Triglycerides as a Risk Factor in Cardiac Heart Disease

MICHAEL MILLER, M.D., F.A.C.C.
Guest Editor

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Supplement II

Triglycerides as a Risk Factor in Cardiac Heart Disease

MICHAEL MILLER, M.D., F.A.C.C., Guest Editor

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ARTICLES IN BRIEF

Original Contributions

II-1 The Epidemiology of Triglyceride as a Coronary Artery Disease Risk Factor
M. MILLER, M.D., F.A.C.C.

Triglyceride (TG) has long been associated as a risk factor for coronary artery disease. A recent meta-analysis of various epidemiologic studies has confirmed this link. An important issue is to assess further the appropriate cutpoints to classify desirable TG because recent data indicate that levels < 200 mg/dl confer elevated risk. The dietary habits of present hunter-gatherer populations reveal the impact of a Westernized diet on both TG and cholesterol and suggest that a desirable TG is < 100 mg/dl. The epidemiologic and observational data in support of this concept are explored.

II-7 Pathophysiology of Triglyceride-Rich Lipoproteins in Atherothrombosis: Cellular Aspects
S. H. GIANTURCO, PH.D. AND W. A. BRADLEY, PH.D.

Elevated plasma levels of triglyceride-rich lipoproteins (TGRLP), including very-low density lipoproteins (VLDL), chylomicrons, and their remnants, are now acknowledged as risk factors for cardiovascular disease. Interactions of TGRLP with lipoprotein receptors on monocytes, macrophages, and endothelial cells may be mechanistically linked to this risk. Triglyceride-rich lipoproteins from hypertriglyceridemic (HTG) subjects have the abnormal ability to bind to low-density lipoprotein receptors via apoE, and plasma chylomicrons from all subjects bind to a new, distinct receptor for apoB48 that is expressed specifically by monocytes, macrophages, and endothelial cells. Receptor binding and uptake of TGRLP by these cells are likely mechanisms involved in the formation of lipid-filled, macrophage-derived “foam cells” of atherosclerotic lesions and for defective fibrinolysis due to endothelial dysfunction. Recognition of the atherothrombogenic potential of TGRLP may lead to improved interventions to lessen or prevent the often fatal sequelae of coronary atherosclerosis and thrombosis associated with elevated plasma triglyceride levels.

II-15 Pathophysiology of Triglyceride-Rich Lipoproteins in Atherothrombosis: Clinical Aspects
H. N. HODIS, M.D., W. J. MACK, PH.D., R. M. KRAUSS, M.D., P. ALAPOVIC, PH.D.

Invasive and noninvasive arterial imaging are important techniques used to study atherosclerosis and, specifically, to evaluate the atherogeneity of triglyceride-rich lipoproteins (TRL). Serial coronary angiography trials show significant benefit from lowering low-density lipoprotein cholesterol (LDL-C) which serves to retard lesion progression. Even with aggressive LDL-C reduction, however, up to half of patients demonstrate continued progression of atherosclerosis. Angiographic studies reveal that lowering LDL-C has the most impact on severe lesions, those ≥50% diameter stenosis, whereas TRL (and their apolipoprotein markers) have been identified as a driving factor behind progression of mild-to-moderate lesions < 50% diameter stenosis. Quantitative coronary angiography (QCA) has demonstrated that progression of mild-to-moderate lesions are among the most significant predictors of clinical coronary events, and that lowering TRL reduces progression of CAD to the same degree as the lowering of LDL-C.

II-21 Measurement of Triglyceride-Rich Lipoproteins by Nuclear Magnetic Resonance Spectroscopy
J. OTVOS, PH.D.

Nuclear magnetic resonance (NMR) spectroscopy is being used to determine the concentrations of very-low density lipoprotein, low-density lipoprotein (LDL), and high-density lipoprotein subclasses of different size. These subclasses have unequal associations with coronary heart disease. Nuclear magnetic resonance distinguishes among the subclasses on the basis of slight differences in the spectral properties of the lipids carried within the particles, which vary according to the diameter of the phospholipid shell. Studies using NMR spectroscopy have shown that individuals with elevated triglycerides are likely to have higher-risk lipoprotein subclass profiles. Triglyceride-rich lipoproteins drive the metabolic reactions that produce LDL of abnormal size and cholesterol content. The quantities of these abnormal LDL particles and the associated risk of coronary heart disease are underestimated by conventional cholesterol measurements. Nuclear magnetic resonance spectroscopy measures lipoprotein subclasses directly and efficiently and produces information that may improve the assessment and management of cardiovascular disease risk.
ARTICLES IN BRIEF

II-28 Postprandial Triglyceride Metabolism in Diabetes Mellitus
A. GEORGOPOULOUS, M.D.

Individuals with diabetes have a two-to-four times higher risk of cardiovascular morbidity and mortality compared with nondiabetics. Patients with both type 1 and type 2 diabetes share a similar risk. Studies in individuals with type 1 diabetes have shown a decreased clearance of postprandial triglyceride-rich lipoprotein particles of abnormal composition. Particles isolated from diabetic individuals show abnormal composition and an increased tendency to cause cholesteryl ester accumulation in macrophages and therefore are potentially atherogenic. Various interventions may alter these abnormalities and improve the atherosclerotic risk. These include adopting a high-carbohydrate diet over a high-monounsaturated diet, improving glycemic control, infusing insulin intraperitoneally, and using pharmacologic therapies such as the statins.

II-34 Brachial Artery Ultrasound: A Noninvasive Tool in the Assessment of Triglyceride-Rich Lipoproteins
R. A. VOGEL, M.D., F.A.C.C.

In recent years, endothelial dysfunction has been identified as an early feature of atherosclerosis. Endothelial function can be measured noninvasively by using brachial artery ultrasound. A variety of factors associated with atherosclerosis also impair endothelial function. Some of these factors are lipoproteins such as various forms of low-density lipoprotein, postprandial chylomicron remnants, fasting triglyceride-rich particles, and free fatty acids. A high-fat diet also has an adverse effect on endothelial function. Several interventions can improve endothelial function and, at the same time, reduce cardiovascular events. Measuring endothelial function may eventually serve as a useful index to determine an individual’s risk for coronary artery disease.

II-40 Nonpharmacologic Treatment of Hypertriglyceridemia: Focus on Fish Oils
W. S. HARRIS, PH.D.

Early studies in Greenland Eskimos stimulated further interest in evaluating the effect of Omega-3 fatty acids on coronary artery disease. Later, subsequent studies showed a significant decrease in triglyceride levels in patients receiving high doses of fish oil containing DHA and EPA. Slight increases in low-density lipoprotein were also observed in patients receiving fish oil supplements. These studies have also shown a dose–response effect which persists as long as supplementation continues. Later trials, specifically the Diet and Reinfarction Trial and the Indian Experiment of Infarct Survival, have demonstrated a reduction in cardiac death rates and in the incidence of cardiac symptoms in patients receiving fish oil.

II-44 Pharmacologic Management of Triglycerides
D. B. HUNNINGHAKE, M.D.

Currently available, cholesterol-lowering pharmacologic agents have been studied for their effect on reducing triglyceride levels. The fibrates increase lipoprotein activity, thereby decreasing the size of triglyceride-rich particles. High doses of niacin can produce decreases in very low-density lipoprotein levels, triglyceride-rich particles, and low-density lipoprotein (LDL) by inhibiting lipoprotein synthesis. By increasing LDL-receptor activity, the statins increase the removal rate of triglyceride-rich particles. Each class of agents produces various degrees of triglyceride lowering, depending on the existing baseline level and other factors. Patients with elevated LDL and who are hypertriglyceridemic should receive statins as first-line therapy. Niacin may be used as an alternative first-line agent in patients with small LDL elevations. Combination therapy, using other agents, may be indicated depending on the patient’s levels of triglycerides and LDL.
In clinical practice, we have all encountered patients with premature coronary disease in whom no “obvious” source of risk could be delineated. In some cases, traditional risk factors, such as hypertension, hypercholesterolemia, and hyperglycemia were absent; in others, mild elevations in these parameters resulted in a disproportionate reduction of coronary luminal diameter than might have otherwise been predicted. Therefore, an exhaustive search has been undertaken in recent years to identify other potential contributors to atherothrombosis. Among the most well recognized of these factors are triglycerides (TGs). At a symposium recently conducted on September 26, 1998, in Washington, D.C., we explored the importance of TG as a Risk Factor in Cardiac Heart Disease. The epidemiology of TG as an independent risk factor for CAD was reviewed and the classification of desirable and elevated TG levels was queried. The seminal work by Drs. Sandra Gianturco and William Bradley on their identification of a novel TG-rich lipoprotein receptor was presented. Dr. Howard Hodis elaborated upon the use of arteriographic studies to evaluate the impact of TG-rich lipoproteins on CAD progression. Lipoprotein subclass differentiation by NMR spectroscopy, a new technique that resolves lipoproteins into 15 distinct subclasses, was discussed by its developer, Dr. James Otvos. Dr. Angeliki Georgopoulos described postprandial studies of TG-rich lipoproteins in diabetics and a putative cellular basis for enhanced atherogenicity. Dr. Robert Vogel explored the use of noninvasive arterial imaging to evaluate the impact of dietary fat perturbation on endothelial vasoactivity. The nonpharmacologic management of hypertriglyceridemia was expanded upon by Dr. William Harris with particular credence to omega-3 fatty acids. Dr. Donald Hunninghake discussed pharmacologic regimens for managing patients with elevated TG and outlined two recently completed clinical trials that may provide new information vis-à-vis the relative impact of TG lowering on CAD event rates. Each presentation was followed by provocative questions and insightful comments. Dr. C. Richard Conti did a masterful job as co-chair.

We are grateful to John Bourgholtzer, Mary Braggin and the outstanding staff of Clinical Cardiology and indebted to Katie McFarland, and to Parke-Davis and Pfizer Laboratories for providing an unrestricted grant for its support and publication.

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The Epidemiology of Triglyceride as a Coronary Artery Disease Risk Factor

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Summary: Triglyceride (TG) has long been associated as a risk factor for coronary artery disease. A recent meta-analysis of various epidemiologic studies has confirmed this link. An important issue is to assess further the appropriate cutpoints to classify desirable TG because recent data indicate that levels < 200 mg/dl confer elevated risk. The dietary habits of present hunter-gatherer populations reveal the impact of a Westernized diet on both TG and cholesterol and suggest that a desirable TG is < 100 mg/dl. The epidemiologic and observational data in support of this concept are explored.

Key words: coronary artery disease, high-density lipoprotein cholesterol, hypertriglyceridemia, risk factor, remnant lipoproteins, triglyceride

Introduction

The role of triglyceride (TG) as a risk factor for coronary artery disease (CAD) has received increased recognition in recent years. The evidence supporting this finding was initially reported in 1959 by Albrink and Mann. Their case-control study compared TG and cholesterol levels in 100 subjects with established CAD with age-matched normal men and women. Significant differences were observed between the two groups as the mean TG in the CAD group (~180 mg/dl) was significantly higher than that in age-matched controls (~100 mg/dl). Epidemiologic data, notably from the Framingham Heart Study, observed that TG was an especially important predictor of CAD in women (Fig. 1). These early endorsements were nullified by Hulley et al. who suggested that while TG appeared to be an important CAD risk factor in univariate analysis, its predictive power was diminished following adjustment for other covariates such as high-density lipoprotein cholesterol (HDL-C). In 1984, the National Institutes of Health adopted the following TG cutpoints based on a Consensus Conference. They included desirable (< 250 mg/dl), borderline-high (250–500 mg/dl), high (500–1,000 mg/dl), and very high TG (> 1,000 mg/dl). Following release of the 1984 NIH guidelines, several other studies were published. Criqui et al. reviewed the Lipid Research Clinics’ population study of approximately 7,500 individuals (4,000 men and 3,500 women). While an association between plasma TG and mortality from CAD was observed in men and women, the effect was minimized after adjusting for body mass index (BMI) and HDL-C and abolished when fasting blood glucose was included. While the study concluded that TG was not an independent risk factor, 43% of the study cohort were hyperlipidemic, thereby limiting generalizability of the results. The Prospective Cardiovascular Munster Study (PROCAM) conducted by Assman et al. in Germany demonstrated an early linear relationship between TG and CAD; however, once TG exceeded 800 mg/dl, event rates were lower and approximated those observed with TG between 200 and 399 mg/dl (Fig. 2). This contrasts with CAD rates and total cholesterol (TC) or low-density lipoprotein cholesterol (LDL-C); that is, as TC rises, CAD rates increase in a curvilinear fashion. It is of interest that a similar distinction in CAD rates between serum cholesterol and TG-rich lipoproteins (Sf 20–400) was observed in the Framingham Heart Study nearly 30 years ago (Fig. 3). Thus, statistical models evaluating the impact of TG (as a continuous variable) on CAD events may be misleading. Further complicating this picture is that very high TG (> 1,000 mg/dl) may be associated with pancreatitis, resulting from environmental factors superimposed on genetic predisposition (e.g., excessive alcohol consumption) or inherited structural mutations in lipoprotein lipase (LPL) or apolipoprotein C-II, a cofactor for LPL. While it has been theorized that very high TG is less likely to lead to atherothrombosis because large uncatabolized TG-rich lipoproteins are impervious through the vascular endothelium, recent data suggest that selected individuals with familial chylomicronemia may be at increased risk for premature atherothrombotic disease. Moreover, elevated TG is associated with considerable heterogeneity of circulating TG-rich particles. They include less atherogenic very-low-density lipoprotein cholesterol (VLDL-C) and very high TG (VH-TG). The VH-TG phenotype is characterized by elevated TG and HDL-C, which may have important implications for the development of CAD.

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lipoprotein cholesterol (VLDL-C) and, to a smaller extent, chylomicrons (t1/2 is ~ 5 min in the absence of genetic deficiency states) to more atherogenic partially catabolized remnant particles. As CAD rates increase as TG exceeds 100 mg/dl (see also below), the atherogenic potential of TG-rich lipoproteins in an individual becomes exquisitely challenging when solely evaluating fasting plasma TG concentration.

Nevertheless, this challenge was overcome by the meta-analysis conducted by Hokanson and Austin.10 They included the Lipid Research Clinics’ Study and PROCAM. In univariate analysis, increases of 76% in women (n ~ 10,800) and 32% in men (n ~ 46,000) were observed for each mmol/l (88.5 mg/dl) increment in TG. Following adjustment for HDL-C, there remained a 37% increased risk in women (n~ 6,300) and 14% enhanced risk in men (n~ 22,300). Two recently published studies, the Copenhagen Male Study11 and the Physicians’ Health Study12 are consistent with the conclusions drawn from the meta-analysis.

In 1993, the National Cholesterol Education Program reassessed its classification of TG and lowered the cutpoints of desirable and borderline-high TG to < 200 mg/dl and 400 mg/dl, respectively.7 Designation of high and very high TG remained unaltered. Despite a similar designation of desirable cutpoints for TC and TG, there are, however, important contrasts. For example, the cutpoint for TC is based on epidemiologic data from the Multiple Risk Factor Intervention Trial (MRFIT) which demonstrated that as levels of TC exceed 200 mg/dl, the risk of coronary events significantly increases.13 Epidemiologic studies have not confirmed similar results for
TG. In fact, in the Framingham Heart Study, increases in CAD events were observed as TG exceeded 100 mg/dl.9 Second, whereas median TC in the Lipid Research Clinics’ population was 200 mg/dl, median TG was only 100 mg/dl (Table I).14 These data raise the possibility that a different cutpoint to designate a desirable TG may be more appropriate.

The Baltimore Coronary Observational Long-Term Study (COLTS) examined the relationship between TG levels and CAD events in patients with arteriographic CAD. Between 1977 and 1978, 740 consecutive patients underwent diagnostic coronary arteriography. Prior to the procedure, each patient completed an extensive questionnaire evaluating CAD risk factors and provided a fasting blood sample. The study included 350 patients with documented CAD at baseline. During the 18-year follow-up period, there were 199 new cardiovascular events. Cox regression analysis revealed that a baseline TG > 100 mg/dl was associated with a 50% increased risk of new events.15 Baseline TG and HDL-C were also studied in relationship to CAD event rate using cutpoints of 100 mg/dl and 40 mg/dl, respectively (Table II). As expected, the lowest number of CAD events occurred with TG < 100 mg/dl and HDL-C > 40 mg/dl. Of interest, however, was the finding that HDL-C did not confer protection if baseline TG exceeded 100 mg/dl. These results are consistent with the Copenhagen Male Study.11

**Lipoproteins in Hunter-Gatherer Societies**

Are there additional data supporting a lower cutpoint for “desirable” TG? Anthropologic research by Eaton and Konner has evaluated the dietary habits of societies that closely resemble our paleolithic ancestors.16 For example, some modern day hunter-gatherer societies consume beans, nuts, and fruits as their most common nutrient sources, yielding a fiber intake of >100 grams daily (compared with a fiber intake of ~25–30 g/day in the average American). Meat consumed in these societies is typically higher in protein and lower in total and saturated fat. For example, carcass fat in wild game is 3.9% with a small proportion contributed by omega-3 fatty acids (e.g., eicosapentanoic acid). In contrast, domesticated livestock contain ~ 30% fat with no detectable omega-3 fatty acids. Nutritional differences between the paleolithic diet and contemporary westernized diet are shown in Table III. Although a large amount of dietary cholesterol was consumed, the impact on raising serum cholesterol levels was considerably lower when viewed in the context of total and saturated fat intake. This is well illustrated in the cholesterol levels obtained in preliterate societies. Both TC and TG appear to be well within the physiologic range (Table IV).17

**Does Dietary Perturbation Impact on Preliterate Societies?**

McMurray et al. examined the impact of a westernized diet in a group of Tarahumara Indians. Caloric intake was increased from 2700 to 4100 kcal/day, and they were switched from a low-fat (20%) diet to a highly saturated, high total fat (43%) diet. After consuming this diet for 4 to 5 weeks, TC rose from a mean 121 → 159 mg/dl with significant increases in

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**TABLE I** Normal plasma cholesterol and triglyceride concentrations from the Lipid Research Clinic’s Population Study (U.S. and Canada)

<table>
<thead>
<tr>
<th>Plasma cholesterol (mg/dl)</th>
<th>Plasma triglyceride (mg/dl)</th>
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<tbody>
<tr>
<td>Males</td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>125 (105)</td>
</tr>
<tr>
<td>5–9</td>
<td>130 (105)</td>
</tr>
<tr>
<td>10–14</td>
<td>127 (105)</td>
</tr>
<tr>
<td>15–19</td>
<td>120 (105)</td>
</tr>
<tr>
<td>20–24</td>
<td>130 (105)</td>
</tr>
<tr>
<td>25–29</td>
<td>143 (105)</td>
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<tr>
<td>30–34</td>
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<td>45–49</td>
<td>169 (105)</td>
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<td>169 (105)</td>
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<tr>
<td>55–59</td>
<td>167 (105)</td>
</tr>
<tr>
<td>60–64</td>
<td>171 (105)</td>
</tr>
<tr>
<td>65–69</td>
<td>170 (105)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>162 (105)</td>
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</tbody>
</table>

| Females                    |                            |
| 0–4                        | 120 (105)                  |
| 5–9                        | 134 (105)                  |
| 10–14                      | 131 (105)                  |
| 15–19                      | 126 (105)                  |
| 20–24                      | 130 (105)                  |
| 25–29                      | 136 (105)                  |
| 30–34                      | 139 (105)                  |
| 35–39                      | 147 (105)                  |
| 40–44                      | 154 (105)                  |
| 45–49                      | 161 (105)                  |
| 50–54                      | 172 (105)                  |
| 55–59                      | 183 (105)                  |
| 60–64                      | 186 (105)                  |
| 65–69                      | 183 (105)                  |
| >70                        | 180 (105)                  |

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**TABLE II** Coronary artery disease event rates in the COLTS study based on comparisons made between baseline TG and HDL levels

<table>
<thead>
<tr>
<th>HDL</th>
<th>Triglyceride</th>
<th>p Value</th>
</tr>
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<tbody>
<tr>
<td>&lt;40</td>
<td>&lt;100</td>
<td>50%</td>
</tr>
<tr>
<td>&gt;40</td>
<td>&gt;100</td>
<td>62%</td>
</tr>
</tbody>
</table>

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Abbreviations: TG = triglyceride; HDL = high-density lipoprotein.

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Another study of 10 diabetic Australian Aborigines evaluated the impact of switching from a Western diet to a hunter-gatherer diet for a period of 7 weeks. Body mass index decreased from 27.2 kg/m² to 24.5 kg/m², while mean TC decreased from 219–193 mg/dl and TG was dramatically reduced (356–102 mg/dl).

Normocholesterolemic Populations at Increased Risk for Coronary Artery Disease

Certain subgroups appear to be at exceptionally high risk of premature CAD even when there is no prominence of traditional cardiovascular risk factors. This is perhaps best exemplified by Asian Indians who have the highest rates of premature CAD. Typically, Asian Indians with CAD have similar TC but elevated TG and Lp(a) levels compared with their Caucasian counterparts. These differences may be the result of alterations in certain candidate genes regulating lipid and lipoprotein metabolism or reflect the gene–environmental interactions.

Pathophysiology of Triglyceride Rich Lipoproteins and Coronary Artery Disease

There are both direct and indirect mechanisms that subserve the enhanced atherothrombogenicity proposed for TG-rich lipoproteins. The direct impact may be enhanced uptake of remnant lipoproteins by the TG-rich lipoprotein receptor (TGR1P) present in macrophages and selected tissues, as reported by Gianturco and Bradley (see Dr. Gianturco’s manuscript). Indirect effects include the transfer of TG from chylomicrons and VLDL-C to HDL-C and LDL-C mediated by the cholesterol ester transfer protein (CETP). Triglyceride-rich HDL-C and LDL-C particles may be subsequently hydrolyzed by hepatic lipase to small, dense particles. Small LDL-C particles (also referred to as phenotype B—see below) are more susceptible to LDL-C oxidation and unregulated uptake by macrophages. The relationship between TG concentration and LDL-C phenotype was described by Austin et al. Phenotype A LDL-C particles are large, buoyant, and less susceptible to LDL-C oxidation than phenotype B. In general, individuals with low TG (e.g., 50 mg/dl) contain a preponderance of phenotype A subclass LDL-C particles, whereas subjects with borderline-high TG (e.g., 250 mg/dl) possess a significant proportion of LDL-C (80–90%) within the phenotype B subclass. It is noteworthy that the shift from phenotype A to B particles accelerates as TG concentration exceeds 100 mg/dl (Fig. 4).

Conclusions

Triglyceride-rich lipoproteins enhance atherothrombotic risk and are underrecognized contributors to the elevated CAD rate observed in Westernized societies. The genetic architecture to which we have evolved suggests that a “desirable” TG may be considerably lower than the

Table IV Comparison of cholesterol (TC) and triglyceride (TG) levels in selected preliterate societies

<table>
<thead>
<tr>
<th></th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New Guinea Melanesians</td>
<td>135</td>
<td>95</td>
</tr>
<tr>
<td>Rural Chinese</td>
<td>127</td>
<td>100</td>
</tr>
<tr>
<td>Tanzanian Villagers</td>
<td>114</td>
<td>81 (fish diet) -116 (vegetarian diet)</td>
</tr>
<tr>
<td>Tarahumara Indians</td>
<td>125</td>
<td>120</td>
</tr>
</tbody>
</table>

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phenotypes A and B. Reprinted from Ref. No. 22 with permission.

els with corresponding prevalence of low-density lipoprotein (LDL) atherogenicity associated with a high TG level is the interaction of insulin resistance and hyperinsulinemia. Part of the other risk factor for CAD. Insulin resistance is probably what tolerance. High fasting serum insulin (insulin resistance) is another risk factor for CAD. Insulin resistance is probably what stimulates plasminogen activator inhibitor (PAI-I). Part of the atherogenicity associated with a high TG level is the interaction of insulin resistance and hyperinsulinemia.

Miller: That’s correct. However, the one exception to this rule are the Pima Indians who, for reasons not well understood, have a fairly low incidence of CAD despite elevated TG, adiposity, and insulin resistance.

Abrams: In terms of the risk from TG, it has been suggested that if one reduces LDL substantially, this risk is minimized or disappears. Is this true? If it is, perhaps the data need to be viewed in a different way since cholesterol levels were not factored into the analyses.

Miller: In the five randomized clinical trials using statins, LDL lowering was associated with marked reduction of primary or secondary CAD events. However, these studies did not assess the impact of TG lowering above and beyond LDL reduction. A study underway in Europe is investigating whether fenofibrate in combination with cerivastatin reduces CAD events in diabetics to a greater extent than either one alone. This study will help to answer the relative impact of TG reduction compared to LDL reduction in high-risk patients. In the U.S. and abroad, the TNT (treat to new targets) is examining whether aggressive LDL-C lowering with atorvastatin (e.g., to below 70 mg/dl) reduces the CAD event rate to a greater extent than reducing LDL-C to the NCEP goal of 100 mg/dl in men and women with preexisting CAD. The TNT study may help to answer your question, although high doses of atorvastatin are also associated with TG reductions which may complicate such analyses.

Bachorik: There was a mention that, in the presence of high TG levels, higher concentrations of partially metabolized TG-rich lipoproteins are found. These become cholesterol-enriched lipoproteins more similar to LDL. There is also evidence that these particles are in fact atherogenic. The risk associated with TG goes up to some degree. Then, when the particles become much larger, they are apparently not as atherogenic. Viewed in this way, can TG, at least within certain concentration ranges, be viewed as surrogate measures of atherogenic particles that have yet to be reduced to the size of LDL (e.g., chylomicron remnants, VLDL remnants)?

Miller: One of the challenges is how to differentiate between atherogenic remnant lipoproteins and less atherogenic TG particles. Perhaps, Dr. Otvos will cover this area in his talk. Presently, I am not aware of any foolproof commercial method that can discriminate between these lipoproteins. Perhaps, maintaining TG levels below 100 mg/dl would circumvent this problem.

Criqui: The Copenhagen data confirm a finding found in the Lipid Research Clinics’ Study and the PROCAM study. Above some level of TG, the risk drops, making it not a curvilinear or even a linear relationship, but rather an upside-down U. In the PROCAM study, the level was 800 g/dl, while in the Copenhagen Male Study, it was 222 mg/dl when the risk dropped rather sharply. When multivariate analysis was conducted by category in the Copenhagen Male Study, a very strong TG risk was shown at every level of HDL. This is consistent with the old observation that patients with Type I and Type V hyperlipidemia apparently do not have an increased risk of atherosclerosis even though their TG levels are very high. The Copenhagen and COLTS data show that, at high levels of HDL, TG continues to be a risk factor. In the Miller data, the risk was higher when the HDL was > 40 mg/dl than when the HDL was < 40 mg/dl and the TG was > 100 mg/dl. This is the direct opposite of all previous population studies, including the Lipid Research Clinics’ Study, the Honolulu Heart Study, the PROCAM Study, and the Helsinki Subgroup Study. All of these showed that TG was the most potent as a risk factor when the HDL was low. If the HDL was high (>40–50 mg/dl), there was no risk for TG. How can this inconsistency in the data be resolved?

Miller: With regard to your first comment, the upside-down U was also observed in Framingham (see Fig. 3). Both the Copenhagen Male Study, which evaluated initial CAD event rates, and the COLTS, which studied recurrent CAD events, showed that TG was important even at high HDL levels. These studies found significant risk at lower TG levels than previously considered. In Copenhagen, elevated risks were observed at lower TG levels; compared to the lowest tertile (<97 mg/dl) levels between 97–140 mg/dl conferred a 50% increased risk of CAD while the highest tertile (>140 mg/dl) more than doubled event rate. In COLTS, we observed a threshold effect with an increased rate of events observed as TG exceeded 100 mg/dl. It would be interesting to re-do the analyses of some of these earlier studies to determine whether

**Discussion**

Glueck: Hunter-gatherers were thin individuals with a much lower fasting insulin and insulin response to oral glucose tolerance. High fasting serum insulin (insulin resistance) is another risk factor for CAD. Insulin resistance is probably what stimulates plasminogen activator inhibitor (PAI-I). Part of the atherogenicity associated with a high TG level is the interaction of insulin resistance and hyperinsulinemia.

Miller: The epidemiology of TG as a CAD risk factor II-5

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**Fig. 4** Cumulative distribution of adjusted plasma triglyceride levels with corresponding prevalence of low-density lipoprotein (LDL) phenotypes A and B. Reprinted from Ref. No. 22 with permission.
lower TG cutpoints also impact on the results. However, one must consider that some of the populations studied (e.g., Lipid Research Clinics’) primarily evaluated hypercholesterolemic subjects. Thus, the impact of TG in this cohort may be very different.

**Conti:** Today, the life expectancy is 84 years for women and 78 for men. What would have been the speculated life span of a hunter-gatherer who did not succumb to an infectious disease or some other untimely death, such as being eaten by a dinosaur?

**Miller:** It is difficult to speculate, although it is presumed they would have lived at least that long, perhaps longer. We have no way of knowing what type of lifespan we could achieve if cardiovascular disease (and cancer) was eliminated. Additional information from present hunter-gatherer societies may provide useful information in this regard.

**Acknowledgment**

Dr. Miller’s original work cited in this review was supported by Grant HL52663 from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland, USA.

**References**

12. Hokanson JE, Austin MA: Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: A meta-analysis of population-based prospective studies. *J Cardiomyol Lipid Research Clinics’* primarily evaluated hypercholesterolemic subjects. Thus, the impact of TG in this cohort may be very different.

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Pathophysiology of Triglyceride-Rich Lipoproteins in Atherothrombosis: Cellular Aspects

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Summary: Elevated plasma levels of triglyceride-rich lipoproteins (TGRLP), including very low-density lipoproteins (VLDL), chylomicrons, and their remnants, are now acknowledged as risk factors for cardiovascular disease. Interactions of TGRLP with lipoprotein receptors on monocytes, macrophages, and endothelial cells may be mechanistically linked to this risk. Triglyceride-rich lipoproteins from hypertriglyceridemic (HTG) subjects have the abnormal ability to bind to low-denisty lipoprotein receptors via apoE, and plasma chylomicrons from all subjects bind to a new, distinct receptor for apoB48 that is expressed specifically by monocytes, macrophages, and endothelial cells. Receptor binding and uptake of TGRLP by these cells are likely mechanisms involved in the formation of lipid-filled, macrophage-derived “foam cells” of atherosclerotic lesions and for defective fibrinolysis due to endothelial dysfunction. Recognition of the atherothrombogenic potential of TGRLP may lead to improved interventions to lessen or prevent the often fatal sequelae of coronary atherosclerosis and thrombosis associated with elevated plasma triglyceride levels.

Key words: atherosclerosis, thrombosis, apoB48, Apo E, triglycerides, cholesterol, hypertriglyceridemia, low-density lipoprotein, macrophage, monocyte, very low-density lipoprotein, chylomicron

Introduction

Elevated plasma triglycerides (TG) and TG-rich lipoproteins (TGRLP), including very low-density lipoproteins (VLDL), postprandial plasma chylomicrons, and their remnants, are emerging as risk factors for atherothrombotic disease. Monocyte-macrophage (MM)-derived, lipid-filled “foam cells” are a hallmark of both early fatty streak and more advanced, rupture-prone, thrombogenic atherosclerotic lesions. Foam cells also are found in the bone marrow, spleen, and skin in certain hypertriglyceridemic (HTG) subjects that have persistent chylomicrons (CM) and/or CM remnants in the postabsorptive state. Diabetic subjects with fasting CMs also develop MM-derived foam cells in eruptive xanthomas that are filled with TG and cholesteryl ester due to uptake of CM-sized lipoproteins by MMs. Modified and oxidized low-density lipoprotein (LDL) can produce foam cells in vitro, and much attention has focused on these as a probable cause of atherosclerosis, since native, nonmodified LDL do not induce foam cell formation in vitro. Certain TGRLP are the only native, nonmodified lipoproteins that can cause rapid, receptor-mediated macrophage lipid accumulation in vitro and endothelial cell dysfunction in vitro. The TGRLP that can induce these potentially atherothrombogenic cellular effects include plasma CMs, from normal as well as hyperlipoproteinemic subjects, and VLDL from subjects with elevated plasma TGs (HTG-VLDL).

Another cell type critical to atherothrombogenesis is the endothelial cell (EC), which is antithrombotic due to its complex and dynamic role in fibrinolysis. Endothelial cells bind and internalize TGRLP from subjects with HTG but not VLDL from normal subjects, with deleterious effects in vitro, including diminished fibrinolytic capacity. Hypertriglyceridemic-VLDL inhibits plasminogen binding to ECs and EC-mediated cell surface fibrinolysis. It also modulates the EC expression of PAI-1, the inhibitor of tissue plasminogen activator (t-PA) and thus inhibitor of plasmin production, in a PAI-1 genotype-specific manner. These may be among the mechanisms to account for the increased thrombotic risk in subjects with elevated plasma TGs.
As TGRLP-induced monocyte-macrophage lipid accumulation and EC dysfunction may be in part responsible for coronary atherosclerosis and subsequent myocardial infarction in subjects with elevated TG and/or prolonged postprandial lipemia, a known risk factor for coronary artery disease (CAD), mechanisms to account for the cellular uptake of TGRLP are the focus of this paper. Potential mechanisms for the rapid uptake of TGRLP by monocyte-macrophages and ECs include the LDL receptor and other members of the LDL receptor family, facilitated binding by proteoglycans and/or by ancillary molecules such as lipoprotein lipase, and a unique monocyte-macrophage- and EC-specific receptor that binds TGRLP via apoB-48. As TGRLP do not bind to scavenger receptors, that receptor family is not discussed here.

Permeability of Lipoproteins into the Intima

Foam cells are located within the arterial intima, where the largest TGRLP (Sf > 400) are thought to be excluded because of their size. The permeability of lipoproteins into and, conversely, out of the intima is inversely proportional to their diameters. Thus, high-density lipoproteins (HDL) are more freely permeable than LDL, which are more so than intermediate-density lipoproteins (IDL) and VLDL. Since larger lipoproteins, once in the intima, are retained longer than small lipoproteins, this too may enhance the atherogenicity of TGRLP once in the intima. There, apoB-containing lipoproteins interact with extracellular matrix, enhancing their entrapment and their potential interactions with intimal cells to induce foam cell formation.

Each TGRLP transports more cholesterol than does each LDL particle, five-fold in large VLDL to 20-fold in CM, so each TGRLP that enters the intima delivers 5- to 20-fold more cholesterol than does each LDL and is therefore potentially more atherogenic on a particle basis. Moreover, when plasma TGRLP are elevated, as in subjects with HTG, the large VLDL are enriched in cholesterol relative to the same particles from subjects with normal plasma levels of TG. Thus, when TGRLP are elevated, they are potentially more atherogenic than LDL because, first, TGRLP are retained longer in the intima, where they can be taken up by macrophages, and second, each TGRLP carries 5 to 20 times more cholesterol than does each LDL.

Finally, studies in humans indicate TGRLP do pass the endothelial barrier where they can interact with intimal cells. Havel et al. demonstrated that VLDL-sized lipoproteins are indeed found in the intima in humans and, moreover, that progression of atherosclerosis in humans is related to plasma levels of VLDL and IDL rather than to levels of LDL. Early electron microscopy studies indicate CM-sized particles are found associated with tissue macrophages in humans, that is, out of the vascular system, indicating that in some circumstances these large particles do exit the lumen, most likely at sites of EC dysfunction and/or greater permeability. Moreover, several cell types intimately involved in atherosclerosis and thrombosis are exposed to even the largest circulating CM: circulating blood cells, including monocytes (the precursors of foam cells) that are known to take up dietary particles in vivo, and endothelial cells that play a critical role in hemostatic balance. Thus, there is ample opportunity for interaction of TGRLP with cells involved in atherothrombogenesis.

Atherogenic Properties of Triglyceride-Rich Lipoproteins

A series of early studies provided evidence for the atherogenic properties of VLDL in individuals with elevated plasma TGs. Very low-density lipoprotein Sf > 60 from normal patients did not contribute cholesterol to cultured human fibroblasts or bovine aortic ECs containing upregulated LDL receptors, but VLDL from subjects with hypertriglyceridemia effectively delivered cholesterol through receptor pathway(s), leading to suppression of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase activity in both fibroblasts and ECs. Very low-density lipoprotein obtained from subjects with hypertriglyceridemia, regardless of their size, bind with high affinity to LDL receptors and to a distinct TGRLP/apoB48 receptor expressed specifically by monocytes, macrophages, and endothelial cells. In contrast, the large VLDL (Sf > 60) from persons with normal plasma TG levels do not bind to LDL receptors or to any receptors on monocyte-macrophages or ECs. The large normal VLDL require lipolysis of the core lipids to produce smaller, denser (Sf 20–60 density range) particles resembling IDL before they acquire the ability to bind to the LDL receptor, a process that is mediated by apoB100 in small VLDL, IDL, and LDL. These small apoB100-containing particles do not cause massive lipid accumulation in MM or impair EC-mediated fibrinolysis in vitro. Large HTG-VLDL, however, bind to both LDL receptors and to the TGRLP/apoB-48 receptor of monocyte-macrophages and ECs, causing massive lipid accumulation in MM and impaired EC-mediated fibrinolysis in vitro (Fig. 1).

Lowering plasma TG levels in subjects with hypertriglyceridemia with coronary heart disease by bezafibrate or by lovastatin lowers the number of large VLDL particles in plasma and, moreover, normalizes their cholesterol content. Thus fibrates or statins may play an important role in reducing coronary heart disease (CHD) risk in subjects with moderate hypertriglyceridemia by reducing the number of potentially atherothrombogenic TGRLP present and by normalizing the TGRLP cholesterol content.

Low-Density Lipoprotein Receptor Family and Atherosclerosis

The LDL receptor is expressed at low levels in confluent ECs and monocytes, where it further declines upon differentiation into macrophages. As the LDL R is suppressed by sterol, both the receptor and its normal smaller apoB lipopro-
tein ligands are not thought to be involved in the conversion of macrophages into foam cells or in EC dysfunction. However, HTG-VLDL Sf > 60 bind to the LDL R and could be a mechanism for lipid delivery. The role of other LDL R family members in atherosclerosis and thrombosis is unclear, but apoE appears to mediate binding of TGRLP to all members of this family of receptors. The LDL R related, α-macroglobulin receptor (LRP) is induced upon differentiation of monocytes into macrophages and may be involved in apoE-mediated lipid accumulation (foam cell formation) and endothelial cell (EC) dysfunction in vitro. HDL = high-density lipoprotein.

Fig. 1 Schematic of metabolism and potentially atherothrombogenic interaction of very low-density lipoprotein (VLDL) from normal subjects versus hypertriglyceridemic subjects (HTG-VLDL) with LDL receptors, expressed by all cells, particularly the liver, and with a reticuloendothelial (monocyte-macrophage- and endothelial cell) -specific TGRLP/apoB48 receptor. Normally, large VLDL do not bind to the LDL R or the apoB48 R and are lipolyzed to smaller VLDL, intermediate-density lipoprotein (IDL), and LDL before the particles can bind and be removed by LDL receptors, primarily in the liver. In contrast, HTG-VLDL has the abnormal ability to bind to both the LDL receptor, via apoE, and the apoB48 receptor of reticuloendothelial cells, which results in rapid, massive monocyte-macrophage lipid accumulation (foam cell formation) and endothelial cell (EC) dysfunction in vitro. HDL = high-density lipoprotein.

Mechanisms of Uptake of Triglyceride-Rich Lipoprotein by the Low-Density Lipoprotein Receptor

ApoE is required for the uptake of large (Sf >60) TGRLP by members of the LDL receptor gene family 30–36 and for hepatic uptake of CM remnants and β-VLDL. 37

The mechanisms of the apoE-mediated uptake of HTG-VLDL by the LDL R have been the most clearly documented (Fig. 2). Early studies focused on whether apoB or apoE mediated the abnormal binding of HTG-VLDL Sf > 60 to the LDL R, and demonstrated conclusively that only apoE of the correct conformation (or orientation on the surface of VLDL) was required for binding of HTG-VLDL to the LDL R and that this apoE conformer was not present in normal VLDL. 30, 31 This LDL R-accessible apoE conformer was initially identified by its specific susceptibility to cleavage by thrombin, which produced two major apoE fragments and abolished LDL R binding. This same apoE conformer on HTG-VLDL binds to a monoclonal antibody (1D7) against apoE that also blocks the binding of HTG-VLDL to the LDL R. The E22 fragment is only loosely associated, at all, to the thrombin-inactivated (TI) particle and is lost upon reflotation (spin-up) of the TI-HTG-VLDL. When intact apoE (E34) is then added back to the TI-HTG-VLDL, it restores full LDL receptor binding activity. Addition of E22 with or without E12 does not restore activity, indicating the intact thrombin-accessible apoE is necessary for binding to the LDL R. Abbreviations as in Figure 1. Reprinted with permission of the author.

Fig. 2 Schematic of studies which determined that apoE of a thrombin-accessible conformation is required for the binding of HTG-VLDL to the LDL receptor. Thrombin specifically cleaves 1–2 mole apoE/mole HTG-VLDL into the N-terminal 22 kDa fragment that contains the LDL receptor-binding domain and the C-terminal 12 kDa particle that contains the lipid binding domain that is necessary to anchor the apoE to the particle surface. This abolishes binding to the LDL receptor. The E22 fragment is only loosely associated, at all, to the thrombin-inactivated (TI) particle and is lost upon reflotation (spin-up) of the TI-HTG-VLDL. When intact apoE (E34) is then added back to the TI-HTG-VLDL, it restores full LDL receptor binding activity. Addition of E22 with or without E12 does not restore activity, indicating the intact thrombin-accessible apoE is necessary for binding to the LDL R. Abbreviations as in Figure 1. Reprinted with permission of the author.
These studies led us to propose a two-domain model for the receptor-accessible apoE of HTG-VLDL (Fig. 3 and Table 1). The carboxy terminal 12 kDa fragment of apoE is the major lipid-binding region of the molecule and anchors the LDL R-, ID7-, and thrombin-accessible conformer of apoE to the VLDL surface. Although E12 does not bind to the LDL receptor, add back studies indicated that it was necessary to anchor intact apoE to the VLDL and for the correct orientation of the receptor-binding domain of intact apoE.30 The two-domain hypothesis was subsequently detailed38 and confirmed by crystallography.39

In normal VLDL, all of the apoE present (1–2 mol/mol VLDL Sf > 60) is inaccessible to the LDL receptor, is not cleaved by thrombin, and is also not accessible to the 1D7 antibody against apoE that inhibits LDL receptor binding. In normal subjects, most plasma apoE is associated with HDL (~75%) and only ~25% is associated with VLDL. In hypertriglyceridemia, however, most of the plasma apoE is associated with TGLRP, indicating that there is a redistribution of apoE from HDL into the VLDL in HTG states. We therefore hypothesized that the apoE associated with “nascent” VLDL upon hepatic secretion (as found in normal subjects) is of the inaccessible conformation(s), which would effectively limit immediate reuptake by hepatocyte LDL Rs and would ensure the delivery of the VLDL’s nutrients to the periphery. When the number of VLDL particles is elevated, as seen in hypertriglyceridemia or the postprandial state, apoE can transfer to the TGLRP and mediate their binding to LDL receptors. Indeed, HTG-VLDL Sf >60 contain 1–2 moles inaccessible apoE per mole particle in addition to the accessible apoE (≥ 1 mol/mol VLDL) described above. In conclusion, extra intact apoE of the appropriate conformation is required for the binding of large TGLRP Sf > 60 to the LDL receptor (at least 1 mol accessible apoE/mol VLDL), and this accessible apoE is not present in normal VLDL but can be acquired by adding apoE to VLDL in vitro.25, 30, 33

Table I  Summary of the accessible (LDL R active) and inaccessible (LDL R inactive) conformations of apoE in VLDL

<table>
<thead>
<tr>
<th>Two conformations of apoE in VLDL Sf 60–400</th>
<th>Normal VLDL (mol/mol)</th>
<th>HTG-VLDL (mol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accessible apoE</td>
<td>0</td>
<td>≥1</td>
</tr>
<tr>
<td>To the LDL receptor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>To thrombin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>To 1D7 antibody</td>
<td>1–2</td>
<td>1–2</td>
</tr>
<tr>
<td>Inaccessible apoE</td>
<td>1–2</td>
<td>1–2</td>
</tr>
<tr>
<td>To the LDL receptor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>To thrombin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>To 1D7 antibody</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The accessible conformation is required for LDL receptor binding and can be created in vitro.

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Abbreviations: VLDL = very low-density lipoprotein, HTG = hypertriglyceridemic, LDL = low-density lipoprotein.

Fig. 4  Schematic representing studies that determined where in the metabolic cascade from HTG-VLDL to LDL does the LDL receptor recognition site switch from apoE to apoB? Detailed studies from two independent laboratories determined that the switch occurs in the small VLDL subclass (Sf 20–60), which comprises about 70% of VLDL.
particles in normal subjects. At this size and smaller, apoE is not required for binding to the LDL receptor (Fig. 4), and apoB is folded in the appropriate conformation that exposes the receptor binding domain so that it can mediate binding to the LDL receptor.\textsuperscript{31, 32} This makes sense metabolically. Under normal circumstances, newly secreted large VLDL are not immediately retracted by the liver because they lack apoE of the accessible conformation to bind to the LDL receptors; these are lipolyzed in the periphery to VLDL $S_t$ 20–60, IDL, and LDL before these residual lipoproteins can be removed by the LDL R pathway. In the normal individual, then, apoB100 is the physiologically important ligand for the LDL receptor.\textsuperscript{26} Triglyceride-rich lipoproteins are not normally catabolized by this receptor pathway. In patients with elevated plasma TG levels, as in hypertriglyceridemia, however, the redistribution of apoE into VLDL produces particles which have the abnormal ability to interact, via this apoE, with the LDL receptor and perhaps other LDL R family receptors.

**The ApoB-48 Receptor of Monocytes, Macrophages, and Endothelial Cells**

While most circulating dietary TGRLP (chylomicrons) are converted into remnants that are taken up rapidly by the liver via apoE, primarily by the LDL R and secondarily the LRP,\textsuperscript{42} animal studies indicate that normally a fraction (20–40\%) of chylomicrons are rapidly taken up by cells of the reticuloendothelial system,\textsuperscript{43, 44} such as accessible macrophages in bone marrow and spleen, which are among the sites where foam cells are observed in humans with elevated plasma TGRLP. More recent animal studies suggest that apoE is not involved in this peripheral uptake, since infused apoE instead diverts CM to the liver.\textsuperscript{45, 46} Recent studies in humans have shown that a fraction of CMs $S_t > 400$ are specifically removed in toto by the peripheral tissues (muscle and adipose), likely by ECs.\textsuperscript{47}

Furthermore, apoE-deficient (apoE$^-$) mice develop extensive atherosclerosis and accumulate apoB-48 VLDL particles which, being devoid of apoE, cannot bind to the LDL R family,\textsuperscript{58, 49} indicating that a monocyte-macrophage pathway independent of apoE exists. We and other investigators have hypothesized that the apoE$^-$ apoB48 particles bind to the apoB-48 R (see below), which is not regulated by sterol,\textsuperscript{11, 13, 14} overwhelming it and leading to foam cell formation and the observed spontaneous, extensive human-like atherosclerosis seen in apoE-deficient mice.\textsuperscript{50, 51} Indeed, the apoE null VLDL from E$^-$ mice bind to the apoB48 receptor, described below, on ligand blots and induce rapid, massive lipid accumulation in macrophages in vitro via this receptor (S. Gianturco, W. Bradley, J. Breslow, and J. Smith, unpublished data).

We have identified, cloned, and characterized a receptor in monocytes, macrophages, and ECs that has the ligand binding characteristics suggested by the above in vivo studies and, for reasons discussed below, have called it the TGRLP /apoB48R (apoB48R for short). It is an apoE$, \text{lipoprotein lipase (LpL)},$ and heparin sulfate proteoglycan (HSPG)-independent TGRLP R pathway that differs from the LDLR and the scavenger receptor families in several respects: (1) it is constitutively expressed during differentiation of monocytes into macrophages, (2) it has retarded intracellular ligand degradation, (3) distinct ligand specificity, (4) different apparent mass of the candidate receptor proteins (Mr 200, 235 kDa), and (5) unique, restricted cellular distribution. Of note, this receptor is not regulated by sterol and could therefore continue to mediate TGRLP uptake when regulatable LDL Rs could not.\textsuperscript{11, 13, 14} Plasma chylomicrons (Sr $> 400$), HTG-VLDL $S_t 100–400$, and tryptsinized VLDL $S_t 100–400$ (tryp-VLDL) immunochemically devoid of apoE, which was used as a model lipoprotein to study apoE-indepenent mechanisms, all bind to the R with high affinity (nM Kds) and cause rapid ($\leq 4$ h), massive lipid accumulation and foam cell morphology of these cells in vitro.\textsuperscript{11, 14} In contrast, normal VLDL and LDL did not compete for this site and did not bind with high affinity or cause macrophage lipid accumulation. Acetyl LDL also did not compete for this site. Two major TGRLP membrane-binding activities with apparent Ms on SDS-PAGE of $\sim 200$ and $\sim 235$ kDa (MBP 200, 235) were identified as likely receptor candidate proteins in normal human blood-borne, THP-1, and U937 monocyte-macrophages, and human umbilical ECs but not in fibroblasts, hepatoma cells, or Chinese hamster ovary cells (CHO).\textsuperscript{11, 52} The receptor proteins have the identical ligand specificities as the cellular site.\textsuperscript{11, 14} Limited proteolysis of THP-1 monocyte-macrophages, but not heparinase or heparitinase treatment, abolished both the receptor proteins and the high-affinity cell binding site; both activities recovered in parallel when proteolysis was quenched, strongly implicating the proteins as the cellular receptor.\textsuperscript{53} MBP 200, 235 are cell-surface proteins with a common protein subunit (MBP 200) that contains the ligand binding domain.\textsuperscript{53} The receptor proteins exhibit the same high affinity (nanomolar Kds), saturable, specific ligand-binding characteristics of the cellular pathway.\textsuperscript{11, 53} MBP 200R, the reduced yet active species, has been purified\textsuperscript{54} and the interrelations of these MBPs further characterized biochemically\textsuperscript{53} and immunochemically with antibodies against a 10-residue synthetic peptide that mimics an internal tryptic peptide of MBP200R determined by microsequence analysis.\textsuperscript{52}

Competitive cell binding and ligand blotting studies demonstrated that the receptor binding domain in TGRLP is within apoB-48 (or an equivalent in apoB-100) near the lipoprotein lipase (LpL) binding site, but not a heparin-binding domain.\textsuperscript{12} Uptake of TGRLP by this mechanism could provide essential nutrients to reticuloendothelial cells or, in hypertriglycerideremia or prolonged postprandial lipemia, cause excess macrophage lipid accumulation and foam cell formation and/or EC dysfunction.

**Conclusion**

Because of the potential importance of the apoB48R pathway in health and disease, we have cloned and characterized the receptor’s unique cDNA sequence and identified corresponding mRNA in THP-1 monocytes, placenta, peripheral
mononuclear leukocytes, bone marrow, spleen, tonsil, lymph
node, and appendix by Northern blotting, a distribution like
that of foam cells in vivo in subjects with persistent CMs. The
cDNA encodes a new, unique protein (no matches in GenBank) that induces TGRLP uptake and foam cell forma-
tion when transfected into R-negative CHO-K1 cells. 55 Im-
munohistochemical studies with anti-R antibodies (generated against fusion proteins) demonstrate that the apoB48 R colo-
calizes with macrophage-specific markers and is expressed in
foam cells in early, human aortic fatty streaks as well as in
foam cells of advanced human coronary and carotid lesions and
in several immune tissues (Bradley, Gianturco, Tanaka,
Watanabe, unpublished data).

Studies in vivo with TGRLP/apoB-48 receptor-deficient
mice and transgenic tissue-specific overexpressing mice, and
with crosses of these mice with murine models of atheroscle-
rosis and hypertriglyceridemia, are necessary to determine the
receptor’s normal function and its potential role in athero-
thrombogenesis in vivo. Understanding of the mechanisms by
which TGRLP contribute to the formation of atherosclerotic
lesions and thrombosis may provide a rational basis for inter-
ventions designed to lower TGRLP levels and reduce cardio-
vascular risk.

Discussion

Khachadurian: Are the triglyceride-rich particles that
bind to the TGRLP/apoB receptor exclusively exogenous or
do they also bind to endogenous, apoB100-containing par-
ticles? What about B48-containing particles?

Gianturco: The triglyceride-rich particles that have
apoB48 as the only apoB species bind to the receptor.
The apoB100-containing particles do not need to be present.
ApoB48 is sufficient for binding. If the equivalent domain is
accessible in apoB100 of HTG-VLDL, it could mediate bind-
ing, but apoB100 of normal VLDL does not bind to this recep-
tor. Antibodies against apoB inhibit binding to the receptor.
However, antibodies against apoCIII, the major protein in
these large particles, do not inhibit this binding.

Margolis: It was indicated that a lot more cholesterol accu-
ulated in macrophages with HTG-VLDL than with modi-
fied LDL. Later, it was mentioned that much more cholesterol
accumulated from modified LDL than from the triglyceride-
rich VLDL. Which is correct?

Gianturco: In the first instance, THP-1 macrophages were
exposed to lipoproteins for 4 hours. During this time, neither
acetyl LDL nor oxidized LDL caused significant lipid accu-
mulation in the cells. Postprandial triglyceride-rich lipopro-
teins, however, caused massive accumulation. In the other in-
stance, P388D1 murine macrophages were used. These were
exposed to lipoproteins overnight (16 hours). By this time
en up in an atherogenic manner through this new receptor. Has
apoCI been examined as another possible protein that interacts
with this macrophage receptor?

Gianturco: Anti-apoCI antibodies did not inhibit binding
of TGRLP to this receptor, suggesting apoCI is not involved in
receptor binding. All specific binding could be inhibited with
anti-apoB antibodies, indicating apoB is sufficient to
mediate receptor binding. There is no information on whether
or not apoCI would inhibit binding. If apoCI binds to the
receptor binding domain of apoB, which is in the B48
region, it might conceivably inhibit binding of the TGRLP to
the apoB48 receptor.

Georgopoulos: Regarding atherosclerotic lesions showing
the receptor being present in foam cells, were these from
hypertriglyceridemic patients? If they were not, might post-
prandial lipoproteins be involved in these individuals?

Gianturco: Yes, we believe it is a postprandial phenome-
non. Postprandial VLDL-sized remnants can contribute chol-
esterol to macrophages via the apoB48 receptor and cause lip-
ad accumulation in vitro. These particles are small enough
to get into the artery wall and could therefore cause macro-
phage lipid accumulation there.

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Pathophysiology of Triglyceride-Rich Lipoproteins in Atherothrombosis: Clinical Aspects

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Summary: Invasive and noninvasive arterial imaging are important techniques used to study atherosclerosis and, specifically, to evaluate the atherogenicity of triglyceride-rich lipoproteins (TRL). Serial coronary angiography trials show significant benefit from lowering low-density lipoprotein cholesterol (LDL-C) which serves to retard lesion progression. Even with aggressive LDL-C reduction, however, up to half of patients demonstrate continued progression of atherosclerosis. Angiographic studies reveal that lowering LDL-C has the most impact on severe lesions, those ≥ 50% diameter stenosis, whereas TRL (and their apolipoprotein markers) have been identified as a driving factor behind progression of mild-to-moderate lesions < 50% diameter stenosis. Quantitative coronary angiography (QCA) has demonstrated that progression of mild-to-moderate lesions are among the most significant predictors of clinical coronary events, and that lowering TRL reduces progression of coronary artery disease to the same degree as the lowering of LDL-C.

Key words: angiography, apolipoprotein C-III, atherosclerosis, cholesterol, coronary artery, lipoprotein, low-density lipoprotein, triglyceride, ultrasonography

Introduction

Arterial imaging is an important tool that can be used to study the atherogenicity of triglyceride-rich lipoproteins (TRL). Arterial imaging may be performed as an invasive or noninvasive procedure.1 An invasive approach uses angiography to examine atherosclerosis at the later stages of development. Noninvasive techniques, such as high-resolution B-mode ultrasonography, are used to measure carotid artery intima-media thickness (IMT), the earliest anatomically quantifiable stage of atherosclerosis.

The Low-Density Lipoprotein Cholesterol Hypothesis and Atherosclerosis

Many studies have examined the low-density lipoprotein cholesterol (LDL-C) hypothesis of atherosclerosis; specifically, if LDL-C is lowered, can the progression of atherosclerosis be reduced? More than a dozen cholesterol-lowering and several risk-reduction studies have enrolled a total of 3,558 men and women (approximately 10%) who underwent serial coronary angiography.1 Overall, the data revealed that LDL-C lowering resulted in twice the amount of regression, 1½ times more stabilization, and approximately half as much progression of coronary atherosclerotic lesions compared with control subjects.1 These changes were associated with a reduction in coronary artery disease (CAD) events, including revascularizations, myocardial infarctions, and deaths from cardiovascular disease. Despite aggressive LDL-C reduction in these trials however, 20 to 50% of subjects continued to show progression of atherosclerosis. Clearly, factors in addition to LDL-C contributed to the progression of atherosclerosis in these subjects.

The MARS Study

Through ancillary analyses, data from the Monitored Atherosclerosis Regression Study (MARS) have been used to study factors in addition to LDL-C that may contribute to the progression of atherosclerosis.3-5 MARS was a 2-year, randomized, double-blind, placebo-controlled study in which coronary angiography and carotid ultrasonography were used to measure atherosclerosis progression.6-7 Both smoking and nonsmoking male and female subjects, 37 to 67 years of age, were enrolled. Cholesterol levels ranged from 190 to 295 mg/dl, while triglyceride levels were < 500 mg/dl. Of the 270 randomized subjects, 247 underwent baseline and 2-year angiograms.6 After 2 years of treatment with lovastatin, it was observed that LDL-C was associated with significant regression of lesions ≥ 50% diameter stenosis. However, LDL-C lowering did
not affect regression in the lesions < 50% diameter stenosis. This and other studies that have reported trial results dichotomized by lesion severity, have shown that LDL-C lowering most significantly impacts severe lesions. Therefore, it was hypothesized that different risk factors might differentially affect lesion progression based on lesion severity.

**Driving Factors behind Lesion Progression**

Multivariate analysis was performed to determine the relative risks for coronary artery lesion progression in the MARS trial (Table I). The largest correlate of lesion progression in the placebo group, untreated individuals with elevated on-trial LDL-C (150–180 mg/dl) was an increase (from study baseline) in the total cholesterol (TC)-to-high-density lipoprotein cholesterol (HDL-C) ratio. The TC/HDL-C ratio was associated with both mild and moderate lesion progression in the placebo group. In the subjects in the lovastatin-treated group who had LDL-C lowered to <90 mg/dl, progression of severe lesions (≥50%) was primarily associated with higher on-trial levels of the LDL-C/HDL-C ratio. Progression of mild-to-moderate lesions was associated with higher levels of apolipoprotein C-III (Apo C-III), a marker of TRL.

Additional evidence that supports the Apo C-III marker as a risk factor for CAD progression comes from the Cholesterol Lowering Atherosclerosis Study (CLAS) Study. In this clinical trial, a bile acid sequestrant and niacin were used to lower the cholesterol level. In ancillary analyses of this study, Apo C-III was also found in multivariate analysis to be the significant risk factor for lesion progression among drug-treated subjects with LDL-C reduced to <100 mg/dl. Apo C-III appears to be a very important risk factor for lesion progression. Apo C-III is distributed between HDL and very low-density lipoprotein (VLDL). Therefore, Apo C-III levels indicate recent chylomicron and VLDL clearance, making it a useful marker for measuring the metabolism of TRL.

Another large database collected on the MARS cohort consisted of the whole density range of lipoprotein particles determined by analytical ultracentrifugation. Similar effects were observed in the lovastatin, placebo, and combined treatment groups. Progression of mild-to-moderate lesions was associated with levels of TRL. In all lesions combined, a higher on-trial level of small VLDL was associated with lesion progression. In the untreated placebo group, a higher level of intermediate-density lipoprotein (IDL) was found to be the strongest risk factor for lesion progression. This is one of two independent methods, based on different physiochemical properties used in the MARS study to measure TRL. One method used protein composition, specifically electroimmunoassay for Apo C-III, and the other method used lipoprotein density. Both methods demonstrated a relationship between progression of lesions <50% diameter stenosis and TRL.

**Particle Composition**

Chylomicrons, VLDL, IDL, and LDL are all within the range of 0.92 to 1.063 g/ml. In general, these classes are divided into TRL and cholesterol ester-rich lipoproteins. In reality, however, there is a mixture of TRL and cholesterol ester-rich lipoproteins within this whole density range. Within these classes, Apo B-containing lipoproteins can be separated out by using immunofinity column separation.

Particles within the family of ApoB-containing lipoproteins can be examined in this manner. For example, LP-B is cholesterol ester-rich with very little triglyceride components. On the other hand, other particle families (e.g., LP-B:C, LP-B:E, LP-B:C:E) have very little or no cholesterol-ester within the particles. Instead, they are triglyceride-rich. When a subset of MARS subjects was examined according to lesion progression, the major lipoprotein particle discriminating between progressors and nonprogressors was not the cholesterol ester-rich LP-B, but rather the LP-A-II:B:C:D:E particle (Table II). This large particle is insensitive to lipoprotein lipase, with a possibly longer residence time in the circulation when compared with the other TRL particles.

**Importance of Triglyceride-Rich Lipoproteins**

When evaluated by serial quantitative coronary angiography, progression of coronary atherosclerosis over a 2-year period is significantly predictive of clinical coronary events during the subsequent 8 to 12 years among CLAS subjects (Fig. 1). This relationship is evident whether percent diameter stenosis or minimum lumen diameter is used as the angiographic measure of atherosclerosis progression. The mild-to-moderate lesions <50% diameter stenosis were predictive of clinical coronary events, whereas the more severe lesions ≥50% diameter stenosis were not significantly related to events (Table III). As noted above, mild-to-moderate lesions exhibit the strongest relationship with TRL.

Plaque fissuring, lesion disruption, and thrombus formation predominantly occur in small, soft plaques with a central

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**Table I** Multivariate relative risks (95% confidence interval) for coronary artery lesion progression (quantitative coronary angiography change in percent diameter stenosis >0)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Relative risk</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50%S</td>
<td>ApoC-III HP</td>
<td>1.35</td>
</tr>
<tr>
<td>≥50%S</td>
<td>LDL-C/HDL-C</td>
<td>4.09</td>
</tr>
<tr>
<td>Placebo group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50%S</td>
<td>δTC/HDL-C</td>
<td>1.82</td>
</tr>
<tr>
<td>≥50%S</td>
<td>δTC/HDL-C</td>
<td>2.66</td>
</tr>
</tbody>
</table>

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Abbreviations: CI = confidence interval; %S = percent diameter stenosis; ApoC-III HP = apolipoprotein C-III heparin precipitate, which is the apo C-III content of low-density lipoprotein, very-low-density lipoprotein, intermediate-density lipoprotein; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; δTC/HDL-C = total cholesterol/high-density lipoprotein cholesterol.
H. N. Hodis et al.: Triglyceride-rich lipoproteins in atherothrombosis

The Significance of Intermediate-Density Lipoprotein

High-resolution B-mode ultrasonographic measurement of arterial wall thickness is an excellent tool for the noninvasive evaluation of atherosclerosis, specifically subclinical atherosclerosis. Since atherosclerosis is an intimal-medial process, IMT measurements focus directly on the atherosclerotic process. Therefore, analyses of risk factors for atherosclerosis can be made directly using carotid artery IMT measurements. Less than 1% of all carotid artery lesions occur in the common carotid artery where blood flow is laminar. Most atherosclerotic lesions occur in the internal and external carotid arteries where blood flow turbulence occurs. Therefore, the distal common carotid artery is an excellent site for examining the relationship between atherosclerosis and risk factors independent from blood flow characteristics.

In the MARS and CLAS studies, a number of correlations were found between the annualized rate of change in the distal common carotid artery far wall IMT and on-trial risk factors (Table IV). Several of these risk factors, such as Apo A, Apo C-III, and Apo E, which are components of TRL, are also correlates of CAD progression. The strongest risk factor found in relation to the progression of carotid IMT was the total IDL mass (Table IV), defined by density fraction ($S_f 12–20$). Progression of common carotid artery IMT is significantly correlated with the progression of CAD as measured by quantitative coronary angiography (QCA). Furthermore, progression of the distal common carotid artery far wall IMT over a 2-year period is significantly predictive of clinical coronary events over the subsequent 12 years (Fig. 2). Thus, carotid wall thickness studies are consistent with coronary angiography results.
**TABLE IV** Correlations (p values) of on-trial lipoprotein subclass levels with annualized rate of change in the distal common carotid artery far wall intima-media thickness.

<table>
<thead>
<tr>
<th>Combined sample (n = 180)</th>
<th>Placebo (n = 83)</th>
<th>Lovastatin (n = 97)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total LDL mass (Sf 0–12)</td>
<td>0.09 (0.26)</td>
<td>0.08 (0.47)</td>
</tr>
<tr>
<td>Total IDL mass (Sf 12–20)</td>
<td>0.21 (0.005)</td>
<td>0.23 (0.04)</td>
</tr>
<tr>
<td>Total VLDL mass (Sf 20–400)</td>
<td>−0.09 (0.24)</td>
<td>−0.01 (0.91)</td>
</tr>
<tr>
<td>Total HDL mass (F1.20 0–9)</td>
<td>0.04 (0.59)</td>
<td>0.08 (0.46)</td>
</tr>
</tbody>
</table>

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*Abbreviations: Sf = Svedberg flotation rate, F1.20 = flotation rate, IDL = intermediate-density lipoprotein. Other abbreviations as in Table I.*

**Effect of Reducing Triglyceride-Rich Lipoproteins**

The Bezafibrate Coronary Angiographic Trial (BECAIT) was a randomized, double-blind, placebo-controlled, 5-year coronary angiographic trial designed to study the effects of bezafibrate, a triglyceride-lowering agent, on the progression of CAD.

The angiographic results of BECAIT were comparable with those from the Regression Growth Evaluation Statin Study (REGRESS) and the Multicenter Anti-Atheroma Study (MAAS) angiographic trials that studied the LDL-C lowering agents pravastatin and simvastatin, respectively, on the progression of CAD. The greatest lipid effect of bezafibrate was on triglyceride levels, resulting in an approximate 30% reduction of this lipid from baseline. This compared with a reduction of approximately 10 to 20% in the triglyceride levels in REGRESS and MAAS.

The largest difference in the BECAIT, REGRESS, and MAAS trials was in LDL reduction. A nonsignificant reduction of approximately 2% was observed in the BECAIT study whereas in REGRESS and MAAS, baseline LDL-C was reduced by about 30%. The effect of bezafibrate on the progression of CAD fell into the middle of the effects seen in REGRESS and MAAS. It should be noted, however, that bezafibrate also significantly lowered fibrinogen levels. The effect of bezafibrate on the clotting system may have been a contributing factor in achieving the angiographic results.

**Conclusion**

Coronary artery disease progression persists in 20 to 50% of patients, even with aggressive LDL-C lowering. This has now been observed in more than a dozen angiographic trials.

Reduction of LDL-C preferentially alters the progression of severe lesions (≥50% diameter stenosis). Triglyceride-rich lipoproteins correlate with the progression of CAD, specifically mild-to-moderate lesions (<50% diameter stenosis).

Progression of mild-to-moderate lesions predict clinical coronary events. These observations are very consistent with the current hypothesis of plaque rupture, mild-to-moderate severity of atherosclerotic disease, and coronary events. The lowering of TRL appears to result in a reduction of CAD progression to the same degree as the lowering of LDL-C.

One other trial, the Lopid® Coronary Angiographic Trial (LOCAT) has also shown similar effects in coronary artery bypass grafts. Several ongoing studies will also explore the relationship between TRL reduction and progression of atherosclerosis.

**Discussion**

**Rackley:** Everyone is familiar with the ability of a lesion <50% to rupture, causing thrombus formation and, subsequently, an acute event. A lesion <50%, however, does not really impair blood flow considerably. Even if a lesion ruptures, a thrombus is not going to form unless peripheral blood flow is decreased. Triglycerides have the potential to release free fatty acids (FFAs) by acting directly on the endothelium to decrease blood flow. When triglyceride changes are more carefully examined, one will find that a reduction in the distal blood flow occurs probably before the plaque ruptures. When this plaque does rupture, there is sufficient reduction in flow for the thrombus to form. After meals, patients get more angina than they do otherwise. While this has been blamed on the blood flow of the intestine, it may very well be the effect of the triglycerides, specifically the FFAs on vascular flow.

**Hodis:** I agree that there is some physiological mechanism at work here. However, there is not a lot of good data available yet to support this view.

**Abrams:** One can set up a triglyceride-rich construct versus a high LDL. The overwhelming clinical evidence is in favor of LDL-C reduction. Evidence from the fibrate analysis and the meta-analysis has been underwhelming with respect to clinical events. Did the patients in the BECAIT study have a different baseline lipid profile than the patients who participat-
ed in the statin trials? Perhaps different populations are being compared here. Did BECAIT patients also have a different lipid profile than patients who participated in other trials? If so, this may suggest that they might have responded differently and better to fibrate.

**Hodis:** Patients in the BECAIT study were considered premature CAD patients selected for myocardial infarction before the age of 45. Most had a mixed lipoprotein pattern. This may have been a little different from that found in some of the other studies since they were more focused on LDL selection criteria. The triglyceride level selection criterion in this study was below 500 mg/dl, just as it has been in every LDL-C hypothesis-driven angiographic study. In this sense, these studies were similar.

**Blumenthal:** In the Johns Hopkins’ Sibling Study, where people with a family history of premature disease were studied, African-Americans were found to have a much lower triglyceride level, on average, than Caucasians. When predictors of carotid intima-media thickness were examined, lipids were found to be predictive in Caucasians. Hypertension, however, was found to completely wipe out the lipids as predictors in African-Americans. Are there any other data about African-Americans? Do triglycerides play as much of a role in this population as they appear to in Caucasians?

**Hodis:** There are remarkable and very interesting differences in the risk factors among different populations and ethnic groups, especially as they pertain to hypertension and early CAD. There is no doubt that genetic background needs to be the focus for our studies. There are going to be triglyceride differences, not only among various racial populations as a whole, but also among individuals within these racial populations.

**Hunninghake:** One of the concerns regarding BECAIT is that it has only studied 75 patients. In the Bezafibrate Infarct Prevention (BIP) Trial involving 3,000 patients, these individuals had moderately elevated LDL-C. This placebo-controlled trial enrolled patients with triglyceride levels <300 mg/dl. There was no overall difference in the primary endpoint for this trial, and no particular effect on HDL-C was shown. It was suggested that patients with triglyceride levels >200 mg/dl may have received some benefit from the fibrates. Overall, however, the fibrates had not resulted in much of a reduction in clinical events.

**Hodis:** My understanding is that benefit was most pronounced in subjects with the higher triglyceride levels. But I do not believe that anyone has ever suggested that high-grade lesions should be ignored. Any coronary artery lesion has the potential of being fatal. Recent data only suggest that mild-to-moderate lesions, perhaps because of their greater number, as Dr. Conti suggested, seem to result in a predominant number of coronary events. As lesions slowly progress, there is time for collaterals to develop and, hence, the myocardium in jeopardy from the progressing lesion may be diminished over time. It is the sudden rupture of a lesion resulting in acute obstruction of blood flow that we have to be concerned with in relation to coronary events. It is hopeful that noninvasive techniques will be developed so that plaques susceptible to rupture can be discerned from those that are not eminently destined to do so.

References


Measurement of Triglyceride-Rich Lipoproteins by Nuclear Magnetic Resonance Spectroscopy

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Summary: Nuclear magnetic resonance (NMR) spectroscopy is being used to determine the concentrations of very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) subclasses of different size. These subclasses have unequal associations with coronary heart disease. Nuclear magnetic resonance distinguishes among the subclasses on the basis of slight differences in the spectral properties of the lipids carried within the particles, which vary according to the diameter of the phospholipid shell. Studies using NMR spectroscopy have shown that individuals with elevated triglycerides are likely to have higher-risk lipoprotein subclass profiles. Triglyceride-rich lipoproteins drive the metabolic reactions that produce LDL of abnormal size and cholesterol content. The quantities of these abnormal LDL particles and the associated risk of coronary heart disease are underestimated by conventional cholesterol measurements. Nuclear magnetic resonance spectroscopy measures lipoprotein subclasses directly and efficiently, and produces information that may improve the assessment and management of cardiovascular disease risk.

Key words: coronary heart disease, very low-density lipoproteins, low-density lipoproteins, high-density lipoproteins, nuclear magnetic resonance spectroscopy, lipoprotein subclasses

Introduction

Historically, inferences about the roles played by lipoproteins in atherogenesis have come from large population studies in which lipids, not lipoproteins, were measured. The focus on lipids was dictated by analytical considerations, as it is much easier to measure the amount of lipid (typically cholesterol or triglyceride) in plasma or in a particular lipoprotein fraction than to measure the lipoprotein particles themselves. All lipoproteins contain cholesterol and triglyceride, but cholesterol is carried mainly in low- and high-density lipoproteins (LDL, HDL), while triglycerides are found predominantly in very low-density lipoproteins (VLDL). Very low-density lipoproteins and LDL are positively associated with coronary heart disease (CHD) (higher levels confer increased risk) and thus are considered atherogenic lipoproteins, whereas HDL is considered antiatherogenic because of its negative association with CHD (higher levels confer protection). Since it is now recognized that patients with widely differing amounts of VLDL, LDL, and HDL (and hence different risks of CHD) can have exactly the same cholesterol level, clinical attention has shifted away from total cholesterol to LDL and HDL cholesterol as the prime risk factors.

What is not widely known is the degree to which LDL and HDL cholesterol levels can fail in many patients to reflect accurately the number of LDL and HDL particles and their atherogenic potentials. There are two sources of this problem and both are related to triglycerides. The first is variability of the lipid composition of LDL and HDL because of a process of cholesterol ester-triglyceride exchange driven by elevated plasma triglyceride (VLDL) levels. When this occurs, HDL and especially LDL become cholesterol-depleted compared with normal. The second is variability in LDL and HDL particle size because of lipoprotein metabolic reactions that are driven in large part by elevated triglycerides. These reactions produce particles that are smaller than normal. By not taking account of the compositional and structural variability of lipoproteins, conventional lipid tests may be failing to provide critical information needed for the accurate diagnosis and management of the CHD risk of many patients.

New technology using nuclear magnetic resonance (NMR) spectroscopy has recently become available to allow lipoprotein particles of different size to be directly quantified. The NMR LipoProfile™ measurement is automated and efficient, and can be performed on either fresh or frozen (−70°C) plasma specimens. Physicians and researchers will now have ready access to information that previously could be obtained only by using time-consuming and laborious laboratory separation procedures.
Associations between Particle Subclasses and Coronary Heart Disease

Very low-density lipoprotein, LDL, and HDL each comprise a heterogeneous group of particles that differ in size and, in many cases, their associations with CHD (Fig. 1). The best known example is small, dense LDL, which confers at least a 3-fold higher risk compared with large LDL. Elevated intermediate-density lipoprotein (IDL), which is included as part of the LDL fraction as measured by standard methods, is also associated with increased risk. Since persons with the same LDL cholesterol level often have very different amounts of small LDL and IDL, it is not surprising that they may differ markedly in their susceptibility to developing CHD. Differing associations of HDL subclasses with CHD have also been noted in several studies. The three largest subclasses separable by gradient gel electrophoresis exhibit the expected inverse correlation with CHD incidence and severity (and therefore are truly antiatherogenic), whereas the two smallest subclasses show the opposite association. Thus, the subclasses that contribute to "good" cholesterol (HDL) are, in fact, not all good! In the triglyceride-rich VLDL category, a recent study showed that levels of the largest particles (many of which may be chylomicron remnants) have a strong positive relationship with an arteriographic endpoint, independent of total plasma triglycerides and other lipid risk factors.

Despite research evidence suggesting that clinical decision-making could be improved by direct measurements of lipoprotein subclasses, there has been no practical way to get at this information until now. Gross separation of LDL or HDL from the other lipoproteins is relatively simple and takes only a few minutes using chemical or immunoprecipitation methods. For this reason, LDL and HDL cholesterol measurements have become routine in clinical practice. Further subfractionation to give LDL and HDL subclass distributions requires far more time and effort and is performed in only a limited number of specialized laboratories. Ultracentrifugation and gradient gel electrophoresis are the methods used most often, and several hours to days are required to complete each analysis. Accuracy and precision are unavoidably limited by the many sources of analytical error introduced during the subclass separation process.

The Value of Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopy gathers lipoprotein subclass information in a manner that is completely unique and inherently far more efficient than existing methods. The technique relies on the spectroscopic distinctness of lipoprotein particles of a particular size and requires no physical separation of one group of particles from the others. Refined over a 10-year period, the NMR process measures 15 subclasses of VLDL, LDL, and HDL simultaneously. The measurement requires only about 1 min, uses no chemical reagents, and is completely automated. By simply adding up the concentrations of the various subclasses, one obtains all of the information provided in a standard lipid panel (total cholesterol, triglycerides, LDL and HDL cholesterol). In addition, average VLDL, LDL, and HDL particle sizes, as well as the total concentration of LDL particles are calculated. From the LDL particle size value, patients are categorized according to LDL subclass phenotype: pattern A (predominantly large LDL, signifying lower CHD risk), pattern B (predominantly small LDL, signifying higher CHD risk), or pattern AB (intermediate category).

When used for clinical decision-making purposes, the NMR data are presented as a 2-page NMR LipoProfile report. This format simplifies interpretation of the new NMR variables by relating them in percentile terms to values observed in the general population (based on soon-to-be published data from 3,437 participants in the Framingham Offspring Study). The names of individual subclasses (V3, L2, H4, etc.) are based on a simple nomenclature scheme that assigns a larger number to a larger particle. Thus, V3 refers to a VLDL subclass that is smaller than V4, L2 to an LDL subclass smaller than L3, H4 to an HDL subclass smaller than H5, and so forth. Check boxes on page 2 of the NMR LipoProfile report help identify patients who are at higher risk than would be inferred from a standard lipid panel, as a result of a clustering of metabolic abnormalities associated with insulin resistance: the so-called metabolic syndrome. This clustering of nontraditional risk factors includes a predominance of small LDL, elevated numbers of LDL particles, low levels of large HDL particles, and elevated levels of triglycerides (carried in large VLDL particles, as will be described later). Among the terms used to refer to the clustering of these variables are the atherogenic lipoprotein phenotype, atherogenic dyslipidemia, or the lipid triad. The unique clinical value of NMR spectroscopy is its ability with a single, low-cost test to identify those patients who do (and do not) have an underlying lipid metabolism that confers higher CHD risk, and for whom different therapeutic choices might be indicated.

Origin of the Nuclear Magnetic Resonance Subclass Data

A detailed description of how the NMR lipoprotein measurement process works has been published and will not be...
repeated here. In brief, though, it is useful to draw an analogy between the different NMR signals emitted by lipoprotein particles of different size and the ringing sounds produced by bells of different size. The actual source of the NMR signals used for subclass quantification are the protons of the terminal methyl groups of the various types of lipid carried in the particles (mainly the cholesterol ester and triglycerides of the particle core, and the phospholipid of the particle shell). The signals from all these different lipids combine to produce a “bulk lipid” signal that has a characteristic frequency and shape that is directly dependent on the size of the particle (specifically, the diameter of the phospholipid shell, excluding the influence of the apolipoproteins attached to the particle). Obtaining the NMR spectrum of a plasma sample is analogous to simultaneously ringing a collection of bells of varying size and recording the composite sound output. With sufficient prior knowledge of the exact sound expected to be produced by each of the different size bells in the collection, one could imagine it might be possible to work backward from the composite sound signal to deduce how big was the signal coming from each set of bells of a given size. In this way, the number of bells (lipoprotein particles) of each size could be determined, since there is a direct proportionality between the amplitude of sound (bulk lipid signal) emitted by each set of bells of a given size (particles of a given diameter) and the number of bells (subclass particles). The great efficiency of NMR lipoprotein analysis derives from the fact that it takes less than a minute to collect the plasma NMR spectrum and only seconds to perform the linear least-squares deconvolution that produces the subclass concentrations. Results are accurate and precise because no particle separation steps are involved and the automated process includes built-in quality control checks.

There is one analytical point that needs to be stressed because of its relation to the topic of triglycerides. Nuclear magnetic resonance directly quantifies the lipoprotein particles themselves (on the basis of their bulk lipid mass), whereas traditional methods measure the amount of one particular type of lipid in the particles (usually cholesterol). This distinction is very important, since the cholesterol content of a particular lipoprotein can vary substantially from person to person. If, for example, two people have the same number of LDL particles, but one has particles containing less cholesterol than normal, their chemically measured LDL cholesterol levels will differ. In contrast, an NMR measurement will show them to have the same LDL concentration. The chief source of cholesterol compositional variability is a metabolic reaction catalyzed by cholesterol ester transfer protein (CETP), in which triglyceride molecules from the core of triglyceride-rich lipoproteins (mainly VLDL) exchange one-for-one with cholesterol ester molecules in the core of LDL and HDL. The result, in people with elevated VLDL levels, is the production of LDL and HDL that are cholesterol-deplet-
ed and triglyceride-enriched compared with normal. In such circumstances, traditional cholesterol measurements (but not NMR) will underestimate the true concentrations of circulating LDL and HDL.

Influence of Triglycerides on Coronary Heart Disease Risk

Several suggestions have been made to explain the observed association between plasma triglyceride levels and CHD. The one most frequently cited is the inverse relation between triglycerides and HDL cholesterol levels. Another is the association of high fasting triglyceride levels with delayed postprandial clearance of chylomicrons and their remnants, which in several studies is an independent predictor of CHD. A relationship to be explored here is that between plasma triglyceride level and the levels of individual VLDL, LDL, and HDL subclasses, which themselves appear to have different strengths of association with CHD. The other relationship to be discussed—which has been cited by many investigators but is still largely unappreciated—is that between triglyceride level and the content of cholesterol in LDL. When the ratio of the concentration of LDL particles (approximated by a plasma apoB measurement) to LDL cholesterol is higher than normal (termed hyperapobetalipoproteinemia), it can be for two reasons. First, the core lipid content of the LDL is relatively enriched in triglyceride and depleted of cholesterol ester. This condition was described in the previous paragraph and might be termed “LDL masking syndrome” since standard LDL cholesterol measurements will give a falsely low impression of the actual amount of LDL present in the patient’s circulation. The second reason for hyperapobetalipoproteinemia is the presence of LDL particles that are smaller than usual (LDL subclass pattern B). There is less cholesterol per particle in this case simply because the volume of each particle is smaller. Since NMR measures the whole spectrum of subclass particles directly and efficiently, it is particularly well suited to provide insights into the origin(s) of the relationship between triglycerides and CHD risk.

Subclass Concentrations and Triglyceride Levels

Nuclear magnetic resonance spectroscopy has been used for the past 2 years to examine large numbers of frozen plasma specimens from various clinical trials and observational studies. Some of the most useful data have come from analyses of over 3,400 samples from men and women participating in the Framingham Offspring Study (Cycle 4). These data, which are not yet published, show the powerful influence exerted by triglycerides on lipoprotein subclass levels and average apolipoprotein particle size. For LDL, it is well established using methods other than NMR that, as triglyceride levels increase in the plasma, LDL size decreases. What NMR shows is that this reduction in particle size is the result of a steady decrease in the level of large LDL (L3) as triglyceride levels rise above 100 mg/dl. Concomitantly, the levels of the more atherogenic small LDL particles (L1) increase. Concentrations of IDL also are observed to be generally higher as triglyceride levels increase. The very high prevalence of the lower-risk LDL pattern A phenotype when triglycerides are < 100 mg/dl and the correspondingly high prevalence of the higher-risk pattern B phenotype when triglycerides are > 250 mg/dl are explained quantitatively by changes in the individual subfractions.

There are also triglyceride-related changes in HDL subclass levels. As triglyceride levels increase, average HDL particle size decreases. This change is explained almost entirely by the response to triglyceride levels of the largest HDL subclasses, H4 and H5. Above a triglyceride level of 200 mg/dl, the H4 and H5 subclasses are no longer the predominant species. These subclasses are thought to be primarily responsible for protection against CHD. Thus, at high triglyceride levels much of the protection afforded by HDL is absent.

Average VLDL particle size is also strongly dependent on plasma triglyceride levels. Above a triglyceride level of about 100 mg/dl, the levels of the largest subclasses (V5 and V6) increase more steeply than those of intermediate-size VLDL (V3 and V4). Virtually the entire increment of triglyceride beyond a level of 300 mg/dl is carried in V5 and V6. It is not clear how many of these largest triglyceride-rich species are chylomicron remnants as opposed to true VLDL, since NMR does not differentiate between liver- and intestinally-derived particles.

Low-Density Lipoprotein Composition and Triglyceride Levels

As mentioned earlier, elevated concentrations of triglyceride-rich lipoproteins (VLDL) drive the production of cholesterol-depleted, triglyceride-enriched LDL particles by promoting cholesterol ester-triglyceride exchange. When large LDL becomes enriched in triglyceride, it becomes a good substrate for hepatic lipase (HL) and may, as a result of core triglyceride hydrolysis and structural remodeling, become transformed into small, dense LDL. Depending on the triglyceride level and CETP activity, the small LDL particles may end up with a normal cholesterol content (cholesterol/triglyceride > 4) or become significantly cholesterol-depleted (cholesterol/triglyceride < 4). Four different types of LDL particles will therefore be encountered depending on the patient’s lipid metabolic circumstances (Fig. 3): large LDL with a normal lipid content, small LDL with a normal lipid content, and abnormal large and small LDL with cholesterol-poor and triglyceride-rich cores. Based on a detailed compositional analysis of LDL isolated from 118 healthy subjects, approximately 65% had large LDL with a normal lipid content. Virtually every subject with a plasma triglyceride level < 100 mg/dl fell into this category (Fig. 4). The other 35% of subjects were fairly equally divided among the other 3 LDL categories. For these individuals, a standard LDL cholesterol measurement does not accurately reflect the true amount of LDL present, at least when compared with individuals with large LDL of normal composition.
To illustrate the disconnect between LDL cholesterol levels and LDL particle concentrations observed in individuals with elevated triglyceride levels, the 118 subjects were grouped according to triglyceride ranges. Low-density lipoprotein cholesterol was measured by beta-quantification (ultracentrifuge) and LDL particle concentration by NMR. At relatively low triglyceride levels, LDL cholesterol levels rise in step with LDL particle numbers (Fig. 5). At higher triglyceride levels, the LDL particle concentrations are shown to be much higher than would be inferred from the LDL cholesterol values. With triglycerides > 250 mg/dl, there is a very large discrepancy between the cholesterol and particle concentrations. For these subjects, standard measurements of LDL cholesterol will seriously underestimate the number of circulating LDL particles, most of which are of the more atherogenic small variety (Fig. 4), and thereby seriously underestimate their risk of developing CHD.

Conclusion

Individuals with elevated triglycerides are observed to have higher-risk lipoprotein subclass profiles. They have a dis-
proportionate amount of large VLDL, higher levels of small, dense LDL, and small, even more dense HDL. The triglyceride-rich lipoproteins drive the metabolic reactions that produce LDL particles with abnormal composition. As a result, the quantities of these particles and their associated CHD risk are underestimated by conventional cholesterol measurements. Direct measurement of lipoprotein particles by NMR or other methods will help to clarify the roles played by triglycerides in atherogenesis.

Discussion

Bachorik: There are a few circumstances where conventional beta quantification should be used. One is the detection of Type III hyperlipoproteinemia. How are the patterns affected by the presence of floating beta-lipoprotein? Another risk factor in certain populations is Lp(a). Where does this risk factor fall into these subclass patterns? If NMR patterning were substituted for conventional beta quantification, is there any way to flag that the patient might be a Type 3 or is less responsive to conventional LDL-lowering agents since their Lp(a), not their LDL, is elevated?

Otvos: We don’t yet have enough experience with the Type III situation to know whether NMR can detect it well. As for Lp(a), remember that NMR differentiates particles on the basis of differences in the diameter of the phospholipid shell. What distinguishes Lp(a) from LDL is the extra apo(a) protein molecule covalently attached to the apoB protein, which makes Lp(a) particles physically bigger and their density greater than normal LDL. However, the diameter of the phospholipid shell is unchanged when the apo(a) protein is attached, so NMR is unable to distinguish Lp(a) from LDL. We wondered whether this might impair our ability to measure LDL size accurately. To investigate this question, we examined plasma samples containing both large and small LDL from which Lp(a) was selectively removed by a lectin binding process. It was shown that when LDL is small, the phospholipid shell diameter of the Lp(a) particles in that sample were also small. The same thing was shown for samples containing large LDL; the diameter of the Lp(a) phospholipid shell was also large. We therefore believe that even high concentrations of Lp(a) do not impair the ability of NMR to assess LDL size accurately. However, it is also true that Lp(a) information is important to have in certain clinical situations, and NMR is unable to supply it.

Glueck: Antiphospholipid antibodies, anticardiolipin antibody, and lipid anticoagulant are known to bind to LDL among other things. Do these affect the NMR signal in any way since this signal is dependent on the phospholipids to which these antibodies bind? In addition, how much does the equipment cost to perform these NMR procedures?

Otvos: Although the equipment is not being made available commercially, the test is being made available. Currently, it is available for clinical research studies. In early 1999, it will become available for patient care. There is no direct evidence that suggests these antibodies and other factors interfere with the NMR signal.

Krauss: For clinicians, a measurement of apo B gives an estimate of particle number within the whole atherogenic range. While it does not provide the specificity across the subfractions, it does unmask some of the risk masked by LDL cholesterol. A VLDL subfractionation was used to document the size distribution across V1 through V6, with V1 being the smallest. It appears to be relatively flat and at low concentration across the triglyceride range. When mass is measured using analytical ultracentrifugation and other techniques, first smaller and then larger particles are measured. It appears that nothing was happening to V1. Is the VLDL subfractionation measuring V1 triglyceride? If so, is it a function of the composition of those particles?

Otvos: V1 is really a very minor constituent; it is very close to IDL. The concentration is expressed in triglyceride units since it is the most abundant lipid in the particles. What is really being measured is the signal intensity that comes from the particle, which is then converted to triglyceride equivalents. Large triglyceride-rich particles are the most sensitive to change at higher triglyceride concentrations.

Krauss: The signal on the phospholipids is the index of particle size. Can the procedure be sensitive to alterations in phospholipid composition or to pharmacologically induced changes in surface lipid or proteins that might affect this signal?

Otvos: We have not tried to generate a defined population where something like that existed, though it might be worthwhile. In these particles, the bulk of the signal amplitude comes from what is in the core and not what is found in the shell. The shell signal becomes more important in the HDL regime, where the core-to-shell ratio is very different. There is no modulation due to the tremendous compositional heterogeneity. The methyl group does not feel this chemical heterogeneity.

Pek: In diabetic patients with hyperlipidemia, when blood sugar goes up, many proteins get glycated, including LDL. Does this glycated LDL influence particle size?

Otvos: It does not influence particle size as far as we know. Glycated LDL does not have a spectral perturbation and cannot be seen. This is occurring on the protein moiety to which the NMR is not sensitive.

Abrams: This is a small particle size group for the most part. There has been controversy over whether it is necessary to interrogate the issue of pattern A versus pattern B. Some clinicians claim one only needs to look at triglycerides and HDL in order to make assumptions about particle size. Can this same assumption be made with respect to the total number of LDL particles in anyone with a high triglyceride and a low HDL? Can one assume that the LDL measurement, whether it is done by the direct or conventional subtraction, is likely to be wrong?

Otvos: It is likely to be wrong reproducibly when the triglyceride levels are quite high, such as above 250 to 300 mg/dl. Most people, however, have triglyceride levels lower than this. There is really no good ability to predict, on the basis of triglyceride level, the extent to which a particle becomes triglyceride enriched and cholesterol depleted. Enzyme activity levels have a lot to do with this. It is a very complex meta-
bolic milieu. The efficiency of NMR spectroscopy will, hopefully, allow it to substitute at approximately the same price for the standard lipid panel.

**Abrams:** In a patient with a triglyceride level of > 350 mg/dl and an abnormal HDL, can one conclude that the particle size will be small (pattern B) and that the particle number will be greater than one might anticipate from the LDL concentration?

**Otvos:** This can be concluded with reasonably good certainty. If the particles are small, and the frame of reference is the large particle with normal lipid composition, the particle number is greater than if the particles were large. This is why the apo B to LDL cholesterol ratio is useful in this regard. If one is only interested in the number of LDL particles, then a plasma apo B measurement is a useful parameter. If small particles on a per-particle basis are believed to be inherently more atherogenic, then it would be good to know both the number of particles and how big these particles are. Both pieces of information are desired. The Quebec Cardiovascular Study has shown that both apo B and LDL size, when taken together, help to discriminate the at-risk population much better than either one alone.²

**References**


Postprandial Triglyceride Metabolism in Diabetes Mellitus

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Summary: Individuals with diabetes have a two to four times higher risk of cardiovascular morbidity and mortality than nondiabetics. Patients with both type 1 and type 2 diabetes share a similar risk. Studies in individuals with type 1 diabetes have shown a decreased clearance of postprandial triglyceride-rich lipoprotein particles of abnormal composition. Particles isolated from diabetic individuals show abnormal composition and an increased tendency to cause cholesteryl ester accumulation in macrophages and are therefore potentially atherogenic. Various interventions may alter these abnormalities and improve the atherosclerotic risk. These include adopting a high-carbohydrate diet over a high monounsaturated diet, improving glycemic control, infusing insulin intraperitoneally, and using pharmacologic therapies such as the statins.

Key words: atherogenic, diabetes, postprandial lipoproteins, macrophages, triglycerides

Introduction

Patients with diabetes have an increased risk of cardiovascular morbidity and mortality. Early studies have examined whether or not these individuals had an increased number of risk factors when compared with nondiabetics. Data from the Multiple Risk Factor Intervention Trial (MRFIT) showed that although a higher number of risk factors was associated with an increased mortality in patients with type 2 diabetes, diabetes itself was associated with additional risk.1 Work conducted by Krolewski et al. evaluated cardiovascular mortality in patients with type 1 diabetes compared with age- and gender-matched controls.2 Patients with type 1 diabetes were followed for 20 to 40 years. This cohort was then compared with a nondiabetic cohort from the Framingham heart study. Those individuals with diabetes had a significantly higher mortality at any given age than did the nondiabetic Framingham cohort. Data provided by the Carter Center showed that diabetic individuals above the age of 20 years die primarily of cardiovascular disease.3 Patients with both type 1 and type 2 diabetes were included in the study. To investigate the effect of diabetes, patients with type 1 diabetes are preferable, because they are usually nonobese, have normal fasting lipids, and have little insulin resistance. Type 2 diabetic patients, on the other hand, are usually obese, display fasting dyslipidemia, and have insulin resistance.

Postprandial Lipoprotein Metabolism in Diabetes

We hypothesized that in patients with type 1 diabetes, “hidden” abnormalities in the metabolism of postprandial triglyceride-rich lipoproteins (TRL) contribute to their increased risk for atherosclerosis. This hypothesis was tested in individuals with type 1 diabetes who had no confounding factors. Is remnant accumulation in the postprandial state due to decreased clearance of TRL in type 1 diabetic subjects compared with nondiabetic controls? The longer residence time facilitates TRL interaction with the endothelium, which may, in turn, contribute to the development of atherosclerosis.

The study compared type 1 diabetic men and controls, matched for age and normal body weight.4 Both groups possessed normal cholesterol and triglycerides, as well as similar high-density lipoprotein (HDL) levels. Subjects in the diabetic group had poor control of their diabetes, as evidenced by high glycohemoglobin and a high blood glucose level. After a 12-h fast, the subjects ingested a milkshake. After 4½ to 5 h, they underwent plasmapheresis in order to harvest enough TRL particles in the chylomicron remnant and very low-density lipoprotein (VLDL) range (Sf > 100). These particles were isolated by density gradient ultracentrifugation of the plasma and labeled with radioactive iodine (125I). After characterization, filtration, and pyrogen testing, they were reinjected into the same individuals 3 days later. The disappearance of the radioactivity in the apo B moiety was then measured over time. Blood samples were collected and three lipoprotein subfractions were isolated: the large chylomicron remnant and chylomicron particles (Sf > 400), the VLDL/remnant range of particles (Sf 100–400), and the

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smaller-range VLDL particles (S<sub>f</sub> 20–100). Following polyacrylamide gradient gel electrophoresis, apo B bands were isolated and their activity measured.

A model of metabolism of injected postprandial 125I TRLs was designed, taking into account two particle clearance mechanisms. One mechanism is the lipolysis of the bigger particles to the smaller-sized particles; the other mechanism is the uptake of the particles by tissue receptors, predominantly in hepatocytes. Due to metabolic heterogeneity of the particles, two pathways were established: a fast and a slow cascade.

When the turnover of postprandial lipoproteins (S<sub>f</sub> 100–400) was measured, the rate in the diabetic subjects was approximately 30% lower to that observed in the control subjects (Table 1). The rate of turnover in the control subjects was due mostly to decreased tissue uptake and not to lipolysis. Lipolysis was not impaired in normolipidemic type 1 diabetic subjects. This was true for both the fast and the slow cascade particles.

This study showed that particle clearance was impaired in normolipidemic, nonobese patients with type 1 diabetes. One reason for the decreased clearance may be that the composition of particles might have been altered. We have shown previously that, compared with particles from normal subjects, postprandial lipoprotein particles from both men and women with type 1 diabetes were cholesterol enriched and phospholipid depleted. These abnormalities could alter the surface of the particle in a way that the apolipoprotein ligand was less readily accessible for binding to the hepatic receptors.

In summary, based on the studies described above, in type 1 diabetes there is decreased clearance and abnormal composition of postprandial triglyceride-rich particles. Because increased residence time can alter particle composition, it remains unclear which of these two processes is the initiating event.

### Cholesteryl Ester Accumulation in Macrophages

Another study examined whether or not particles isolated from diabetic individuals have an increased tendency to cause cholesteryl ester accumulation in vitro in macrophages. This study enrolled 13 control subjects and 14 type 1 diabetic subjects matched for age, gender, and race. In addition to being matched for body mass index, these individuals also had similar cholesterol, triglyceride, HDL, and LDL levels. All diabetic subjects were in poor glycemic control with elevated glycated hemoglobin. The objective was to identify which of the postprandial TRL subfractions (S<sub>f</sub> >400, S<sub>f</sub> 100–400, S<sub>f</sub> 20–100) were atherogenic in subjects with type 1 diabetes by measuring cholesteryl ester synthesis and accumulation in macrophages.

The subjects ingested a milkshake containing corn oil (60 g/m<sup>2</sup> of body surface). Triglyceride-rich lipoprotein subfractions were isolated by density gradient ultracentrifugation from plasma and harvested 4.5 h and 7 h following ingestion of the shake. Normal and diabetic lipoproteins were incubated with THP-1 macrophages. C-oleate incorporation into cellular cholesteryl ester and triglyceride were determined by thin layer chromatography. The mass accumulation of cellular cholesteryl ester and free cholesterol was measured by gas chromatography.

Lipoprotein subfractions obtained from the diabetic subjects in the postprandial state caused higher accumulation of cholesteryl esters in macrophages. In every experiment, TRL subfractions from a normal and a diabetic patient (matched for age, gender, and race) were incubated with the macrophages.

There was also a matching of the number of particles that were present in the incubation media. This was done by normalizing for apo B concentration of each subfraction being incubated. Compared with postprandial control TRL, diabetic postprandial TRL caused an increased degree of accumulation. This increase was similar to that observed for malondealdehyde LDL (Fig. 1).

### Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fast cascade</th>
<th>Slow cascade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>L*</td>
<td>0.680 ± 0.242</td>
</tr>
<tr>
<td>Diabetic</td>
<td>L*</td>
<td>0.170 ± 0.126</td>
</tr>
</tbody>
</table>
|           | Lipolysis    | 0.617 ± 0.328 | p<0.05 | NS
|           | Lipolysis    | 0.064 ± 0.087 | p<0.05 | NS
| Control   | Lipolysis    | 0.084 ± 0.082 | p<0.05 | NS
| Diabetic  | Lipolysis    | 0.087 ± 0.069 | p<0.05 | NS

**Abbreviation:** NS = not significant. Reprinted from Ref. No. 4 with permission. *L = rate constant = min<sup>-1</sup>.

![Fig. 1 All triglyceride-rich lipoprotein subfractions from patients with type 1 diabetes increase cholesteryl ester accumulation in THP-1 macrophages when compared with lipoproteins from normolipidemic control subjects. The extent of cholesteryl ester accumulation was similar between postprandial diabetic lipoproteins and oxidized MDA LDL. N = normo lipidemic controls, D = diabetic, LDL = low-density lipoprotein, MDA = malondealdehyde. Reprinted from Ref. No. 6 with permission.](image-url)
Comparison of Fasting to Postprandial Triglyceride-Rich Lipoproteins

In another experiment, the atherogenic potential of fasting and postprandial TRL was compared by measuring the amount of cholesteryl ester accumulation in macrophages. In normal subjects, there was no significant difference in cholesteryl ester accumulation between fasting and postprandial lipoproteins. In type 1 diabetic subjects, however, postprandial TRL increased cholesteryl ester accumulation to a greater extent than fasting TRL. To assess the duration of this effect, postprandial lipoprotein particles, harvested 7 h after fat ingestion, were studied. In normal subjects, the 7-h sample did not cause much accumulation at all in macrophages; but the effects of diabetes persisted for at least 7 h.

The mechanisms accounting for the increased cholesteryl ester accumulation in macrophages were studied using radiolabeled lipoproteins that were incubated with macrophages. Diabetic TRL was associated with higher cell-associated uptake and degradation by macrophages than that in controls.

In summary, the data described above are consistent with the initial concept that despite a normal lipid profile, normal weight, and lack of insulin resistance, patients with type 1 diabetes may exhibit abnormalities in the metabolism of postprandial TRL. These could contribute to the development of atherosclerosis.

Impact of Improved Glycemic Control/Intraperitoneal Insulin

Several factors, such as improved glycemic control, changes in diet (high-carbohydrate vs. high monounsaturated fat) or statin therapy, could help improve these abnormalities. Improved glycemic control lowers triglycerides in the postprandial state. A paired study was conducted with young patients with type 1 diabetes before and following treatment with an implantable insulin pump, infusing insulin intraperitoneally. These patients were nonobese with normal lipid profiles. Postprandial lipids were monitored before and 6 months following insulin pump treatment. Subjects ingested a milkshake containing corn oil (60 g) plus vitamin A (60,000 U/M²), which was used as a probe to examine retinyl ester metabolism. A marker of intestinal particles, retinyl esters are not resecreted by the liver after they are taken up and cleared. Two subfractions corresponding to chylomicrons and VLDL remnants (Sf > 100) and small VLDL (Sf 20–100) were assessed for 10 h following the fatty meal. After treatment with intraperitoneal insulin delivery through the implanted insulin pump, retinyl esters were lowered in the same individuals (Fig. 2). There was a more pronounced effect in the Sf > 100 than in the Sf 20–100 subfraction. Both improved glycemic control and intraperitoneal infusion are factors which aid in reducing levels of postprandial TRL.

Impact of Dietary Changes

A different study has been conducted to determine the effect of a high-monounsaturated fat or a high-carbohydrate diet on lipoproteins. In type 2 diabetes, it has been suggested to use a high-monounsaturated fat instead of a high-carbohydrate diet in these individuals. The former results in less triglyceride production in the liver when compared with a high-carbohydrate diet. The randomized, crossover study placed patients with type 1 diabetes on each diet for 4 weeks. Fasting lipoproteins were measured at three separate intervals, during the last week of the study. Postprandial lipoproteins were also measured for 10 h after ingestion of a fatty meal on the last day of the study. Glycemic control parameters were also measured. Diets were matched for protein intake, saturated and polyunsaturated fat, omega-3 fatty acids, and fiber content. The only differences between the two diets were the percentages of monounsaturated fat (24 vs. 9) and carbohydrate (45 vs. 61); these were the parameters being tested. There were no changes in weight and glycemic control. Exercise and required medications were maintained throughout both dietary phases of the study.

When the fasting lipid profile was examined (total cholesterol, triglyceride, LDL, HDL, apo A1 or apo B), no difference was found between the high-monounsaturated fat diet and the high-carbohydrate diet. However, a consistent difference was observed in postprandial triglycerides between both groups (Fig. 3). In all TRL ranges tested (Sf > 400, Sf 100–400, and Sf 20–100) there was a significant increase in triglycerides in the high-monounsaturated fat diet when compared with the high-carbohydrate diet. The level of retinyl esters was significantly lower also in the high-carbohydrate diet in all subfractions except Sf 20–100 where no difference was observed.

Differences in triglycerides and retinyl esters were examined to determine whether they represented differences in particle numbers. Apo B was measured in the subfractions of Sf 100–400 and Sf 20–100. In both subfractions, apo B
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was lower during the high-carbohydrate diet than during the high-monounsaturated fat diet (Fig. 4). This indicates that particle number rather than size was affected by the diets. In conclusion, diet plays a role in normolipidemic, nonobese patients with type 1 diabetes. No differences were observed in weight, glycemic control, insulin dose, fasting lipids, or thrombosis tendency between the two diets. Postprandial lipoprotein triglycerides, retinyl esters, and apo B were lower during the high-carbohydrate diet than during the high-monounsaturated diet. A high-carbohydrate diet might, therefore, aid in decreasing the levels of postprandial TRL compared with a high-monounsaturated diet.

Impact of Pharmacotherapy

Drugs may also help decrease postprandial lipoproteins. Low-density lipoprotein receptors are important in the clearance of postprandial triglyceride-rich lipoproteins mediated through apo E binding. Statins could affect the clearance of postprandial lipoproteins. Studies reviewed by Havel have shown that approximately 50% of chylomicron clearance is mediated through the LDL receptor. A placebo-controlled, randomized trial of simvastatin was conducted in a small...
number of patients with type 1 diabetes with elevated LDL cholesterol. The objective was to lower the LDL to < 130 mg/dl. Simvastatin or placebo was titrated up to the level necessary to achieve this goal or until the number of pills equivalent to 40 mg of simvastatin was reached.

Lipids were monitored 4 weeks after the LDL level of 130 mg/dl was achieved or maximum number of pills was administered. At that time, patients ingested a fat load, and postprandial triglycerides, retinyl esters, and apo B were measured in two TRL subfractions (Sf > 400 and Sf 20–400). A decrease of postprandial lipoproteins was consistently found when triglyceride was measured in plasma and in the two subfractions (Fig. 5). This decrease was also observed when apo B and retinyl esters were measured in the TRL subfractions. In conclusion, statins decrease the postprandial TRL in patients with type 1 diabetes.

Abnormalities in Type 2 Diabetes

Are these abnormalities observed in type 1 diabetes also seen in type 2 diabetes? In type 2 diabetes, postprandial TRL levels are elevated. This occurs even when fasting triglyceride levels are normal. The composition of the particles in type 2 diabetes is also abnormal. Obesity and hypertriglyceridermia worsen these observed abnormalities. Questions have been raised as to the value of improved glyemic control by subcutaneous insulin administration in patients with type 2 diabetes. It might not be as beneficial as in type 1 diabetes. There is also disagreement as to the optimal diet for type 2 diabetes, as the data are conflicting.

Conclusion

Triglyceride-rich lipoproteins in the postprandial state are elevated and abnormal in diabetes. Studies of TRL have shown increased accumulation of cholesteryl esters in macrophages. Most of these abnormalities are common to both types of diabetes. In type 2 diabetes, however, there is a considerably more pronounced postprandial effect due to the presence of obesity and insulin resistance.

Discussion

Miller: What was the dose of simvastatin used in the study? High doses of simvastatin will lower triglycerides.

Georgopoulos: The goal was not to lower the triglyceride, but rather to titrate for the LDL to fall below 130 mg/dl. The dose varied from one individual to another, ranging from 10 to 40 mg.

Miller: There was an increased peak and a delayed clearance in the monounsaturated diet. Might this have potential clinical implications for diabetic patients?

Georgopoulos: Perhaps. When the monounsaturated fat study was conducted, the hypothesis was that this type of diet is better. The finding was just the opposite.

Glueck: Insulin-sensitizing drugs lower fasting triglyceride levels substantially and probably also lower postprandial triglycerides. These drugs will play a very major role in the issue of high triglycerides in diabetes. In a study about to be released in the United Kingdom, the group that had a significant reduction in macrovascular cardiac events and all-cause mortality was the metformin group. The insulin-sensitizing drugs have an antiatherogenic effect as well.

Bradley: In the patients who received interventions, such as drugs, to lower the particle number, is the inherent abnormality of the particle being changed or is there just a lowering of the particle numbers?

Georgopoulos: These studies have only looked at actual particle composition. More elaborate studies have not been done. It has been shown that, with improved glycemic control, the composition of the particles can be normalized. They are more phospholipid depleted than cholesterol enriched. When diet studies were conducted, no difference was found in the particle composition; only the number was affected.

Bradley: Were the glycation levels of subjects being treated in the early studies checked?

Georgopoulos: These were not checked. If their blood sugar was higher, it was assumed they would have a higher glycation.

Bradley: This higher glycation would alter their ability to interact, either with the LDL receptor or with receptors in the macrophage.
Georgopoulos: Some glycation studies of apo E were conducted. No difference was found in the binding. It is not known if glycation is the answer or if it is the way the particle surface is altered so that the binding site of apo E might be more or less available. The answer to this is not known.

References

Brachial Artery Ultrasound: A Noninvasive Tool in the Assessment of Triglyceride-Rich Lipoproteins

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Summary: In recent years, endothelial dysfunction has been identified as an early feature of atherosclerosis. Endothelial function can be measured noninvasively by using brachial artery ultrasound. A variety of factors associated with atherosclerosis also impair endothelial function. Some of these factors are lipoproteins such as various forms of low-density lipoproteins, postprandial chylomicron remnants, fasting triglyceride-rich particles, and free fatty acids. A high-fat diet also has an adverse effect on endothelial function. Several interventions can improve endothelial function and, at the same time, reduce cardiovascular events. Measuring endothelial function may eventually serve as a useful index to determine an individual’s risk for coronary artery disease.

Key words: brachial artery, coronary artery disease, endothelium, high-density lipoprotein, low-density lipoprotein, ultrasound, very low-density lipoprotein

Introduction

In recent years, endothelial function has been used as a surrogate or intermediate biological marker for coronary atherosclerosis and cardiovascular events. Traditionally, atherosclerosis has been viewed as a disease of low-density lipoprotein (LDL) deposition in subintimal spaces. Through a sequence of events (platelet adherence, foam cell formation, collagen formation, etc.), there is eventual plaque formation and rupture leading to thrombosis and, ultimately, to a cardiovascular event. This process falls short, however, in the understanding of why so many different risk factors interact in a multiplicative fashion to yield exactly the same disease. This is true for lipids, dietary habits, and a number of environmental issues.

In the last two decades, a second atherosclerosis pathway has evolved that focuses on endothelial dysfunction. The basic tenet of this hypothesis is that all coronary risk factors (e.g., LDL, hypertension, smoking, triglyceride-rich particles, homocysteine, Chlamydia, etc.) lead to endothelial dysfunction. Such dysfunction then leads to a vasculopathy associated with the attraction of platelets and monocytes. Growth factors are subsequently released leading to smooth muscle cell migration and proliferation. This proposed pathway helps explain why the diverse risk factors lead to coronary artery disease (CAD).

Endothelium Function

The endothelium is the body’s largest endocrine organ. It weighs approximately 1.5 kg and would cover two tennis courts with a surface area of 600 m². The endothelium carries out a number of functions. For example, it regulates vasoactivity, vascular cell growth, thrombotic and fibrinolytic properties, leukocyte and platelet adhesion to its surface, and vascular permeability. It also modulates lipid oxidation and mediates inflammatory and immune mechanisms. One of the most important endothelial functions is the maintenance of vascular tone and structure. Specifically, this is short-term vasodilation, predominately brought about by nitric oxide, prostacyclin, and hyperpolarizing factor. Vasoonstriction, on the other hand, is brought about by endothelin, thromboxane, angiotensin-II, oxygen free radicals, and other substances. Individuals constantly live in a balance between vasoconstriction and vasodilation, which are, respectively, proatherosclerotic and antiatherosclerotic. In addition to short-term factors, the endothelium elaborates a number of growth factors, thrombogenic factors, thrombolytic factors, inflammatory factors, immune factors, and so forth. In conclusion, the endothelium is much more than the simple, semipermeable membrane it was originally thought to be.

Measuring Endothelial Function

Clinically, it is relatively easy to measure endothelial function using the vasodilator capacity of the endothelium. Most investigations have used vasodilation as an index of endothelial function. Figure 1 is an example of abnormal endothelial
function based upon an arteriographic study using an arteriographic section of a coronary artery.1 This in vivo vascular ring experiment shows a vasoconstrictive response (A) to an endothelium-dependent agonist, acetylcholine, in a hypercholesterolemic patient before cholesterol lowering. After a cholesterol-lowering intervention, there is now a mild vasodilator response to the same agonist (B). This shows that endothelial function is not a consequence of the atherosclerosis but vice versa. Endothelial function can be normalized, even in the face of significant atherosclerosis, by changing the risk factor milieu. Coronary narrowing is not simply a disease of fixed stenoses. In response to exercise, cold exposure, or emotional stress, patients may vasoconstrict if they have endothelial dysfunction in addition to atherosclerosis.

Another way to investigate endothelial function is to use an intrinsic stimulus, such as increased shear stress or blood flow. The endothelium regulates the linear velocity of blood to approximately 15–20 cm/s under basal conditions throughout the body. It has a shear receptor on the surface that can sense an increase in flow. Acute vasodilation and chronic vessel growth occur in response to increased shear. This is why all vessels turn out to have exactly the right dimensions under healthy conditions. When dysfunction is present, however, the response to the same increase in coronary blood flow is an absence of vasodilation and, perhaps, vasoconstriction.2 One of the components of ischemia is a dysfunctional endothelium responding to increased flow inappropriately vasoconstricting the vessel.

Flow-mediated vasodilation is mostly nitric oxide dependent. Experiments conducted with nitric oxide synthase inhibitors show a reactive hyperemia with little vasodilation in response to this hyperemia (Fig. 2).3 Of importance is the fact that nitric oxide availability reveals something about the antiatherosclerotic state of the vessel. In an experimental animal, inhibiting nitric oxide synthase with a specific eNOS inhibitor will result in about twice as much atherosclerosis compared with control.1,5 When L-arginine, a precursor of nitric oxide, is given, there is approximately half as much atherosclerosis compared with control.

### Factors and Interventions Affecting Endothelial Function

A variety of factors are clearly associated with endothelial dysfunction (Table I).6 Every risk factor that is associated with atherosclerosis also impairs endothelial function. This observation is supporting evidence that the endothelium is also an important mediator of vascular and other diseases such as congestive heart failure and hypertension.

There are interventions which can improve endothelial function (Table I). Some of these interventions have been shown to reduce cardiovascular events, while some, such as

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<tr>
<th>Factors associated with endothelial dysfunction</th>
<th>Interventions improving endothelial function</th>
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<tr>
<td>Increased age</td>
<td>L-arginine</td>
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<td>Male sex</td>
<td>Estrogen</td>
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<td>Family history of CAD</td>
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*Abbreviations: CAD = coronary artery disease, HDL = high-density lipoprotein, ACE = angiotensin-converting enzyme.*

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**Fig. 1** Abnormal endothelial function in the coronary artery of a hypercholesterolemic patient. A vasoconstrictive response is shown to acetylcholine (A). After a cholesterol-lowering intervention, there is a mild vasodilator response to the same agonist (B). Reprinted from Ref. No. 1 with permission.

**Fig. 2** The effect of a nitric oxide synthase inhibitor (L-NMMA) on flow-mediated vasodilation. There is a reactive hyperemia but no vasodilation in response to this hyperemia after the administration of L-NMMA. RH = reactive hyperemia. Reprinted from Ref. No. 3 with permission.
lowering homocystine, have not yet been verified. Estrogen, on the other hand, has been clearly shown to improve endothelial function. Hormone replacement therapy, in the form of estrogen and progestin, however, may not improve endothelial function.

The Effect of Age on Endothelial Function

Endothelial cells live about 30 years. Rejuvenated endothelial cells do not elaborate as much nitric oxide as the original cells. As men and women approach the ages of 40 and 50, there is a progressive decline in endothelial function. At age 40, there is a slow decline in men in endothelial function. In women, function is preserved on the basis of estrogen availability. This results in a longer plateau but a steeper decline.

There are increasing data, however, to suggest that age is not an immutable risk factor. At age 50 or 60, however, individuals can no longer tolerate this risk-factor burden that they were once able to tolerate at age 10 or 20. For example, aging data from Australia and China show that Caucasians in Australia have a progressive decline in endothelial function (endothelial-dependent dilation). In the Chinese population studied, this progressive decline in endothelial function is not observed.

At our institution, work has also been conducted on master athletes. These are defined as individuals over the age of 65 who run marathons. One of these individuals studied was an 80-year-old male marathon runner who had CAD. He was placed on an aggressive exercise, lipid reduction, and antioxidant program. After this program, he showed normal endothelial function. This demonstrates that it is not the atherosclerosis that causes causing endothelial dysfunction, but rather the risk factors, which can be reversed even at this advanced age.

The Effect of Smoking

Cigarette smoking is a potent risk factor for atherosclerosis. Endothelial function data look much the same. Short-term changes in endothelial function have been measured immediately following cigarette smoking. Within 5 min of smoking one cigarette, there is no nitric oxide availability (Fig. 3). This is quite interesting, since cigarette smoke contains nitric oxide; something must break this nitric oxide down very quickly. This effect lasts upward of an hour to an hour and a half. It also occurs whether the person is a smoker or is simply breathing in secondhand smoke.

Lipoproteins and Endothelial Dysfunction

A number of lipoproteins produce endothelial dysfunction. Endothelial dysfunction can be used as biological markers to identify the lipoproteins which are predominantly atherogenic. Lipoproteins which have been identified as depressing endothelial function include LDL, oxidized LDL, lipoprotein(a), small dense LDL, postprandial chylomicron remnants, very low-density lipoprotein (VLDL) remnants, free fatty acids, and low levels of high-density lipoprotein (HDL).

The data for total and LDL cholesterol are scattered but statistically significant. Correlations tend to be 0.3 to 0.4 in most studies, suggesting individual biological variability. These data also suggest a biological ideal LDL cholesterol to be in the range of 50–75 mg/dl.

There is an inverse association between HDL and endothelial function: the higher the HDL cholesterol, the better the endothelial function. There is also an association between LDL and endothelial function. We conducted a study on medical school faculty members who were about 50 years of age. An LDL of 125 mg/dl was associated with an impairment in endothelial function, which was a value roughly equivalent to that of a cigarette smoker. When the LDL was lowered to slightly below 100 mg/dl, endothelial function improved. When it was lowered to the range of 75–80 mg/dl, it improved even further.

The Effect of Diet on Endothelial Function

The contribution of diet to endothelial function has also been examined. Around the world, there is a very different incidence of heart disease depending on factors much more complex than total or LDL cholesterol. For example, a Japanese person with a cholesterol of 200 mg/dl has far less disease than a Scandinavian person also with a cholesterol of 200 mg/dl. Several questions have been asked in this regard. Is there an immediate effect of a high-fat diet on the endothelium? Is there a direct association between diet and endothelial function?

Endothelial function has been studied in relationship to diet. In one study, participants received one of two meals. The first was a high-fat, fast-food breakfast consisting of 900 calories and 50 g of fat. The other meal consisted of a cereal-type breakfast containing 900 calories and 0 g of fat. Endothelial function was examined hourly for 6 h after ingestion of the breakfast. Within 2 h, there was a significant decrease in nitric oxide availability. Reprinted from Ref. No. 9 with permission.
Oxidative stress of these fatty meals. An exception to fats that confer any impairment on endothelial function; it also found to have the same impairment to endothelial function as the rest of these high-fat meals. This impairment, however, was also totally eliminated when vitamins C and E were given. As with antioxidant vitamin supplementation, olive oil, and other risk factors that injure the endothelium are oxidatively stressed modified. The study involved the same 20 subjects who ate in fast-food restaurants and the 20 subjects who ate a cereal-based breakfast. The subjects eating a fast-food breakfast were pretreated with 800 U of vitamin E and 1 g of vitamin C. A significant reduction in nitric oxide availability impairment was noted in the group receiving the supplements (Fig. 4).

**The Impact of Specific Foods**

The direct impact of specific meals on triglycerides and endothelial function was also studied. Three-hour rises in triglycerides and 3-h declines in flow-mediated vasodilation have been observed with a traditional meal of a hamburger and fries, as well as with cheesecake (Fig. 5). Olive oil was also found to have the same impairment to endothelial function as the rest of these high-fat meals. This impairment, however, was also totally eliminated when vitamins C and E were given. As with antioxidant vitamin supplementation, olive oil, eaten with vinegar on a salad, did not impair endothelial function. Some societies that use the Mediterranean diet may have learned to provide the natural antioxidants which buffer the oxidative stress of these fatty meals. An exception to fats that impair endothelial function is fish oil. Salmon (50g) does not confer any impairment on endothelial function; it also results in half the rise in triglycerides. Other studies show that Omega-3 fatty acids improve endothelial function.

There has been additional study of the effect of two high-fat meals on coagulation factor VIIa. Both olive oil and butter show, in essence, the same increase in factor VIIa. The coagulant effects of fat are the same; they only differ in the way they elevate LDL cholesterol.

Other studies have looked at links between various foods and CAD. For example, an association has been demonstrated in different countries between animal fat and the incidence of CAD. This study showed a rise in the incidence of CAD with an increased intake of animal fat. The clinical community has used these observations to convey the message about stopping excessive fat intake. In the same study, fruit showed an inverse relationship between fruit intake and CAD. For example, an association has been demonstrated in different countries between animal fat and the incidence of CAD. This study showed a rise in the incidence of CAD with an increased intake of animal fat. The clinical community has used these observations to convey the message about stopping excessive fat intake. In the same study, fruit showed an inverse relationship between fruit intake and CAD compared with the adverse effect of fat. Both of these phenomena are based on the balance between the direct impairment of fat on the endothelium and the protection given by concomitant antioxidant-rich foods.

**The Effect of Transient Triglycerides on Vascular Reactivity**

Another study has looked at the effect of transient triglycerides and vascular reactivity. Plasma concentrations of triglyceride, cholesterol, and free fatty acids were measured before and during an infusion of 0.15 g/kg body weight $\cdot$ h $^{-1}$ of intralipid. A profound decrease was observed in flow-dependent vasodilation, as was a significant decrease in flow-independent vasodilation. Such data support the cautious use of parenteral nutrition with lipids in acute settings where endothelial or vascular dysfunction may be an issue (e.g., shock lung).

In addition to postprandial chylomicron remnants being injurious to the endothelium, VLDL remnants have also been found to cause injury. Data obtained from research in Japan demonstrate that non-apo A, non-apo B VLDL remnants impair endothelial function. This remnant fraction has the strongest association with endothelial dysfunction of any par-
article or of any other risk factor described to date, including LDL, age, and smoking.

**Pathophysiologic Mechanisms of Nitric Oxide Availability**

Endothelial dysfunction may be associated with increased nitric oxide destruction or impaired synthesis as occurs with hypercholesterolemia or hypertriglyceridemia. Experimental atherosclerosis is associated with increased supersoxide production and increased nitric oxide production. Superoxide deactivates nitric oxide more rapidly than does superoxide dismutase. In human advanced atherosclerosis, the prevailing data at the moment, however, show that there is decreased, not increased, nitric oxide production. This may be an advanced stage of atherosclerosis. Oxidized LDL has been shown to impair endothelium nitric oxide synthase directly, and L-arginine may have decreased microdomain availability in hypercholesterolemia. Inhibitors of nitric oxide synthesis, such as ADMA and caveolin, are also increased in hypercholesterolemia.

**Conclusion**

Westernized individuals live in a world of coronary risk factors. Oxidative stress, among others, contributes to endothelial dysfunction. Since endothelial function is relatively easy to measure, it is hoped that it will serve as a useful intermediate index. Such endothelial dysfunction may eventually lead to atherosclerosis and subsequent cardiovascular events.

**Discussion**

**Rackley:** Endothelial interaction offers the opportunity to bring the metabolic aspects of cardiovascular disease into the hemodynamic aspect. One major challenge is how to explain some of the acute events based on chronic metabolic and physiologic abnormalities. About a year ago, the Indianapolis group showed that the infusion of free fatty acids, first in diabetics and then in normal, non-diabetic individuals, induces the same vasoconstriction rate as observed with various lipid components. The source of free fatty acids is triglyceride. We monitored this by giving glucose and potassium in solution. As a metabolic measure, we discovered that solution suppressed the free fatty acids so low that it changed the respiratory quotient at the myocardium. It actually could be shown to increase coronary blood flow. If one wants to elevate free fatty acids, one has to elevate the triglycerides or provide an appropriate intermediate biological marker. What the redox state really will do since superoxide dismutase and other intrinsic antioxidant levels will change. The experimental world is rather mixed on documentation of whether you can chronically improve endothelial function by giving antioxidant vitamins; some studies say yes while others say no. Much of it is stimulus and model dependent. Recent work has shown that a combination of lipid-lowering medication and chronic administration of vitamin E shows a benefit.

**Criqui:** What experimental work has been conducted on the consumption of alcohol or red wine solids and their effect on endothelial function?

**Vogel:** The best data show that a modest intake of alcohol (defined as one drink/day) is probably optimal. In Europe, the data suggest that red wine may have an event advantage over other alcohol. In the United States, the data show no predominance as to alcohol type. The data on endothelial function are far less clear with regard to alcohol. Alcohol has a lot of effects, including changing lipids and coagulation. The direct vasoactive properties of alcohol confound these measurements when it comes to studying endothelial function. Preliminary data suggest that red wine extracts are favorable to the endothelium, but alcohol is not.

**Ferdinand:** Is the use of brachial ultrasound a clinical tool at this point? If we have a transducer, should we start doing brachial studies to see endothelial function in our patients?

**Vogel:** Absolutely not. This is a research tool. Reproducibility depends on many factors in the laboratory and a lot of dedication. The question that really is behind your question is: Is endothelial function an independent predictor of events or atherosclerosis? Early data suggest that endothelial dysfunction is a predictor of future cardiovascular events. The NIH is also looking at different predictors of events. We are also conducting two trials. One is a hormone replacement study and the other is a statin trial. The hormone trial is comparing endothelial function through the use of quantitative arteriography. The statin trial is using intravascular ultrasound. In the future, this will help determine whether this is an appropriate intermediate biological marker.

**Conti:** Does activity in the brachial artery reflect what is going on in the coronary circulation? As far as I know, we don’t have an answer yet. We have given a lot of acetycholine into people’s coronary arteries. Constriction has been seen in one part and dilation in another. While we know what is happening in the coronary circulation with acetycholine, we have not looked at what is happening in the brachial artery.
Vogel: There has been reported an approximate 0.4 correlation that is statistically significant concerning acetylcholine in the coronary circulation versus flow-dependent vasodilation in the brachial artery. The correlation between flow-dependent in the coronary circulation and flow-dependent in the arm is better, in the range of 0.8. This would suggest a reasonable association. At a recent NIH conference, there is some consensus that flow-dependent would be the endothelium index of choice. Clearly, it is wholly endothelium-dependent. It is not yet known, however, whether or not improving endothelial function will lessen cardiovascular events.

References

Nonpharmacologic Treatment of Hypertriglyceridemia: Focus on Fish Oils

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Summary: Early studies in Greenland Eskimos stimulated interest in evaluating the effect of Omega-3 fatty acids on coronary artery disease. Subsequent studies showed a significant decrease in triglyceride levels in patients receiving high doses of fish oil containing DHA and EPA. Slight increases in LDL were also observed in patients receiving fish oil supplements. These studies have also shown a dose–response effect which persists as long as supplementation continues. Later trials, specifically the Diet and Reinfarction Trial and the Indian Experiment of Infarct Survival, have demonstrated a reduction in cardiac death rates and in the incidence of cardiac symptoms in patients receiving fish oil.

Key words: coronary artery disease, DHA, EPA, fish oil, high-density lipoprotein, low-density lipoprotein, Omega-3 fatty acids, triglycerides

Introduction

Interest in Omega-3 fatty acids began in the late 1970s with studies in Greenland Eskimos.1 These studies showed that Eskimos living on diets high in fat, cholesterol, and Omega-3 fatty acids had very little coronary artery disease (CAD). Subsequently, several clinical trials were launched to study this phenomenon further.

One of these trials was published by Harris et al. in 1983.2 At the time, there was little information about the link between Omega-3 fatty acids and triglycerides. This trial focused chiefly on cholesterol. Normal volunteers were asked to ingest 100 g of salmon oil each day for a period of 1 month. This quantity of salmon oil equaled approximately 25 g of Omega-3 fatty acids each day. In addition to eating two meals containing salmon steaks, the volunteers also drank salmon oil. Participants began with a 1-month control period, followed by 1 month on salmon oil and then 1 month on vegetable oil. There was a drop in triglycerides from 78 to 50 mg/dl, (−30%), during the period of salmon oil intake. Total cholesterol and low-density lipoprotein (LDL) also went down during the salmon and vegetable oil diet periods.

The mistaken conclusion was made that salmon oil lowered LDL; in fact, it was the reduction in saturated fat that lowered LDL. As a result, fish oils were mistakenly marketed in the mid-1980s as cholesterol-lowering agents.

A second trial was conducted in patients with type 5 hyperlipidemia.3 At baseline, the triglyceride level for these patients was 1,350 mg/dl. They were given 30% of their calories as fat in the form of fish oil containing approximately 20 g of Omega-3 fatty acids per day. Their total triglyceride levels dropped to <300 mg/dl. This was a remarkable indication that Omega-3 fatty acids exert a very potent triglyceride-lowering effect.

The Effect on Plasma Lipids and Lipoproteins

In 1996, a review of the literature was conducted to summarize the effects of Omega-3 fatty acids on plasma lipids and lipoproteins.4 Placebo-controlled trials, either crossover or parallel, published in English, were included. Only those studies that gave <7 g of Omega-3 fatty acids per day were selected. The selected studies also had to be at least 2 weeks in duration and had to be supplementation rather than food substitution studies. All patient types were acceptable. A total of 72 data sets from 60 separate publications met the inclusion criteria. At least 70 before-and-after trials, which were not placebo-controlled, had to be excluded from the analysis. The results were weighted to give the larger trials a greater impact on the aggregate outcomes. As such, the study resembled a meta-analysis.

The analysis examined the effect of Omega-3 fatty acid supplementation on triglycerides during placebo and then during fish oil ingestion. There was an approximate 25% drop in mean triglycerides with baseline readings <176 mg/dl across all studies. In those individuals with baseline triglyceride
levels > 176 mg/dl, there was a 28% average decrease. In the lower triglyceride group, LDL levels increased approximately 4% compared with placebo. In the higher triglyceride group, the percent increase was 6% overall. Although high-density lipoprotein (HDL) rose slightly with Omega-3 fatty acid feeding, no significant increase in HDL was documented in this large database.

**Dose Response, Duration of Effect, and Low-Density Lipoprotein Changes**

Does ingesting more Omega-3 fatty acids result in a larger drop in triglycerides? The fractional percent reduction in triglycerides versus placebo from each of these trials was plotted. Most of the trials studied gave subjects 3 to 4 g of Omega-3 fatty acids per day. A clear and significant relationship was observed; the higher the dose, the greater the drop in triglycerides. This was more evident in the group of studies that looked at the patients with high triglycerides. In conclusion, a dose–response relationship was observed in individual studies and across the whole spectrum of trials.

Does the reduction in triglycerides persist as long as an individual keeps taking Omega-3 fatty acids? Most of the studies in this analysis were short term; however, one lasted for 120 weeks. In that trial, there was still a 25% reduction in triglycerides versus placebo. The effect of fish oil supplementation, therefore, appears to persist for as long as it is taken.

The fractional change in LDL cholesterol was also examined in these trials. A 30% increase in LDL was observed in some studies. However, in the 120-week trial, LDL levels were not increased at all after 2 years despite lowered triglycerides being maintained. In the larger trials, which enrolled 400 to 500 subjects, an increase in LDL did not persist. While the triglyceride-lowering effect persisted, the LDL-raising effect may diminish over time.

**Studies Using Highly Concentrated Fish Oil**

Omacor™ is a concentrated Omega-3 fatty acid product produced by Pronova in Oslo, Norway. Compared with the typical fish oil product containing 30% Omega-3 fatty acids, Omacor contains 85% DHA and EPA. The fatty acids are provided as ethyl esters, allowing higher concentrations in each capsule. The long-term effects of Omacor have been studied in patients with severe hypertriglyceridemia, defined as 500–2,000 mg/dl. In a double-blind, placebo-controlled trial lasting 4 months, the placebo group received corn oil, while the Omacor group received four capsules/day of Omacor for 4 months. There was a significant lowering of triglyceride levels (25%), while the placebo group showed no change. The rise in HDL (14%) was similar as that to the 4 g/day dose.

In conclusion, both two and four capsules a day of Omacor were well tolerated for 6 to 10 months. The only minor side effect was eructation, which may be controlled by taking the preparation at bedtime. In summary, with 2 g/day, triglycerides were lowered by 25%; HDL was raised by 14%; there was no change in LDL. With 4 g/day, triglycerides were lowered by 43%, and HDL was raised by 18%. There was a 32% increase in LDL; mean values, however, remained in a low-risk range (2.8 mmol/l). Omacor appears to be both a safe and effective treatment for severe hypertriglyceridemia.

The anti-atherogenic effects of Omega-3 fatty acids have been demonstrated in animal models of CAD. Weiner et al. conducted a study using pigs who were given approximately two tablespoons/day of cod liver oil with an atherogenic diet. After 8 months, the animals were sacrificed and coronary lesions examined. There was a significant reduction in the amount of atherosclerosis in the animals given fish oil compared with the control group.
The Diet and Reinfarction Trial (DART)

Two published studies present compelling evidence for the beneficial effects of Omega-3 fatty acid intake in the secondary prevention of CAD. The first study, called the Diet and Reinfarction Trial (DART), was published nearly 10 years ago.9 DART looked at the effects of changes in fat, fish, and fiber intakes on death and myocardial reinfarction. This study was a randomized, controlled, and prospective trial with a factorial design. A total of 2,033 men aged < 70 years who survived a myocardial infarction were allocated to receive advice on their dietary habits. One group was advised to decrease their fat intake to < 30% energy. The second group was advised to increase their intake of fatty fish to 200–400 g/week. Some examples of oily fish include herring, mackerel, salmon, and sardines. This intake of fish amounted to approximately 600 mg/day of Omega-3 fatty acids. Finally, the third group was asked to increase their intake of dietary fiber to 18 g/day. All subjects were then followed for total mortality for a 2-year period.

At approximately 100 days (3 months), there was a divergence in death rates between those men who had been advised to eat oily fish and those who did not (Fig. 3). Those in the group advised to take fish, but who did not want to eat fish were given the option of taking fish oil capsules. When a post hoc subgroup analysis of patients taking capsules was conducted, a 62% reduction in ischemic heart disease deaths and a 57% reduction in total mortality was observed (Fig. 4).10

The Indian Experiment of Infarct Survival

The second study, called the Indian Experiment of Infarct Survival, was published in 1997.11 This was a randomized, double-blind, prospective, placebo-controlled trial of fish oil and mustard oil in patients admitted with suspected myocardial infarction. Mustard oil contains the plant-based Omega-3 fatty acid alpha-linolenic acid. Immediately after admission, patients were randomized into one of three groups. The fish oil group (n = 122) received 2 g/day of the Omega-3 fatty acids EPA and DHA. The mustard oil group (n = 120) ingested 20 g/day in order to receive a 3 gram dose of alpha-linolenic acid. The remaining placebo group (n = 118) received a 100 mg tablet of aluminium hydroxide.

The incidence of heart failure (class 3 and 4) developing over the next year was reduced significantly by the fish oil (Fig. 5). Mustard oil also significantly reduced, as did fish oil, the incidence of arrhythmias and angina. The incidence of cardiac death, nonfatal myocardial infarction, and total events was also significantly reduced with both fish oil and mustard oil (Fig. 6).

Conclusion

Omega-3 fatty acids have the potential to impact many aspects of intracellular metabolism. By incorporating into the cell membranes, these fatty acids can affect the way membrane-bound enzymes work. Omega-3 fatty acids may, therefore, affect a myriad of biochemical pathways. Their beneficial effects in CAD may be due to changes in lipoprotein metabolism, platelet function, blood vessel biology, myocardial function or other mechanisms to be identified.
increase or decreased production of cyclooxygenase products, such as prostaglandins, etc. Two elements determine how much prostaglandin is produced. One is substrate availability, while the other is the availability of the enzyme itself. Whenever there is a significant decrease in substrate, prostaglandin production goes down. In these 3-week experiments, fish oil caused a marked reduction in prostaglandin generation which was verified with HPLC analysis of the tissues.

The initial event in the vascular system that starts atherogenesis is not an increase in cholesterol or LDL. Rather, it is an inflammatory process that starts with macrophages and mononuclear cells infiltrating the area. Prostaglandins are very important in attracting these inflammatory cells into the vascular tissues. The fish oil effect is more likely to be due to the inhibition of inflammation than anything else.

Drevon: What are the mechanisms behind the ability of Omega-3 fatty acids to decrease triglyceride levels?

Harris: The rate of secretion of VLDL from the liver is decreased. Fish oil may also stimulate lipolysis in the bloodstream.

References

Pharmacologic Management of Triglycerides

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Summary: Currently available cholesterol-lowering pharmacologic agents have been studied for their effect on reducing triglyceride levels. The fibrates increase lipoprotein lipase activity, thereby decreasing the size of triglyceride-rich particles. High doses of niacin can produce decreases in very low-density lipoprotein (VLDL) levels, triglyceride-rich particles, and low-density lipoprotein (LDL) by inhibiting hepatic lipoprotein synthesis. By increasing LDL-receptor activity, the statins increase the removal rate of triglyceride-rich particles. Each class of agents produces various degrees of triglyceride lowering, depending on the existing baseline level and other factors. Patients with elevated LDL who are also hypertriglyceridemic should receive statins as first-line therapy. Niacin may be used as an alternative first-line agent in patients with low LDL elevations. Combination therapy using other agents may be indicated depending on the patient’s levels of triglycerides and LDL.

Key words: atorvastatin, coronary artery disease, fenofibrate, fluvastatin, gemfibrozil, high-density lipoprotein, hypertriglyceridemia, low-density lipoprotein, niacin, simvastatin, triglycerides, very low-density lipoprotein

Effect of Pharmacotherapy on Triglyceride-Rich Particles

In terms of pharmacotherapy, the main interest is the effect of these drugs, specifically fibrates, on triglyceride-rich particles. The primary mechanism of fibrate action is to increase lipoprotein lipase activity which, in turn, decreases the size of triglyceride-rich particles. This allows them to be taken up by the receptors and catabolized. Some of the fibrates may also have an effect on inhibiting lipoprotein synthesis or increasing LDL receptor activity. When fibrates are used, the concentration of triglyceride-rich particles is decreased and their composition is changed.

Niacin works primarily by inhibiting hepatic lipoprotein synthesis. If a high enough dose of niacin is given, there will be subsequent decreases in very low-density lipoprotein (VLDL) levels, triglyceride-rich particles, and LDL. In addition, there will also be some increase in high-density lipoprotein (HDL) levels.
The statins have been thought of primarily as inhibiting cholesterol synthesis and increasing LDL-receptor activity. If LDL-receptor activity is increased, then the removal rate of these triglyceride-rich particles is also increased. There is some evidence that when higher doses of statins are reached, some inhibition of VLDL synthesis takes place. In conclusion, although the various classes of drugs have different effects, they are also doing many of the same things. In addition to lowering triglycerides, the important issue is what these different agents are doing to VLDL remnants.

Available Agents in the U.S.

Several triglyceride-lowering drugs are available, both in the United States and abroad. In the U.S., the available agents are gemfibrozil, the fibrates, and fenofibrate. Three different preparations of niacin are available: the crystalline or immediate release, the slower sustained release, and a newer preparation called Niaspan®. There are also several HMG CoA reductase inhibitors. Although the bile acid sequestrants increase triglyceride levels, they may not increase the risk of CAD. In individuals with significant hypertriglyceridemia or type 3 hyperlipoproteinemia, these agents can cause very dramatic increases in triglycerides. As such, they can actually precipitate pancreatitis. Research has found that bile acid sequestrants may actually be good in individuals who have small dense LDL.

The Effect of Fibrates

Individuals with normal triglycerides who are treated with fibrates experience a lowering of LDL and triglycerides. The higher the triglyceride level, the less likely a lowering of LDL will be achieved. As the triglyceride level gets progressively higher, actual increases in LDL are seen. When an individual with a high triglyceride level of 430 mg/dl, for example, is treated with fenofibrate, there is a slight, insignificant increase in LDL cholesterol along with a decrease in the other triglyceride-rich particles. Treating even higher triglyceride levels, for example 700 mg/dl, results in dramatic increases in LDL cholesterol; this is due to the change in the LDL composition. There is no increase in the concentration of apo B and in the number of LDL particles. One of the fibrate mechanisms for decreasing risk is the change in the composition of VLDL: it is switched from small, dense LDL to the larger, buoyant LDL. This may be one of the reasons why there is a decreased risk of CAD for these patients.

The Role of Niacin Preparations

When it comes to niacin and its LDL-lowering properties, the sustained-release preparations are more effective in lowering LDL than the crystalline formulations. In contrast, when changes in HDL are examined, crystalline niacin is much more effective in increasing HDL than the sustained-release form. The sustained-release preparation never increases HDL by more than about 10% in any studies. Crystalline niacin is also more effective in lowering triglycerides. The characteristics of the newest niacin preparation, Niaspan®, are similar to those of the sustained-release preparation as this preparation is very effective in increasing HDL. Niaspan is given once a day at bedtime. It is better tolerated and has less hepatotoxicity than the sustained-release preparations.

Current Efficacy of the Statins

The impact of the statins on lipids, lipoproteins, and triglyceride-rich particles has been underestimated in the past. A good example is their triglyceride-lowering effect with a baseline triglyceride level of <150 mg/dl. It does not make any difference what statin is used or what dose is given; a consistent effect on triglycerides will be not achieved. However, when the baseline triglyceride level is in the range of 200–250 mg/dl, the amount of triglyceride-lowering will equal the amount of LDL lowering. This means that if the LDL is lowered by 30%, then the amount of triglyceride lowering will also be approximately 30%. Since lowering the LDL means cholesterol and lipoprotein synthesis are being inhibited, then all of the statins produce about the same degree of triglyceride lowering.

Newer Uses for the Statins

As the statins continue to be used, the ways in which they are used are also changing. In the past, statins were not used in individuals with triglyceride levels >400 mg/dl. This position has changed, however, because of some of the initial results obtained with atorvastatin. One study looked at individuals with triglyceride levels in the range of 200 to 800 mg/dl and TC >200 mg/dl. The mean triglyceride levels of participants in this study were in the high 300s. A dose of 10 mg of atorvastatin was used.

When the amount of LDL lowering with this dose was examined, it was essentially the same as the amount of triglyceride lowering. With progressively higher triglyceride levels, however, the amount of LDL lowering is less than the 40% reduction normally seen in individuals without hypertriglyceridemia. In conclusion, it is not appropriate to start with a statin in patients with triglycerides of 1,000–2,000 mg/dl.

Several studies have also suggested that statins have little or no effect on changing the LDL composition. There is no decrease in the risk associated with a reduction in triglycerides or any of the triglyceride-rich particles since the LDL composition is not changed. However, the statins do decrease the concentration of many of the triglyceride-rich particles, including remnants. While they do not change the composition of LDL, the statins do change the absolute concentration
of small, dense LDL. Therefore, the benefits of statins extend beyond lowering LDL.

Statins have also been evaluated in patients with type 3 hyperlipidemia. These individuals have primarily beta-VLDL. A comparison was conducted of two different doses of atorvastatin, gemfibrozil, and simvastatin. Very dramatic reductions in VLDL and intermediate-density lipoproteins (IDL) were achieved; these were greater than what was observed with gemfibrozil in the study. The amount of triglyceride lowering was essentially comparable. When apo B levels were measured, however, the decrease in triglyceride-rich particles was also associated with a very significant decrease in the apo B concentration. This suggests that the total number of particles is decreased.

Another statin, fluvastatin, is not potent in lowering LDL. In the original studies of fluvastatin, there was an expected 20% lowering of triglycerides with 20 mg of the drug. When the decreases in VLDL and IDL were examined, there was also a very significant decrease in the concentration of triglyceride-rich particles. The total number of apo B particle concentrations also decreased, demonstrating once again that the statins reduce the concentration of various triglyceride-rich particles.

What implications do the data on the statins have on the use of other drugs, such as the fibrates? In the Helsinki Heart Study, there was a dramatic reduction (34%) in the number of cardiac events. This was partially attributed to decreases in LDL as well as to increases in HDL. Generally, the fibrates also have an effect on platelet function. In addition, some of the fibrates decrease fibrinogen levels, which may be due to the lipid effects of the fibrates.

The Impact of Statins on Cardiovascular Endpoints

The Bezafibrate Coronary Atherosclerosis Intervention Trial (BECAIT) shows that the moderate fibrate formulations have good efficacy. Subjects in the bezafibrate group had a good effect in regard to changes in minimum lumen diameter. There was also a 60% reduction in coronary event rates. However, these numbers are not large enough to make decisions for treating the U.S. population as a whole.

As a result of the data from the BECAIT Trial, there was much anticipation for the Bezafibrate Infarct Prevention (BIP) Trial that enrolled approximately 3,000 patients. The majority of participants had triglyceride levels of < 350 mg/dl. Participants receiving bezafibrate had no significant reduction in primary endpoints, defined as fatal myocardial infarction (MI), nonfatal MI, and sudden death. It also appeared that increases in HDL predicted benefit. Another important trial, the Veterans Affairs High-Density Lipoprotein Intervention Trial (HIT), will be published in 1999. This trial has enrolled 2,500 men with CAD who received gemfibrozil or placebo. Entry criteria included an HDL level of < 40 mg/dl, LDL of < 140 mg/dl, and triglycerides of < 300 mg/dl. Data from this trial, combined with data from the Bezafibrate Trial, will help put into perspective just how rigorously fibrates should be used in these patient populations.

Conclusion

Lowering LDL is the first choice for reducing the risk in hypertriglyceridemic patients. This is especially appropriate for patients with triglyceride levels up to 350 mg/dl. It is questionable whether there will be additional benefit if triglyceride is altered. There is also some suggestive evidence that the risk can be reduced in these patients by lowering triglycerides and by altering the LDL composition. When this is accomplished, the concentration of many triglyceride-rich particles also decreases. Risk can also be lowered by decreasing lipoprotein synthesis, since this decreases all of the triglyceride-rich particles.

In terms of an overall approach to treatment, patients with an elevated LDL and who are hypertriglyceridemic should be treated with statins: these are the initial drugs of choice. Such patients should also be taking aspirin as well as their beta blockers. In addition, many of them should be placed on an angiotensin-converting enzyme (ACE) inhibitor, although cost at this point may become a problem. Pancreatitis should also be taken into account in patients with triglyceride levels >750 mg/dl. These individuals should not be started on statins in order to avoid pancreatitis.

Some patients may have a small elevation of their LDL; in these cases, niacin or statin could be used. Theoretically, niacin is an alternative first-line therapy. Gemfibrozil can be added later in order to treat the high triglycerides. Other patients may have hypertriglyceridemia but do not have LDL elevations. Their LDL may be in the range of 60–70 mg/dl. These individuals do not need any additional LDL lowering. In these patients, the focus should be on agents that control triglycerides. Once the triglyceride level is lowered, then controlling the LDL can be considered.

In summary, the future management of patients with CAD will depend to a large extent on regulating various cholesterol and lipoprotein levels. Lowering LDL levels should receive the highest priority when treating these patients. Individuals with low HDL levels can eliminate their risk simply by lowering their LDL. Once this is achieved, the second most important intervention to consider is the lowering of triglycerides.

Discussion

Abrams: Does fenofibrate offer any advantage over gemfibrozil? With respect to its triglyceride effect, is atorvastatin different? In other words, if a patient took simvastatin for comparable LDL lowering, is the triglyceride effect less than or equal to atorvastatin?

Hunninghake: Some studies suggest that fenofibrate is more effective than gemfibrozil in lowering LDL, especially in patients without hypertriglyceridemia. In patients with severe hypertriglyceridemia, there is not as much of a decrease in LDL when they take fenofibrate. The statins produce the same degree of triglyceride lowering as they do LDL lowering when the same dose is given. Comparisons of atorvastatin with simvastatin have shown the same degree of LDL lowering and triglyceride lowering.
Roche: Are there any studies investigating the effect of fibrates on postprandial hyperlipidemia? If this is reduced by the fibrates, is this due to reduced VLDLs and, therefore, less competition for clearance or increased chylomicron removal?

Hunninghake: Some small studies show a reduction in the incidence of postprandial hyperlipidemia when statins are given. Generally, if one lowers fasting triglycerides, there will also be a decrease in the amount of postprandial hyperlipidemia observed.

Criqui: In one of our population studies which enrolled patients with type 1 and type 5 hyperlipidemia, we found no increase in coronary risk. Yet, in the PROCAM Study, the risk decreased as the triglyceride level rose above 800 mg/dl. In the Copenhagen Male Study, there was a decreasing risk above 225 mg/dl. About 40% of these patients with high triglyceride levels have diabetes. What is the contribution of triglycerides to the risk seen in these individuals with very high triglycerides (>600 mg/dl)? Is it possible that lowering them to a range of 300–400 mg/dl might actually increase their cardiovascular risk?

Hunninghake: Since so few individuals live in this type of situation, there is very limited information available. Less than 5% of patients have triglycerides >400 mg/dl. The majority who do are probably either diabetics or have some other secondary cause. Some of these individuals may be type 3 patients or those with familial combined hyperlipidemia. These patients have small, dense LDLs and very high apo B levels. If these patients could be pulled out of the population being studied, then almost all of the high-risk individuals would be taken out. Some individuals with high triglycerides also have a number of coagulation problems. As triglycerides are lowered in these individuals, their thrombotic tendencies may also decrease. It is unlikely that lowering triglycerides in someone will place them into a higher risk category. Some future data may suggest, however, that the use of cholesterol-lowering drugs may increase the risk of some arrhythmias.

Limacker: What are the reliability and accuracy of triglyceride measurements in the clinical laboratories? It took years to get to a reasonable range for cholesterol. Triglycerides continued to remain a problem.

Hunninghake: A lot of clinical laboratories are not as precise about measuring triglycerides. To a certain extent, they may not be able to measure them within 5 or 10 points. There is not much concern about the exact variability. The same may be true for measuring LDL. Some people are worried about LDL changes from visit to visit in the range of 4–5 points. Laboratories are not that good at detecting the difference.

Bachorik: In serial measurements from the same patient, there will be some variation with a coefficient of variation, on average, of about 7 or 8% for LDL. This gives an overall range of about 30% for 90% limits. This is just simple, normal biological fluctuation due to day-to-day activities. Five-point changes in LDL mean nothing. At one point, direct measurements for LDL seemed to be desirable. Results of a 3-month study of one of the direct LDL methods have changed this view. A direct LDL measurement can be obtained within a 10% range. Most clinicians want to get more than just the LDL, such as triglyceride and HDL levels. With a conventional Fredwald test, most of this information can be obtained in the majority of patients.

Glueck: In our center, we see two to three patients a week with severe hypertriglyceridemia, which we define as >750 mg/dl. One or two rich meals, and a few drinks, can put these individuals into a postprandial triglyceride level in the 1,500 to 2,000 mg/dl range. A surprising percent develop pancreatitis, with a third dying from their first episode. In these individuals, it is important to eliminate the factors that have created these high levels. These include estrogen, alcohol, poorly controlled diabetes, and corticosteroids. Once these are controlled, the triglyceride levels will decline. In addition, the risk of atherothrombotic stroke is much higher in these patients. This is due to a whole series of thrombophilic vascular events. These patients need to be treated aggressively.

Miller: Combined hyperlipidemia is a major problem. For example, a patient with CAD is started on a statin. Even after therapy, the triglycerides remain high (>200 mg/dl). Should the statin dose be increased or should the statin dose be decreased and a second agent added?

Hunninghake: The decision depends on what the absolute level of LDL is. If the LDL is still elevated, the statin dose should not be decreased in lieu of adding either niacin or gemfibrozil. The risk of myopathy is very small. In a high-risk patient where the triglycerides remain high, another agent should probably be added. However, it is unknown if this additional agent reduces the cardiovascular risk or by how much. Adding more agents also increases the cost of treatment.

Conti: We don’t see patients with just hyperlipidemia. They also have CAD or some other related disease. Some clinicians have become quite aggressive with atorvastatin. It is common to see patients receiving 80 mg/day of atorvastatin. What is the risk of this or of using high-dose niacin when liver function studies cannot be done?

Hunninghake: There are large numbers of patients taking 80 mg of atorvastatin, including many who are participating in controlled clinical trials. Many of these patients have LDL between 20 and 50 mg/dl. Going this low may not necessarily increase the benefit. In our post-CABG Study, progression appeared to be linear related to our in-trial LDLs. The group with LDLs between 60 and 80 mg/dl had the best benefit in that study. It is not known how much lower a patient can go and still continue to benefit. There are not enough data to support trying to push the level to below 60 mg/dl.

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