunoaffinity gel is incubated with plasma, which results in binding of HDL, LDL, and the majority of VLDL particles to the gel. Unbound lipoproteins in the supernatant are quantified on the basis of their cholesterol content; this is a result of action of hepatic lipase (HL), cholesterol ester transfer protein (CETP), and plasma phospholipid transfer protein (PLTP). As a consequence, population of circulating apoB-containing lipoprotein particles is marked by heterogeneity on basis of their size, density, and compositional characteristics.

**Plasma RLP-C Concentration in Fasted Normolipidemic Subjects**

Plasma levels of RLP-C in normal healthy subjects have been determined in a number of studies. Levels of plasma RLP-C in healthy normolipidemic whites typically range from 0.16 to 0.24 mmol/L (6.2 to 9.3 mg/dL). RLP-C levels in Japanese subjects have consistently been reported to be lower than those in Caucasians, however, which may reflect ethnic differences or differences in the calibration of RLP-C assays in different laboratories. A number of studies have demonstrated significant differences between RLP-C levels in men and women, with higher levels in men. Plasma RLP-C levels increase with age in both males and females. Additional evidence for the presence of increased levels of plasma RLP-C in postmenopausal women has been reported. Indeed, in the Framingham study, women with diagnosed cardiovascular disease displayed plasma RLP-C and RLP-TG levels that were 16% and 27% higher than in women without cardiovascular disease, of whom 57% were in a postmenopausal state. In the total cohort, the prevalence rate for cardiovascular disease in the upper quartile of plasma RLP-C
levels, with an age-adjusted estimate, was 85 per 1000 women. The lower 3 quartiles yielded an age-adjusted estimate of 32 to 50 cases per 1000 women. Moreover, plasma RLP-C levels, but not total plasma TG levels, were an independent risk factor for cardiovascular disease.55 Hormone replacement therapy with estrogen alone56 or with estrogen-progestin combination56,57 resulted in a significant decrease in plasma RLP-C levels that was accompanied by improvement of endothelial function.

Postprandial Plasma RLP-C Levels in Normolipidemic Subjects

Plasma TRL and RLP accumulate in the postprandial period in dyslipidemic patients. Indeed, an increased postprandial accumulation of TRL is a central feature of the atherogenic lipid phenotype. With the use of an oral fat-load test with cream or a mixed meal, the postprandial lipid profile may be determined. Maximal postprandial RLP-C levels in mildly obese normolipidemic subjects, aged 50 years, were attained between 2 and 4 hours after a meal.58,59 In healthy young male volunteers, plasma RLP-C levels increased from 0.15 to 0.23 mmol/L at 4 hours after eating.60 Atorvastatin intervention for 3 weeks attenuates the postprandial RLP-C increase in contrast to placebo and gemfibrozil intervention. Ai et al61 found a postprandial RLP-C increase from 0.08 to 0.13 mmol/L in normolipidemic Japanese subjects after an oral fat load with only 17 g fat/m2. It has been shown that the amount of fat given orally influences the magnitude of the postprandial response. The chylomicron fatty acid moiety (represented as retinyl palmitate [RP] in the d <1.006 g/L density fraction) in normolipidemic French, nonobese (body mass index 20 to 24 kg/m2) subjects did not increase 3 hours after ingestion of either a nonfat or a 15 g/m2 low-fat meal, whereas significant increases in chylomicron REs were induced 3 hours after inggestion of meals with a fat content of 30, 40, and 50 g/m2.62

Plasma RLP-C Levels in Patients With Primary Dyslipidemia

Heterozygous Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) results from mutations in the LDL receptor gene associated with a lipoprotein phenotype that is characterized by elevated plasma cholesterol and LDL cholesterol levels. In a subset of heterozygous FH patients with elevated fasting plasma TG levels,63 plasma RLP-C levels were increased independently of plasma LDL cholesterol.64 Moreover, a post hoc analysis of this study revealed a significant association between baseline plasma RLP-C levels and carotid artery intima-media thickness, a surrogate marker for atherosclerosis in addition to plasma LDL cholesterol (T.B.T. et al, unpublished data, 2003). Both plasma apoB-48 and RLP-C levels were increased in a heterozygous FH population in Australia.65 Additional kinetic analyses using a stable isotope breath test revealed no difference in chylomicron catabolism in patients with FH compared with controls.66 Earlier studies using ultracentrifugal analyses and RE as a marker for chylomicron clearance showed a delayed chylomicron remnant clearance in the postprandial state.62,67 These observations support the growing interest in plasma RLP-C concentration as an atherogenic lipid component, even in lipid disorders such as FH.

Type III Dyslipidemia and Combined Hyperlipidemia

Elevated plasma levels of remnant particles characterize patients with type III dyslipidemia. These patients display an apo e2/e2 genotype, which results in abnormal apoE-mediated TRL uptake via hepatic lipoprotein receptors and consequently in accumulation of remnant particles in the circulation.68 Only a small proportion of individuals with an apo e2/e2 genotype develop type III hyperlipidemia. It would therefore be advantageous to have an additional clinical marker for the identification of individuals susceptible to the expression of this phenotype. Measurement of RLP-C and the RLP-C/TG ratio, an estimation of the enrichment of cholesterol in the TRL fraction (a characteristic of β-VLDL), provides an alternative for diagnosis of lipid abnormalities in these patients.69,70 Furthermore, plasma RLP-C levels were increased 5-fold in type III subjects. RLP in patients with type III hyperlipidemia exhibited “slow β” VLDL mobility on agarose gels, but their size was smaller (32 nm). In an Asian population, plasma RLP-C levels in type III hyperlipidemia were increased compared with control subjects but were lower than in Caucasian subjects.71

In patients with mixed or type IIb hyperlipidemia, plasma RLP-C levels were increased 2-fold, and in type IV hyperlipidemia subjects, they were increased almost 3-fold compared with control subjects.22,51,69 Plasma RLP were larger, with a particle diameter of 41 nm in patients with severe hypertriglycerideremia (TG >4.5 mmol/L).

Elevated plasma levels of TG, apoB, and/or cholesterol are characteristic features of the atherogenic lipid profile in subjects with FCHL. Lipoprotein metabolism in FCHL is disturbed, which leads to remnant accumulation in the circulation.72–74 Plasma levels of plasma RLP-C may constitute an additional marker for the identification of patients with FCHL. Male FCHL patients displayed a 6-fold increase in plasma RLP-C concentration compared with male normolipidemic controls, together with marked elevation of plasma TG levels, whereas plasma RLP-C concentration was only 2-fold higher in FCHL females compared with controls,75 and in contrast, plasma TG levels were only moderately elevated. Plasma TG was strongly correlated with plasma RLP-C in both men and women with FCHL. Multivariate analyses revealed that plasma RLP-C was the only significant contributor to abnormalities in the number of basal and postocclusive microvascular skin capillaries.75

Postprandial RLP-C Levels in Patients With Primary Hyperlipidemia

In patients with an equivalent increase in fasting plasma TG levels, such as in type IIb, type III, and type IV dyslipidemia, elevation in postprandial serum TG level was in the range of 30% to 50%, without significant differences between the maximal postprandial TG concentration in these dyslipidemic groups.76 Equivalent results were observed for postprandial plasma RLP-C elevations, with the exception of patients with
type III hyperlipoproteinemia, who displayed an increased level of circulating plasma RLP-C in the fasted and the fed state, as was illustrated by a significantly greater RLP-C/TG ratio.76 In FCHL patients, abnormalities in postprandial lipoprotein metabolism have been described,77,78 which indicates the presence of elevated plasma levels of apoB-48–containing and apoB-100–containing TRL fractions. No data on postprandial plasma RLP-C levels in FCHL patients have become available as yet, but we may speculate that RLP-C may constitute an additional biomarker for the abnormal postprandial lipoprotein response in this highly atherogenic dyslipidemia.

Plasma RLP-C in Secondary Dyslipidemia

Insulin Resistance

The insulin-resistant state is associated with a cluster of abnormalities in glucose and lipid homeostasis, including elevated levels of plasma TG, low plasma concentrations of HDL cholesterol, and increased prevalence of small, dense LDL.79 Metabolic defects include impaired free fatty acid metabolism, saturation of TRL remnant removal, and increased hepatic secretion of VLDL particles.80 Hepatic VLDL-1 production and secretion are suppressed by induction of acute hyperinsulinemia in healthy men, whereas in patients with type 2 diabetes mellitus, this feedback mechanism is impaired,81,82 Patients with the metabolic syndrome (ie, patients with visceral obesity, hypertension, and insulin resistance) equally display an atherogenic lipoprotein profile.79 Elevated fasting plasma RLP-C concentrations have been found more frequently in individuals with insulin resistance than in healthy subjects. Moreover, in a multiple regression analysis, the HOMA (homeostasis model assessment) ratio (an index of insulin resistance) was closely related to plasma RLP-C levels.83

Type II Diabetes Mellitus

Type II diabetes mellitus is associated with a marked increase in risk of cardiovascular disease. A characteristic clinical feature of diabetic patients is the prevalence of a dyslipidemia with elevated plasma levels of TG and small, dense LDL particles, whereas plasma HDL cholesterol concentrations are subnormal.84 It is therefore not surprising that a number of studies have investigated the contribution of RLP-C to the atherogenic lipoprotein profile in type II diabetes mellitus. Both fasting and postprandial plasma RLP-C levels were elevated.54,61,85–90 Interestingly, the impact of type II diabetes mellitus on lipoprotein phenotype and on risk of CAD is enhanced in women compared with men. Thus, women with type II diabetes mellitus have a higher proportion of small, dense LDL present that is dependent on plasma TG tertile.91,92 and they have relatively higher plasma RLP-C levels than men.54 Both parameters significantly contribute to the atherogenic lipoprotein phenotype seen in patients with type II diabetes mellitus.

Renal Disorders

In patients with renal disorders, the expression of an atherogenic lipid phenotype is associated with elevated plasma levels of TRL and, consequently, increased plasma RLP-C concentrations. Indeed, glomerular disease and albuminuria (defined as >2 g/24-h collected urine) are associated with an increase in plasma RLP-C levels. Plasma RLP-C concentration was not related to renal function (assessed as calculated creatinine clearance) but was associated closely with plasma TG level.93 Patients with a fatty liver or steatosis had significantly higher plasma RLP-C levels than control subjects (16 versus 5 mg/dL, respectively).94

RLP and Atherogenesis

It is established that elevated plasma RLP-C levels are associated with endothelial dysfunction, a marker for atherosclerotic disease.95 Patients with established coronary heart disease present elevated plasma levels of RLP-C.96,97 The intima-media thickness of the carotid artery was positively related to baseline plasma RLP-C concentrations in a secondary intervention study in patients aged 50 years and older after their first cardiovascular event.95 This association was independent of plasma TG and LDL cholesterol levels. In the Lipid Coronary Angiography Trial (LOCAT),95 the mean on-treatment plasma RLP-C concentration was significantly associated with the reduction of minimum luminal diameter (P<0.004). However, this association was not independent of plasma TG levels. In addition, a significant association was found between plasma RLP-C concentration and the occurrence of new lesions in vein grafts. In patients with vasospastic angina with or without myocardial infarction, plasma RLP-C concentration was a major risk factor in the prediction of myocardial infarction.98 Elevated levels of plasma RLP-C were predictive of future coronary events in Japanese patients with CAD independently of other risk factors.99,100 In the postprandial period, arterial relaxation (as assessed by flow-mediated dilation) in healthy young normolipidemic subjects decreased in parallel with the peak plasma concentration of TG and RLP-C.101 Additional evidence for a relationship between postprandial increase in plasma RLP-C and endothelial function was observed in a small study by Wilmink et al,60 wherein the increase in plasma RLP-C and decrease in endothelial function were attenuated by short-term statin treatment, independently of differences in plasma TG and cholesterol. Human studies therefore suggest a direct effect of RLP on atherogenesis.

In vitro studies have further contributed to elucidation of the role of RLP in the atherosclerotic process. Incubation of RLP with isolated rabbit aortas showed inhibition of arterial relaxation by acetylcholine in a dose-dependent manner.102 Pretreatment of rat aortas with Nω-nitro-L-arginine methyl ester downregulated vasorelaxation of thoracic rat aortas, which indicates the involvement of a nitric oxide–mediated pathway. Moreover, blocking of functional cell-surface glycoproteins with heparin and lactoferrin did not affect RLP-induced impairment of vasorelaxation. These results suggest that RLP exert a direct effect on endothelial function without binding to glycoproteins that are located at the apical surface of the endothelium. Incubation with RLP, but not VLDL (d<1006 g/mL) or LDL (1019<d<1063 g/mL), induced an elevation in expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and tissue factor in a human umbilical vein endothelial cell model in part through...
a redox-sensitive mechanism. Incubation with the antioxidant α-tocopherol dose dependently suppressed the RLP-induced mRNA expression of these proteins. In addition, treatment of subjects with elevated plasma RLP-C levels (RLP-C >5.1 mg/dL) for 4 weeks with α-tocopherol (300 mg/d) prevented the rise in plasma levels of adhesion molecules such as soluble intercellular adhesion molecule-1 and soluble vascular cell adhesion molecule-1. Incubation of RLP with human umbilical vein endothelial cells and a mononuclear cell fraction in a flow-conditioned model resulted in enhanced expression of CD11a, CD18, CD49d, and interleukin-1β, which indicates a role of remnant lipoproteins in the initiation of vascular inflammation.

Inflammation is a key step in atherogenesis, and a proinflammatory phenotype is closely associated with an increase in cardiovascular morbidity and mortality. The interaction of RLP with macrophages and the consequent atherogenic response is still under investigation, but RLP may induce foam cell formation, according to previous studies in animal models (Tomono et al and T.B.T. et al, unpublished observations, 2003).

**Effect of Statin Treatment on Plasma RLP-C**

Extensive evidence from intervention trials has been presented to demonstrate that statin treatment results in reduced cardiovascular morbidity and mortality. By contrast, statin treatment has not been associated consistently with reduction in plasma RLP-C levels. The effects of pravastatin, atorvastatin, or simvastatin on RLP-C levels were investigated in a randomized crossover study in patients with combined hyperlipidemia. Plasma RLP-C reduction did not occur after pravastatin treatment (40 mg/d), whereas simvastatin (20 mg/d) and atorvastatin (10 mg/d) lowered plasma RLP-C levels significantly. Although high-dose simvastatin therapy in homozygous FH patients also reduced plasma RLP-C concentrations significantly, only 25% of FH patients reached plasma RLP-C levels comparable to age- and gender-matched normolipidemic subjects. In a small FH population, treatment with statins resulted in a significant reduction in baseline and postprandial RLP-C levels. In patients with established coronary heart disease, a significant dose-independent decrease in plasma RLP-C (~33%, 20 mg/d; ~34%, 40 mg/d; ~32%, 80 mg/d) with atorvastatin could be demonstrated together with dose-dependent lowering of plasma levels of LDL cholesterol (~38%, ~46%, and ~52%, respectively) and TG (~22%, ~26%, and ~30%, respectively). This is not surprising, because the primary target for lipid lowering with a statin is reduction in plasma LDL cholesterol, whereas a decrease in plasma TG is much less pronounced. Additional intervention studies in distinct patient groups are required to compare different statins with regard to their potency to reduce plasma RLP-C levels. In general, however, it can be concluded that RLP-C lowering by statins depends on their ability to reduce plasma TG levels.

**Effect of Fibrate Treatment on Plasma RLP-C**

The principal pharmacological effect of fibric acid derivatives (fibrates) is to induce reduction in plasma TG and elevation in plasma HDL cholesterol levels. Fibrates are agonists of peroxisome proliferator-activated receptor-α (PPAR-α). Several key genes involved in TG metabolism, such as apoC-III and lipoprotein lipase, have a PPAR-α-regulating element in their promoter. ApoC-III gene expression is thus downregulated by fibrates, whereas lipoprotein lipase gene expression is upregulated. Furthermore, a shift toward more buoyant, less dense LDL particles with diminished atherogenic properties occurs. As mentioned above, plasma RLP-C concentrations are strongly correlated with plasma TG, and therefore it is predictable that fibrate treatment significantly decreases plasma RLP-C levels. In the LOCAT study, patients who had undergone CABG were treated for 2 years with gemfibrozil (1200 mg/d). Median plasma RLP-C concentration was reduced by 33% (from baseline 0.24 [0.17 to 0.42] to 0.15 [0.13 to 0.20] mmol/L). In patients with proteinuric renal disease, no effect of cerivastatin (100 or 200 µg once per day for 2 months) on plasma RLP-C was found, in contrast to fenofibrate (200 mg once per day for 2 months), which resulted in a 35% decrease in plasma RLP-C levels. This reduction was significantly correlated with the drug-induced reduction in plasma TG concentration ($r^2 = 0.58$, $P<0.005$). In line with these results, the total integrated (AUIC [area under the incremental curve]) postprandial plasma RLP response, measured hourly from 8 AM to 4 PM, was significantly (~43%) reduced by gemfibrozil treatment (3 months) in patients with type 2 diabetes and combined hyperlipidemia.

**Diet Intervention and Plasma RLP-C**

The effect of diet intervention on plasma RLP-C levels has not been evaluated extensively. In contrast to long-term intervention studies, short-term dietary intervention studies with either a high-fat or high-carbohydrate intake in healthy adults did not result in changes in plasma TG levels. Moreover, in type IV hyperlipidemic patients who consumed a low-fat diet for 3 months, no changes in fasting plasma TG levels were found. In a short-term dietary intervention study in which carbohydrate was added to mixed meals, no increase in de novo lipogenesis was found. A diet of 20 g of soy protein isolate for 3 weeks reduced baseline plasma RLP-C levels by 9.8%. Replacement of diacylglycerol by triacylglycerol in the oral fat load increased postprandial plasma RLP-C levels. Dietary reduction in lipid content with a concomitant increase in carbohydrate resulted in beneficial effects on the plasma RLP-C profile.

**Conclusion and Perspectives**

There is now considerable evidence to indicate that elevated levels of plasma TRL remnants are associated with increased risk of premature CAD. This association may reflect a direct effect of remnant lipoproteins on arterial lipid accumulation and lesion formation or alternatively may represent a characteristic feature of an atherogenic lipoprotein phenotype associated with elevated concentrations of small, dense LDL and low levels of antiatherogenic plasma HDL. Increased plasma RLP-C levels are clearly associated with increased risk of disease and can be significantly reduced by lipid-lowering therapy. They therefore not only constitute a diagnostic marker but also may be a prospective marker of response to treatment.
lipid-lowering therapy. Despite the large number of publications on RLP-C in the last 10 years, additional work is required to establish prospectively the therapeutic value of reducing plasma RLP-C levels in dyslipidemic patients at high risk of experiencing cardiovascular end points in prospective intervention trials.

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