Lipid Analyses for the Management of Vascular Diseases

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Despite a long history of studies on lipid abnormalities, numerous problems in laboratory technologies and techniques remain unresolved. One of the most commonly tested molecules is low-density lipoprotein (LDL) cholesterol, and a homogenous assay technique for measurement of this molecule has recently been introduced. Although the method represents remarkable technological breakthroughs with great potential for improving lipoprotein analysis, some discrepancies exist among assay protocols. Even for direct measurement of high-density lipoprotein (HDL) cholesterol, which has widely been accepted, there are still large discrepancies among data obtained by different protocols. Oxidatively modified LDL is an independent factor that is considered to directly contribute to the pathogenesis of early atherosclerosis. Lipid peroxidation products, surface charge, and spectrophotometric patterns are all applicable to the evaluation of in vitro oxidation. Only enzyme-linked immunosorbent assays using monoclonal antibodies have a potential for clinical use, but such methods are not yet standardized. There is also increasing evidence for the presence of anti-oxidized LDL autoantibodies in human sera, but the diagnostic utility remains controversial. In addition, small dense LDL has recently attracted much attention as an independent risk factor. Although this is a potential target of oxidization, a robust and simple analytical method does not yet exist. This review presents the current state of laboratory technologies for testing lipid abnormalities. J Atheroscler Thromb, 2004; 11: 190–199.

Key words: LDL cholesterol, HDL cholesterol, Oxidized LDL, Anti-oxidized LDL autoantibodies, Small dense LDL, Triglyceride

Introduction

In human sera, there are a variety of lipid-related molecules that are considered to contribute to the pathogenesis of atherosclerosis. The association between high serum levels of low-density lipoprotein (LDL) cholesterol and the risk of developing coronary heart disease (CHD) has been well established by numerous clinical studies (1). Direct measurement of LDL cholesterol by a homogenous assay, which has recently been introduced, has been recommended for routine usage and to replace the Friedewald calculation (2). However, some important performance issues, such as whether intermediate-density lipoprotein (IDL) cholesterol should be included in the test results, have not been fully discussed.

Low serum levels of high-density lipoprotein (HDL) cholesterol are also correlated with the risk of CHD (1). Although the test is widely accepted, there are still large discrepancies among data measured by various assay protocols. Little consideration has been given to the detailed relationship between the distribution of HDL particle size and the selectivity of assay protocols.

Oxidatively modified LDL is thought to be an independent factor that accelerates the pathogenesis of early atherosclerosis (3, 4). In vitro oxidation of LDL can be easily detected by various methods, such as measuring lipid peroxidation products, surface charge, and spec-
trophotometric patterns. Only enzyme-linked immuno-
sorbert assay (ELISA) methods using monoclonal anti-
bodies against oxidized LDL have the potential for cli-
cal use. However, these methods are not widely ac-
cepted, because different monoclonal antibodies are
used for each assay protocol. There is also increasing
evidence that anti-oxidized LDL autoantibodies exist in
human sera, but their diagnostic utility remains contro-
versial (5). Therefore, some fundamental flaws must ex-
ist in current analytical technologies.

Some clinical studies have presented evidence that in-
dividuals with small, dense LDL particles have a higher
risk of CHD than subjects with large, buoyant LDL par-
ticles (6). However, other studies have conclusively re-
ported that when LDL size was compared in individuals
with and without CHD, no significant differences were
observed, and that LDL size was mostly related to lipo-
protein components in insulin resistance syndrome (7).
Although the use of gel electrophoresis is the most com-
mon approach for the determination of LDL size and sub-
class, this method cannot easily be introduced into cli-
nical laboratories.

Here, we review studies investigating the association
between these molecules and CHD, and discuss the cur-
rent state of analytical laboratory technologies.

**LDL Cholesterol**

**The National Cholesterol Education Program
(NCEP) guidelines**

In 2001, the National Cholesterol Education Program
(NCEP) updated the clinical guidelines for cholesterol
testing and management in adults aged 20 years or older
(Adult Treatment Panel III, or ATP III) (8). The guidelines
recommend that all adults should undergo testing for a
fasting lipoprotein profile of total cholesterol, LDL cho-
lesterol, HDL cholesterol, and triglyceride once every five
years. It also gives criteria to determine which patients
should go on to have further analysis and which patients
should receive cholesterol-lowering treatment on the
basis of the test results. The desirable total cholesterol
level is less than 200 mg/dl, LDL cholesterol less than
100 mg/dl, and HDL cholesterol greater than 40 mg/dl.

In the guidelines, it is assumed that LDL cholesterol is
calculated using Friedewald’s formula as follows: LDL
cholesterol = total cholesterol – HDL cholesterol – (trig-
lyceride/5). However, this calculation has a fundamental
flaw in estimating LDL cholesterol levels, as Friedewald et
al. stated in their initial report (2). They reported that their
method yielded a relatively reliable estimate, provided
that triglyceride levels were lower than 200 mg/dl. LDL
cholesterol cannot always be accurately estimated when
plasma triglyceride concentrations exceed 400 mg/dl. It
is also necessary to obtain serum after at least 12 hours of
fasting in order to accurately assess triglyceride levels.

**Direct methods**

In 1998, three years before the ATP III announcement,
Okada et al. reported a revolutionary method for directly
measuring LDL cholesterol. Using this method, serum or
plasma LDL cholesterol is chemically measured without
any preparative steps, such as immunonephelometry and/
or centrifugation (9). The assay protocol consists of a
two-step reaction, which is performed by adding reagents
1 and 2 to the sample. Reagent 1 removes non-LDL cho-
lesterol via the selective reaction of cholesterol oxidase
and cholesterol esterase supplemented with surfactants
that specifically react with these particles. The resulting
LDL cholesterol is determined spectrophotometrically for
the color produced by adding reagent 2. A different pro-
tocol based on another principle has also been report-
ed (10). This protocol also uses two reagents: one or both
reagents contain blocking agents, such as sugar com-
pounds, to mask cholesterol in chylomicrons, very-low-
density lipoprotein (VLDL), and HDL. Then, LDL choles-
terol is selectively measured via an enzymatic reaction.

Several assay kits are commercially available at present,
each of which is based on one of the two above-de-
scribed assay principles. ATP III comments that these
methods will grow in use but still require careful quality
control and monitoring. This comment is not appropriate
because there is substantial evidence to support the ef-
ficacy of these direct methods. For example, the differ-
ence between test results obtained by the direct method
and those by ultracentrifugation assay was very small, not
only in normotriglyceridemic subjects but also in
hypertriglyceridemic patients. The accuracy and preci-
sion of the direct methods fulfill the criteria of the NCEP
Lipid Standardization Panel. Figure 1 shows differences
between calculated LDL cholesterol levels and those di-
rectly measured in reference to serum triglyceride lev-
els (11).

LDL is simply defined as lipoprotein particles having
density ranges of 1.019–1.063 g/ml and containing ap-
proximately 50% cholesterol, 25% protein, 20% phos-
pholipid, and 5% triglyceride in weight percent. How-
ever, lipoprotein particles that have marginal densities
between IDL and LDL have intermediate physicochemi-
cal characteristics. If we attempt to detect each and ev-
ery LDL particle, the results will inevitably include some
cholesterol in IDL particles, but if not, some of the LDL
cholesterol will be missed. Because of this dilemma, it is
very difficult to develop assay protocols that enable the
accurate measurement of LDL cholesterol. Some com-
mercial kits have been developed to exclude as much
IDL cholesterol as possible, while others have not. Sakaue
et al. summarized the IDL cholesterol recovery rates of
three assay kits (A, B, and C) as follows: A: 31%, B: 47%,
and C: 64% (12). Recovery rates for VLDL cholesterol
were also reported (A: 10%, B: 19%, and C: 16%). It
should be noted that any protocols intended to include
IDL cholesterol inadvertently detect VLDL cholesterol. There is some evidence that IDL is another atherogenic molecule, and direct methods have been criticized for not measuring its levels. However, IDL has three or more times the triglyceride content of LDL. Measurement of triglyceride is more cost-effective and reliable for screening patients with high IDL. Therefore, methods that measure LDL cholesterol should be standardized so as not to include IDL or VLDL cholesterol. Otherwise, the term ‘Testing LDL cholesterol’ will cause confusion in clinical settings.

Is total cholesterol still necessary?
Is measurement of total cholesterol still necessary? The test results include so-called “good” cholesterol and bad cholesterol. Total cholesterol measurement can be hazardous because individuals whose serum levels of good cholesterol are high may be erroneously diagnosed as hyperlipidemic and subsequently medicated. We investigated whether any individuals with abnormally high total cholesterol levels were missed by testing LDL cholesterol only (11) (Fig. 2). We examined 539 subjects whose serum levels of LDL cholesterol were normal (< 130 mg/dl). The total cholesterol levels of 23 of the subjects were greater than 220 mg/dl, but none of these were abnormal because they had higher HDL cholesterol levels (> 85 mg/dl). This observation suggests that measurement of total cholesterol does not provide any additional useful information for patient management.

Direct detection methods can be performed rapidly using automated analyzers and can be implemented cost-effectively in routine lipoprotein testing.

HDL Cholesterol

HDL species
HDL comprises a complex, heterogeneous population of particles, of which there are three species, a minor preβ-electrophoretic migrating species and two major α-migrating species. The latter two species are usually described as HDL2 and HDL3.

One of the major sources for cholesterol detected as HDL is the cell membrane. Cellular free cholesterol is initially transferred to the preβ-migrating HDL, esterified, and then transferred to α-migrating HDL (13). The cholesteryl esters are transferred to HDL2 through the action of cholesteryl ester transfer protein (CETP), causing an increase in the lipid content and size of HDL. CETP also mediates the exchange of cholesteryl esters from HDL2 with triglycerides from chylomicrons, VLDL, and IDL. The remainder of the cholesteryl esters are delivered to the liver by HDL2 itself. Higher serum levels of HDL cholesterol, therefore, show that this reverse cholesterol transport system is functioning well.

The question is which species deserves the title “good cholesterol”? Investigators have studied the relationship between HDL subclasses and CHD risk. However, the results were conflicting; some studies presented data

Fig. 1. Distribution of differences between measured LDL cholesterol levels and those calculated using Friedewald’s formula with reference to triglyceride levels. Calculated LDL cholesterol levels are inaccurate even if triglyceride levels are less than 200 mg/dl (reproduced from Ref. 11 with permission).

Fig. 2. Distribution of total cholesterol levels of subjects whose LDL cholesterol levels were less than 130 mg/dl. None of these indicated hyperlipidemia because the subjects, whose total cholesterol levels were greater than 220 mg/dl, had higher HDL cholesterol levels (reproduced from Ref. 11 with permission).
showing that HDL3 and HDL2 were associated with CHD (14), while other studies did not. One study which reviewed eight reports revealed that seven reports showed an association between HDL2 and CHD and five showed an association between HDL3 and CHD (15). The study concluded that HDL3 and HDL2 were significantly associated with CHD, but multivariate-adjusted data demonstrated no advantage in CHD prediction beyond conventional HDL cholesterol testing. This conflict may be attributable in part to assay characteristics.

Problems with HDL measurement

Apolipoprotein A-I (apoA-I) is a major protein component of HDL3 and HDL2. Apolipoprotein A-II (apoA-II), which is much smaller than apoA-I, is another protein component of HDL3. Larger particles of HDL2 are enriched with apolipoprotein E (apoE), while HDL3 is not. ApoE is a glycoprotein that is known for receptor-mediated interaction. Another remarkable characteristic of apoE is that it is highly lipophilic. Therefore, apoE solubilizes hydrophobic lipids and plays a role in regulating the particle size of lipoproteins. Although apoA-I and apoA-II are found exclusively in HDL, apoE is found in almost all lipoproteins. Accordingly, the presence of apoE can make it difficult to chemically discriminate HDL from other lipoprotein particles.

Direct measurement of HDL cholesterol does not require pretreatment, such as centrifugation, and has been widely introduced in clinical laboratories. However, it has been reported that some of the assay protocols measure cholesterol in every species of HDL, including those enriched with apoE, while others do not (16). As a result, there are large discrepancies among data obtained using various protocols. Further discussion is required for the standardization of commercially available kits.

HDL with preβ-electrophoretic migration consists of three subcomponents, preβ1, preβ2, and preβ3. Preβ1 HDL is the major initial acceptor of free cholesterol from cell membranes. A method for quantifying preβ1 HDL using a monoclonal antibody was recently introduced (17). Because of the in vitro instability of preβ1 HDL, this protocol requires some pretreatment of the samples. The clinical significance must therefore be extensively studied in the future (18).

Oxidized LDL

Free radical attacks

Oxidative modification of LDL plays a key role in the pathogenesis of early atherosclerosis. Oxidized LDL stimulates endothelial cells via an unknown mechanism, resulting in expression of adhesion molecules such as endothelial leukocyte adhesion molecule 1 (ELAM-1), which initializes monocyte/macrophage recruitment (19, 20). These macrophages become foam cells after uptake of oxidized LDL. Subendothelial accumulation of foam cells generates fatty streaks or atheromatous plaques.

An initial step in LDL oxidation is the peroxidation of polyunsaturated fatty acids and phospholipids, as follows:

$$\text{LH} \rightarrow \text{LOO}$$

where

\( \text{L} \): lipid
\( \text{OO} \): peroxy radical

Lipid peroxidation leads to the generation of lipid hydroperoxides,

$$\text{LOO} \rightarrow \text{LOOH}$$

which undergo carbon-carbon bond cleavage, resulting in formation of short-chain unesterified aldehydes. Free-radical species and metal cations promote this reaction. Malondialdehyde (MDA) is a highly reactive dialdehyde produced as a byproduct of this reaction. The most common method for detecting this process is the thiobarbituric acid reactive substances (TBARS) assay, which measures the MDA content in oxidized LDL (21).

Oxidative attack of LDL phospholipids will convert phosphatidylcholine (PC) to lysophosphatidylcholine (lyso-PC) (22). This is another byproduct of LDL oxidation. Fragments of these modified lipids attach covalently to apolipoprotein B (apoB) on the surface of LDL and neutralize the positively charged amino-acid groups, such as lysine (23). The neutralization of positively charged lysine residues inhibits recognition by LDL receptors on endothelial cells. On the other hand, scavenger receptors on macrophages are able to recognize the modified apoB.

In vitro determination

The electrophoretic mobility of modified LDL is a conventional parameter for in vitro determination of changes in surface charge. An increase in spectrophotometric absorption at 234 nm also reflects surface morphological changes caused by the oxidative reaction, as described by Esterbauer et al. (24).

In vitro oxidation of LDL is easily promoted by adding metal cations, such as copper (Cu^{2+}). Lipid peroxidation products increase substantially between 30 and 60 min after the start of incubation, and reach a peak at 120 min (25). The products then continue to decrease for about 6 hours. The electrophoretic mobility of LDL increases during incubation due to the negative charge. Determination of electrophoretic mobility can be performed on 1% agarose gels. The charge changes slightly over the first 60 min of incubation and continues to increase between 60 min and 6 hours of incubation (Fig. 3). Spectrophotometric patterns show clear changes for up to 48 hours of incubation (Fig. 4). By subtracting the spectra of a control solution of native LDL from that of oxidized LDL after 6 hours of incubation, a distinctive
pattern can be obtained (Fig. 5). There is a peak at 232 nm, not at 234 nm, as previously described (24), and a trough at 203 nm. The ratio of absorbance at 232 nm divided by that at 203 nm is a significant indicator of LDL oxidation. These phenomena are exclusively observed in LDL oxidation, because neither isolated apolipoproteins nor artificial lipid-particles without apolipoprotein exhibit these changes (25).

The lipoprotein is composed of apolipoprotein, phospholipid, triglyceride, and cholesterol. All of these sites can be oxidized by free radicals and metal cations. Oxidized cholesterol also induces damage to endothelial cells (26, 27). It has been shown that there are five species of oxidized cholesterol in human plasma, 4-cholesten-3-one, 20 α-OH cholesterol, 7 α-OH cholesterol, 26-OH cholesterol, and 7-keto-cholesterol. The major oxidative product is 7-keto-cholesterol. Some of these are present exclusively in plasma from CAD patients, while others are also present in plasma from control subjects (27). High-performance liquid chromatography and gas-liquid chromatography can be used to identify and quantify oxidized cholesterol species. Although the presence of oxidized cholesterol would be expected to significantly contribute to the pathogenesis of early atherosclerosis, the precise mechanism needs to be further clarified. Existing data merely indicate that the oxidized cholesterol content of LDL is a good indicator of lipoprotein modification and closely parallels changes in electrophoretic mobility (26).

**Circulating oxidized LDL**

Despite the increased awareness of the importance of oxidized LDL, the diagnostic significance of circulating oxidized LDL in human blood is poorly understood. Itabe et al. reported a monoclonal antibody that reacts specifically with oxidized LDL but not with native LDL, MDA-modified LDL, acetyl LDL, or glycated LDL (28). The epitope of this antibody seems to be an oxidized product of PC or oxidized PC-polypeptide complex. Using this antibody in combination with an anti-apoB antibody, Toshima et al. developed an ELISA method that measures oxidized LDL in plasma (29). They reported that plasma levels of oxidized LDL were significantly higher in patients with CHD than in control subjects.

Holvoet et al. reported another ELISA technique in which they used monoclonal antibodies having high affinity for apoB with aldehyde-modified lysines (oxidized LDL) and for MDA-modified LDL (30, 31). They compared plasma levels of oxidized LDL and those of MDA-modified LDL. After correction for age, sex, LDL cholesterol, and HDL cholesterol using multiple regression analysis, they found that elevated plasma levels of MDA-modified LDL were significantly higher in patients with acute coronary syndrome than in patients with stable CHD, but the difference between plasma levels of oxidized LDL in patients...
with stable CHD and those in patients with acute coronary syndromes was less pronounced. They also found that there was no association between plasma levels of oxidized LDL and LDL cholesterol. These data suggest that increases in plasma levels of oxidized LDL are independent of plaque instability and may simply be due to their backward diffusion from stable subendothelial lesions. In contrast, plasma levels of MDA-modified LDL may represent plaque instability or inflammation of the inner surface of the vascular wall.

The above assays are not standardized. Because LDL is a complex molecule and the mechanisms of oxidation are not well understood, several problems remain, including the nature of LDL oxidation, the oxidation sites in LDL that should be measured, and methods for obtaining appropriate antibodies.

**Autoantibodies against oxidized LDL**

In 1992, Salonen et al. reported that the presence of circulating autoantibodies against oxidized LDL was an independent predictor of the progression of carotid atherosclerosis (32). Several investigators have reported evidence supporting this, as follows: anti-oxidized LDL autoantibodies were present in the plasma of patients with CHD (33–35), and patients with severe carotid atherosclerosis specifically develop autoantibodies (36). In contrast, other investigators failed to show any significant differences in the levels of anti-oxidized LDL autoantibodies between patients with CHD and control subjects (37–39). Thus, data on the clinical significance of these autoantibodies are conflicting. It is considered that there are two reasons for this conflict: technical flaws exist in the methods used in these studies, or the presence of circulating autoantibodies is not involved in any pathological processes.

The methods used in the studies were described by Salonen et al. (32). Serum or plasma samples were typically measured by ELISA or occasionally by radioimmunoassay. Antigens for the assays included native LDL and oxidized LDL prepared by incubation with copper sulfate and/or MDA. These were separately coated onto wells of microtiter plates. To obtain antibody titers, the ratio (or difference) between readings of optical density (OD) for the anti-oxidized and anti-native LDL wells was calculated. The problem is that serum or plasma shows non-specific binding to wells coated with antigens, and even the autoantibodies appeared to have a relatively low affinity for the coated antigens. Technical flaws might also lower the sensitivity and specificity of these methods.

In human blood, specific and nonspecific antibodies circulate. Virella et al. developed an ELISA for deleting nonspecific circulating antibodies (37). They prepared two aliquots of each sample; one is absorbed with oxidized LDL and the other is unabsorbed. The differences in OD between the unabsorbed aliquot and that absorbed with oxidized LDL are evaluated. The obtained values correspond to true antibody titers. Using this method, no significant differences were found between patients with CHD and control subjects. It was also found that anti-oxidized LDL autoantibodies are relatively common, even in healthy young subjects. This indicates that the antibodies are involved in a physiological process, such as eliminating modified LDL particles from circulation. They also found that antibody concentrations appear to be greater in hyperlipidemic patients. The data also suggested that hyperlipidemia is associated with the formation of larger quantities of oxidized LDL, which triggers antibody synthesis.

Okada et al. suggested the possibility that antigens might be contaminated by epitopes other than native and oxidized LDL, and that non-specific binding of the IgG molecule could occur (40). They found that some molecules were non-specifically bound to LDL particles and could be easily detached by *in vitro* oxidation. Western blot revealed the presence of a non-specific epitope to IgG (28 kD) in an oxidized LDL solution. The primary structure of this 28-kD fragment was found to be apoA-I. In addition, oxidized LDL particles readily aggregated (Fig. 6) into a non-specific epitope. They attempted to minimize false-positive findings that could occur due to the non-specific reactivity of immunoglobulins, particularly to apoA-I, by purifying the antigens using gel-permeation chromatography. A significant difference in IgG autoantibody titers was confirmed between patients with Achilles tendon xanthoma and control subjects. Thus, antigen contamination is a factor that possibly biased results in previous studies.

**Fig. 6.** Aggregation of oxidized LDL. Oxidized LDL was analyzed by using gel permeation chromatography and monitored with an array-spectrophotometric analyzer. In this analysis, the shorter the elution time was, the larger the eluted particle was. The small peak of the left upper corner is aggregated LDL.
Small, Dense LDL

Clinical aspects

LDL particles are heterogeneous in size and density. In 1988, Austin et al. categorized LDL particles into the A and B patterns and found that a preponderance of small, dense LDL particles (B pattern) was significantly associated with a three-fold increased risk of myocardial infarction (41). They also found that small, dense LDL particles were significantly associated with increased triglyceride and decreased HDL cholesterol levels. Other investigators supported these findings (42–44). Other studies have shown that men generally have smaller LDL particles than women (6, 7), that there are significant increases in the percentage of the B pattern in patients with non-insulin-dependent diabetes mellitus (45, 46) and that the LDL cholesterol concentration shows no significant association with LDL size (42).

Gray et al., however, found no significant difference in LDL size between patients with CHD and control subjects (7). The association between LDL size and the incidence of CHD is controversial. It has also been documented that approximately one-third of the general population has a predominance of small, dense LDL particles (B pattern), and a gene locus of the phenotypic pattern has been identified.

To date, the most important issue in the prevention of vascular diseases is that LDL subclasses are strongly influenced by environmental factors, such as diet, exercise, weight loss, alcohol consumption, and drug regimens. Campos et al. examined LDL particle size distribution using gradient gel electrophoresis and studied the association between dietary intake and LDL subclass (6). They identified seven peaks in the pattern and found that cholesterol and low saturated fat intakes were significantly associated with smaller LDL particles.

Physicochemical aspects

In vivo regulation of LDL particle size occurs as described below. LDL cholesteryl ester is exchanged with an excess of triglyceride from chylomicrons and VLDL, while triglyceride is catalyzed by lipoprotein lipase (LPL), leading to small, dense LDL. CETP mediates the exchange of cholesteryl ester with triglyceride. Therefore, an excess of chylomicrons, VLDL, or CETP will raise the ratio of small, dense LDL to large, buoyant LDL particles. Koba et al. reported that postprandial increases in large VLDL fractions and remnant-like particles contribute to the formation of small, dense LDL (47).

The largest and most buoyant subclass has a size greater than 26.0 nm and a density range of approximately 1.025 to 1.035 g/mL. The other major subclasses are progressively smaller and more dense (41). ApoB is one of the largest monomeric proteins known. Secondary structure analysis indicates that apoB contains 43% α-helices, 21% α-sheets, 20% random structures, and 16% β-turns (48). Although the tertiary structure is still poorly understood, it is considered that apoB consists of parts located on the surface and buried inside the LDL particle.

Figure 7 shows the putative surface structure of two LDL subclasses (original drawing, data not shown). It is considered that the closed interface between the parts of apoB is easily attacked by free radicals and/or oxidative byproducts.

Laboratory technologies

LDL particle size can usually be determined using gradient gel electrophoresis. For example, 2 – 16% polyacrylamide gradient gels are used and analyzed by lipid stain followed by scanning. Particle diameters can be calculated from calibration curves obtained using protein standards of known size. Density gradient ultracentrifugation is another method for quantifying these particles. A variant is the use of ultracentrifugation in combination with apoB immunoassay (49). Existing methods, however, are either semi-quantitative or time-consuming. Recently, Hirano et al. found that the combination of heparin and magnesium cations could be used to assist in the separation of small, dense LDL from other apoB-containing lipoproteins (50). They developed an assay kit exploiting this phenomenon.

Clinical data should be accumulated through the use of standardized methods in the future.

Discussion and Conclusions

Triglyceride as a risk factor should also be considered. Interestingly, existing clinical trials have failed to show that triglyceride contributes to the pathogenesis of early
atherosclerosis. However, there is a possible mechanism that links hypertriglyceridemia and CHD (51). VLDLs induce transcription of the human plasminogen activator inhibitor-1 (PAI-1) promoter in endothelial cells. Increased plasma levels of PAI-1 attenuate fibrinolysis, which results in atherothrombotic disorders, such as CHD. Thus, an increased level of triglyceride-rich VLDL is a possible risk factor for vascular diseases. Therefore, VLDL triglyceride should be measured instead of total triglyceride in the future. Nevertheless, at present, the measurement of total triglyceride is clearly important for preventing development of atherosclerosis.

Although numerous other methods for measuring lipid-related molecules have been developed, only a few have been proven to be clinically valuable in clinically controlled trials. The combination of LDL cholesterol, HDL cholesterol, and triglyceride plays a larger role in the prevention of vascular diseases than any other serum markers. It should be stressed that environmental and lifestyle factors are also important parameters for prevention (52–55).

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