Triacylglycerol-rich lipoproteins and atherosclerosis – where is the link?

P. Cullen
Institut für Arterioskleroseforschung, Domagkstrasse 3, D-48149 Münster, Germany

Abstract
The link between raised levels of LDL (low-density lipoprotein) cholesterol in the blood and atherosclerosis is incontrovertible. The situation with regard to TRLs (triacylglycerol-rich lipoproteins) is not as clear. Nevertheless, there is substantial evidence that TRLs may in some cases be just as atherogenic as triacylglycerol-poor LDL. This review focuses on three aspects of the link between TRLs and atherosclerosis: (i) the epidemiological evidence for an association between raised levels of TRLs and atherosclerosis, with particular reference to the results of the PROCAM study; (ii) the possible pathophysiological contribution of TRL to atherogenesis at the level of the arterial wall; and (iii) the case for specific lowering of triacylglycerol levels to prevent atherosclerosis.

Introduction
The link between raised levels of LDL (low-density lipoprotein) cholesterol in the blood and atherosclerosis is incontrovertible. There is also very strong evidence that this link is causal; lowering of LDL cholesterol by drugs, in particular statins, reduces the morbidity and mortality from cardiovascular disease in general and CHD (coronary heart disease) in particular.

The situation with regard to TRLs (triacylglycerol (triglyceride)-rich lipoproteins) is not as clear. Nevertheless, there is substantial evidence that these may in some cases be just as atherogenic as triacylglycerol-poor LDL. Most of the other papers in the present colloquium are directed to the metabolism or modification of TRLs. This review will focus instead on three aspects of the link between TRLs and atherosclerosis: (i) the epidemiological evidence for an association between raised levels of TRLs and atherosclerosis, with particular reference to the results of the PROCAM study; (ii) the possible pathophysiological contribution of TRLs to atherogenesis at the level of the arterial wall; and (iii) the case for a specific lowering of triacylglycerol to prevent atherosclerosis.

TRLs and atherosclerosis – the epidemiological evidence
The link between raised triacylglycerol levels and ischaemic stroke is, at best, weak and of little clinical significance. In the recently published and careful prospective Atherosclerosis Risk in Communities (ARIC) study, for example, triacylglycerols showed no association with ischaemic strokes in white or black men or women after adjustment for age, systolic blood pressure, smoking, diabetes, left ventricular hypertrophy, fibrinogen and von Willebrand factor [1]. This result is interesting in view of an earlier result from the ARIC study that greater postprandial increases in triacylglycerols and TRLs were found to be an independent risk factor for thickening of the carotid artery [2]. Similar negative results on the effects of triacylglycerol on risk of ischaemic stroke have been reported in other studies [3–7]. In fairness, however, it should also be stated that LDL cholesterol shows an equally poor relationship with ischaemic stroke as triacylglycerols [1], suggesting that, overall, circulating lipids play little role in the genesis of this condition.

Very few prospective studies have focused on the link between TRLs and peripheral vascular disease. A positive association between raised triacylglycerol levels and atherosclerosis of the peripheral arteries was reported in the Speedwell heart disease study [8], but at the present time this evidence must be regarded as preliminary.

In contrast with the relative paucity of studies investigating the role of raised triacylglycerol levels in ischaemic stroke or peripheral vascular disease, many investigations have focused on the link between TRL and CHD. As I wrote previously, however, the evidence for this link also has not been consistent [9]. In the 12-year assessment of the Lipid Research Clinics Follow-up Study, for example, no independent association between fasting plasma triacylglycerol levels and coronary mortality was found [10]. The Quebec Cardiovascular Study also failed to find an association between high triacylglycerol levels and CHD once HDL (high-density lipoprotein) cholesterol was accounted for [11].

In contrast, in a prospective case–control investigation from the Physicians’ Health Study, non-fasting serum triacylglycerol concentrations were a strong and independent predictor of outcome over 7 years of follow-up, even allowing for HDL cholesterol [12]. Similar positive associations...
between hypertriglyceridaemia and CHD were seen in the Boston Area Health Study [13] and in a 14-year follow-up among women in late middle age in the Framingham Heart Study [14]. Data from the Paris Prospective Study support the significance of raised triacylglycerols as a risk factor in patients with Type II (non-insulin-dependent) diabetes mellitus [15], while in the Honolulu Heart Program, the rate of CHD or thromboembolic stroke was increased at triacylglycerol levels above 200 mg/dl (2.27 mmol/l) [16]. The Copenhagen Male Study also showed an increased risk of CHD among middle-aged and elderly men in the middle and highest tertiles of triacylglycerol levels after adjustment for age, body mass index, alcohol intake, smoking, physical exercise, hypertension, Type II diabetes mellitus, social class, LDL cholesterol and HDL cholesterol [17]. Austin and Hokanson [18,19] performed an extensive meta-analysis of 17 prospective population-based studies published between 1965 and 1994 on the effects of triacylglycerol levels on CHD risk. When only the six studies are examined that adjusted for other risk factors, including HDL cholesterol, the relative CHD risk for each 88 mg/dl (1 mmol/l) increase in serum triacylglycerol concentration among some 22,000 men was 1.14. In two studies incorporating some 6000 women, the relative CHD risk associated with each 88 mg/dl (1 mmol/l) rise in serum triacylglycerol concentration was 1.37 [19].

Good evidence for an independent association between circulating triacylglycerol levels and CHD also comes from the Prospective Cardiovascular Münster (PROCAM) study. In the 10-year follow-up of this study, there was a significant and independent association between serum triacylglycerol concentration and the incidence of major coronary events among 4559 middle-aged men. The data in this cohort of men were used to generate an algorithm based on a Cox proportional-hazards model for CHD risk calculation using the eight independent risk variables age, LDL cholesterol, HDL cholesterol, triacylglycerols, family history of myocardial infarction, systolic blood pressure, smoking and diabetes mellitus [20]. This algorithm in turn was used to general a simple point score suitable for use at the desk or bedside (see also the website of International Task Force for Prevention of Coronary Heart Disease at www.chd-taskforce.com). Previous results in PROCAM had shown that the lipid triad, consisting of low HDL cholesterol [\(<0.9 \text{ mmol/l (35 mg/dl)}\), a high total cholesterol/HDL cholesterol ratio (\(>5\)) and a high triacylglycerol level (\(>2.3 \text{ mmol/l (200 mg/dl)}\)], to be particularly atherogenic. Fully 14% of 40–65-year-old men with this constellation developed a coronary event within 8 years. Although this subgroup comprised only 4.3% of middle-aged men in the study, it contained no fewer than 21% of all observed CHD events [21]. Also, among survivors of myocardial infarction in the Münster Heart Study, mixed hyperlipidaemia, with increases in both LDL cholesterol and triacylglycerol, and not hypercholesterolaemia alone, was the rule [21].

Increased levels of triacylglycerol have also been shown to be associated with coronary events in patients with established CHD. In the European Concerted Action on Thrombosis (ECAT) study, the geometric mean triacylglycerol level was 180 mg/dl (2.0 mmol/l) in 106 patients with a major coronary event within 2 years of follow-up, compared with 157 mg/dl (1.8 mmol/l) in 2700 patients who remained free of a recurrent event within that period (\(P = 0.007\)) (A. von Eckardstein, personal communication).

**Contribution of TRLs to atherogenesis in the arterial wall**

Chylomicrons and their remnants, as well as the remnants of VLDL (very-low-density lipoprotein), can penetrate into and be trapped within the arterial wall, where they can be taken up by macrophages. Animal studies have shown that particle size determines the rate of particle entry into the artery [22]. In studies on atherosclerosis of the aorta, visceral and renal artery in humans, about half of all particles recovered from the atherosclerotic lesion by immunadsorption on an anti-apoB (apolipoprotein B) column were found to have the size of VLDL, IDL (intermediate-density lipoprotein) or, especially, VLDL remnants [23,24]. Small TRLs, including postprandial chylomicron remnants, are believed to be the most atherogenic of all TRL particles. Particle for particle, for example, VLDL delivers five times as much cholesterol to the macrophage as does LDL [22].

Lipoprotein lipase may also be pro-atherogenic in the artery wall. Macrophages synthesize lipoprotein lipase, and transgenic mice overexpressing lipoprotein lipase in macrophages show increased atherosclerosis [25]. One mechanism for this may be that lipoprotein lipase releases non-esterified fatty acids from TRL, which increase the permeability of the endothelium to proteins and may also potentiate oxidant stress [26]. Consistent with this hypothesis, lowering of triacylglycerol levels by fenofibrate [27] and the use of niacin in patients with low or normal LDL [28] improves endothelial-dependent vasodilatation. A second mechanism is that lipoprotein lipase acts as a high-affinity molecular tether, linking lipoproteins to proteoglycans within the arterial wall [29]. Indeed, retention of atherogenic lipoproteins by proteoglycans within the arterial wall may be one of the very first steps in the development of the atherosclerotic lesion [30]. This retention is mediated by the interaction of positively charged residues in apolipoprotein E, apoB100 or apoB48 with negatively charged moieties on the proteoglycans [30].

**Specific lowering of triacylglycerols to prevent atherosclerosis**

As noted in the Introduction, an important element in proving that a factor is causally linked to a disease is the demonstration that removal or lessening of this factor reduces the incidence or severity of the disease in question. With regard to risk factors for CHD, this criterion has been fulfilled for LDL cholesterol and cigarette smoking, for example. Thus a major criticism of the postulated link between TRL and CHD is the fact that no clinical trial has
been performed to specifically examine the effects of lowering isolated hypertriglyceridaemia on CHD risk. However, as pointed out by Gotto [31], it may not be possible to design such a trial, as available drugs that lower triacylglycerol levels also alter the concentrations of other lipoproteins. Nevertheless, indirect conclusions can be drawn from a large number of intervention trials on the effects of lowering triacylglycerol levels on CHD risk.

**Triacylglycerol lowering using fibrates: effects on CHD**

Several trials using fibrates provide evidence for a salutary effect of triacylglycerol lowering on CHD risk. In recent years, the molecular action of fibrates has been elucidated by Bart Staels, Johan Auwerx and others [32,33]. Fibrates bind to and activate PPARα (peroxisome proliferator-activated receptor α), a nuclear receptor that forms heterodimers with the retinoid X receptor to exert effects on gene transcription. PPARα is the primary PPAR subtype expressed in the liver, and is a major regulator of the hepatic metabolism of fats, the synthesis and catabolism of lipoproteins and some steps in the HDL synthetic and reverse cholesterol pathways [32]. Binding to the PPARα by fibrates activates genes involved in the cellular uptake, derivatization and β-oxidation of fatty acids [34–38]. Increased diversion of fatty acids into β-oxidation decreases the availability of fatty acyl-CoA substrates for triacylglycerol synthesis in the liver, and thus decreases the triacylglycerol content of VLDL in plasma.

In the Helsinki Heart Study, the group of patients who benefitted most from treatment with gemfibrozil were those with the lipid triad, i.e. high LDL cholesterol, low HDL cholesterol and high triacylglycerol levels [39,40]. In fact, this group accounted for fully 70% of the reduction in CHD risk in that study. The Bezaﬁbrate Coronary Atherosclerosis Intervention Trial (BECAIT) was an angiographic study in which 92 young male dyslipidaemic survivors of myocardial infarction were randomized to receive either bezafibrate or placebo. Mean serum triacylglycerol concentrations fell by 31% and HDL cholesterol concentrations increased by 90% in the treatment group, with no change in the concentration of LDL cholesterol [41–44]. Patients randomized to receive bezafibrate had a lower rate of progression of focal coronary atherosclerosis and fewer coronary events (three compared with 11) after 5 years. The effect size was similar to that observed in statin-based angiographic studies, despite the different effects on lipid proﬁles.

The Bezaﬁbrate Infarction Prevention (BIP) study was a multicentre investigation of the effects of treatment with bezafibrate for at least 5 years on coronary morbidity and mortality in 2855 older men and 267 older women (mean overall age 60 ± 7 years). The participants in the BIP study had moderately elevated lipid levels and clinically manifest CHD [45,46]. In this study, bezafibrate lowered LDL cholesterol by 5% and triacylglycerols by 22%, while HDL-cholesterol increased by 12%. The pre-specified primary end-points of the BIP study were the incidence of myocardial infarction and sudden death. While there was no change in the combined primary end-point (non-fatal myocardial infarction, fatal myocardial infarction or sudden death) or in total mortality under treatment, the subgroup of patients with baseline triacylglycerol levels of ≥2.3 mmol/l (200 mg/dl; 15% of the study population; n = 459) profited from treatment with bezafibrate. The reduction in the combined primary end-point in this subgroup of participants was 40% (P = 0.03) (E. Kaplinsky, personal communication).

The HDL Intervention Trial (HIT) was conducted to investigate if raising low HDL cholesterol levels [mean at entry 0.83 mmol/l (32 mg/dl)] in the presence of normal LDL cholesterol [mean at entry 2.87 mmol/l (111 mg/dl)] and triacylglycerol [mean at entry 1.82 mmol/l (161 mg/dl)] levels confers clinical beneﬁt [48]. A total of 2331 men with a mean age of 64 years were randomized to receive 1.2 g of gemfibrozil or placebo for 5 years. One-quarter of the patients had frank diabetes mellitus. In the gemfibrozil group, there was a 3.6% rise in LDL cholesterol (4.0% rise in controls) and a 7.5% rise in HDL cholesterol (1.8% rise in controls), while triacylglycerols fell by 24.5% (9.6% rise in controls). These changes were associated with a 22% fall in the predefined primary end-point of non-fatal myocardial infarction or death due to CAD (P = 0.006). There was also a 27% fall in the incidence of reported stroke (P = 0.05) and a 58% fall in the incidence of transient ischaemic attacks (P = 0.001) in the gemfibrozil-treated group. The effects of gemfibrozil were the same in diabetic and non-diabetic subjects [48].

A number of trials have been performed to specifically assess the importance of triacylglycerols as a target for lipid modification in patients with Type II diabetes mellitus. In the Diabetes Atherosclerosis Intervention Study (DAIS), fenofibrate reduced triacylglycerol levels by approx. 30%, increased HDL cholesterol by 7% and increased the diameter of LDL particles by 4%. These changes were associated with 40% less progression of coronary stenosis [49,50]. Results from the Collaborative Atorvastatin in Diabetes Study (CARD) [51] and the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trial are expected in 2005 [52]. The outcome of the Lipids in Diabetes (LDS) study, which uses cerivastatin, is uncertain following the withdrawal of this drug from the market.

**Triacylglycerol lowering using statins: effects on CHD**

Up to now, drug treatment of patients with high triacylglycerol levels and mixed hyperlipidaemia has focused on the use of fibrates. However, evidence is emerging that statins, drugs that inhibit 3-hydroxy-3-methylglutaryl-CoA reductase, the enzyme controlling the rate-limiting step of cholesterol synthesis, may also be effective under these circumstances.

In the Monitored Atherosclerosis Regression Study (MARS) using lovastatin [53], the progression of mild-to-moderate coronary lesions was correlated best with the on-treatment circulating concentration of lipoprotein remnant particles. This progression was correlated with the presence of a high proportion of apoC-III in VLDL and LDL rather
than in HDL. An increased amount of apoC-III in HDL relative to that in LDL and VLDL indicates recent clearance of chylomicrons and VLDL. Thus a high proportion of apoC-III in VLDL and LDL suggests impaired metabolism of lipoprotein remnant particles [54]. Blankenhorn and colleagues [55] conducted a within-group analysis of the results of the Cholesterol Lowering Atherosclerosis Study (CLAS), showing that HDL apoC-III was negatively related to disease progression in drug-treated subjects, supporting the concept that removal of TRLs is anti-atherogenic.

Subgroup analysis of the Cholesterol and Recurrent Events (CARE) study [56] suggested that patients with a baseline triacylglycerol level of <143 mg/dl (1.62 mmol/l) experienced a 32% reduction in the risk of a coronary event (P < 0.001) on pravastatin, while those with a baseline triacylglycerol level of ≥143 mg/dl (1.62 mmol/l) did not [56]. Baseline triacylglycerol levels were also predictors of risk in the West of Scotland Coronary Prevention Study (WOSCOPS) of pravastatin (57); cited in [31]).

In the Scandinavian Simvastatin Survival Study (4S), increases in the baseline triacylglycerol level in the placebo group, but not in the simvastatin-treated group, were associated with increases in the coronary event rate [58]. In 4S, the increased risk associated with increased triacylglycerol levels appeared to be abolished by lipid-lowering treatment with simvastatin (reported at the European Society of Cardiology meeting, Stockholm, Sweden, August 24–28, 1997, and cited in [31]).

**Conclusion**

There is consistent evidence that raised levels of TRLs are associated with coronary atherosclerosis. Evidence for an association with other forms of atherosclerotic disease is much less certain. TRLs enter the wall of the coronary artery and adhere there to proteoglycans, either directly or via the enzyme lipoprotein lipase, which acts as a molecular tether. Lipoprotein lipase also liberates non-esterified fatty acids from TRLs, and these in turn may increase the development of atherosclerosis by contributing to endothelial toxicity. The main effects of fibrates are a fall in circulating triacylglycerol levels and an increase in HDL cholesterol. The results of the fibrate trials are inconclusive with regard to an effect on overall coronary morbidity and mortality, but seem to influence the concept that removal of TRLs is anti-atherogenic.

The references are as follows:


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