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Chapter II

ATHEROSCLEROSIS: RISK FACTORS, PREVENTION AND TREATMENT

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1. INTRODUCTION

Over the past 150 years, there have been numerous efforts to explain the complex events leading atherosclerosis. In this endeavor, several hypotheses and the risk factors have emerged that currently are under active investigations. However, these hypotheses are not mutually exclusive, but rather emphasize different concepts as the necessary and sufficient events to support the development of atherosclerotic lesions. In this review, the combination concept of ‘‘response-to-injury’’ [1] and ‘‘oxidative modifications’’ [2] for the initiation of atherosclerosis, rather than the progression of atherosclerosis associated with risk factors, is mainly discussed for the prevention and treatment.

HMG-CoA reductase inhibitors (statins) are well known to decrease cellular cholesterol synthesis and consequently reduce the hepatic production of VLDL and increase expression of LDL receptor [3]. Clinical trials have shown that improvements in plasma LDL-C levels are associated with retardation of atherosclerosis and reduction in coronary artery morbidity and mortality [4, 5]. The major mechanism of this therapeutic effect has been recognized as the increase of LDL receptor expression in liver to remove elevated LDL-C in plasma. However besides LDL-C, remnant lipoproteins (RLP) have been increasingly implicated in progression of atherosclerosis, with elevated fasting RLP-Cholesterol (RLP-C) levels shown to predict clinical events independently in coronary artery disease patients [6]. Oxidized lipoproteins, notably oxidized low-density lipoproteins (Ox-LDL) and RLP-C in plasma emerged as the new risk factors after lowering LDL-C. Both risk factors are paid attention by the clinical laboratories as diagnostic tools for the cardiovascular disease with the progress of

new technology. Accordingly, attempts are made to provide an insight into the atherogenecity of remnant lipoproteins and Ox-LDL including their contribution to endothelial cell dysfunction, for example through the lectin-like oxidized LDL receptor-1 (LOX-1 receptor) [7] which has been discovered as an Ox-LDL receptor. It was recognized that activation of LOX-1 receptor by remnant lipoproteins plays a key role for the initiation of endothelial cell dysfunction [8] and may present a major factor in atherogenesis which is independent from plasma LDL concentration. The new concept on prevention and therapeutic target and its associated risk factors in cardiovascular disease are described in this review recognized from our previous sudden cardiac death research performed during last two decades.

2. TWO MAJOR RISK FACTORS OF ATHEROSCLEROSIS

2.1. The Oxidized LDL Hypothesis Associated with High Plasma LDL-C

In 1989, Steinberg et al. [2] put forward the original oxidative modification hypothesis based on the notion that oxidation represents a biologic modification analogous to chemical modification discovered by Goldstein et al. [9] that gives rise to foam cells. Since then, numerous studies have supported the Ox-LDL hypothesis which says Ox-LDL can promote foam cell formation through the so-called ''scavenger receptor'' pathways [9, 10]. Scavenger receptor, SRA, in macrophage was first characterized in 1988 by Kodama et al. in Krieger's laboratory [11], but it should be noted that macrophages express more than one scavenger receptor.

Several new receptors for Ox-LDL in macrophage such as CD36 [12], LOX-1[7] and SR-PSOX [13], etc., have been discovered after Steinberg proposed Ox-LDL hypothesis. Sawamura et al. [7] noticed the absence of scavenger receptors for Ox-LDL in endothelial cells which may cause the endothelial dysfunction to initiate atherosclerosis in elevated LDL-C cases. They found a new receptor for Ox-LDL in endothelial cells and named LOX-1 receptor.

The Steinberg's hypothesis was proposed under the situation that Ox-LDL receptor (SRA) was the only one receptor found in macrophage associated with the formation of atherosclerotic lesions, but no other receptors ware yet found in endothelial cells. The Ox-LDL hypothesis was proposed mainly for the formation of foam cells from macrophages via scavenger receptor as the major cause of atherosclerosis, but not for the dysfunction of endothelial cells which most probably associated with the initiation of the formation of atherosclerotic lesions. However, the present concept is that atherosclerosis represents a state of heightened oxidative stress characterized by lipid and protein oxidation in the vascular wall generated by LDL penetrated through between the endothelial cells from plasma. The current oxidative modification or stress hypothesis of atherosclerosis predicts that LDL oxidation is an early, essential event in atherosclerosis and that Ox-LDL does contribute to both initiation and progression of atherosclerosis. But besides Ox-LDL, the possibility still exists that other lipoproteins which are oxidized in plasma with normal LDL concentration may cause to form the atherosclerotic lesions.

As shown in Figure 1, we have proposed the oxidative modification hypothesis of remnant lipoproteins on athegenesis.

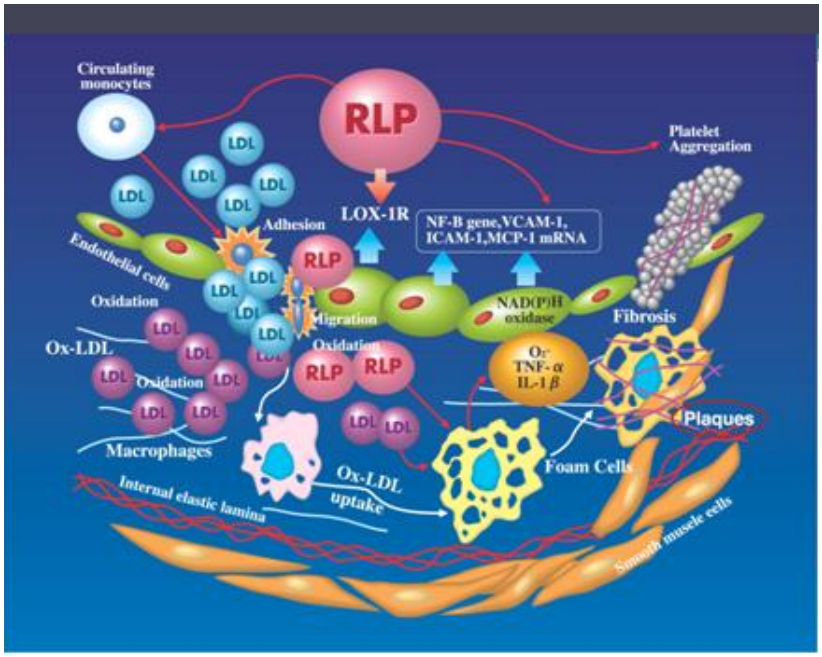

Figure 1. Effect of RLP and Ox-LDL on the formation of atherosclerotic plaque. The endothelial cell dysfunction is initiated by RLP in plasma (not oxidized LDL) followed by the induction of LOX-1 receptor. The associated pathway of various cytokines and enzymes are activated and promote for the endothelial cell damage. After increased permeability of endothelial cell by damage, a large amount of LDL move into the subendothelial space and form Ox-LDL which are incorporated into macrophages and foam cells and promote the progression of atherosclerotic plaques in blood vessel wall.

The endothelial cell dysfunction is initiated by RLP in plasma followed by the induction of LOX-1 receptor and the associated pathway of various cytokines and enzymes. Ox-LDL promotes the progression of atherosclerosis in subendothelial space after a large efflux of LDL from plasma and form foam cells and atherosclerotic plaques. As small dense LDL-C is a part of LDL, or a kind of Ox-LDL and the similar target of cardiovascular disease by statins, we have not described in this review.

2.2. Characteristics of Ox-LDL and Remnant Lipoproteins on Atherogenesis

The oxidative modification hypothesis focuses on the concept that LDL in its native form is not atherogenic [14]. However, LDL modified chemically is readily internalized by macrophages through the so-called scavenger receptor pathway [9]. Exposure to vascular cells in medium that contains transition metals also results in modification of LDL such that it serves as a ligand for the scavenger receptor pathway [10]. Therefore, it is now clear that only one mechanism whereby cells in vitro render LDL a substrate for the scavenger receptor pathway is via oxidation of LDL which results in modification of apo B-100 as well [15]. These observations form the basis for the oxidative modification hypothesis of atherosclerosis in which LDL traverses the subendothelial space of lesion-prone arterial sites. During this process, LDL lipids are subject to oxidation and modifications of the lysine residues on apo B-100 leads to an increase of the net negative charge on the lipoprotein particles [16]. This

modification of apo B-100 renders LDL susceptible to macrophage uptake via a number of scavenger receptor pathways producing cholesterol ester-laden foam cells [17]. The presence of Ox-LDL in atherosclerotic lesions has been studied using antibodies that recognize specific epitopes on Ox-LDL that are not present in its native, non-oxidized form. The oxidation of polyunsaturated fatty acids can lead to the formation of aldehydes that modify lysine residues in apo B-100 [18]. Adducts of lysine residues with malondialdehyde and 4-hydroxynonenal have been characterized extensively and antibodies raised against these species. These antibodies avidly stain atherosclerotic lesions in LDL receptor-deficient rabbits [19, 20], apo E deficient mice [21], and humans [22, 23] with no demonstrable staining in normal arteries. As the oxidative modification hypothesis would predict, these epitopes largely co-localize in macrophages, although one might argue that they are not specific for LDL and could represent modification of other proteins and phospholipids in the atherosclerotic lesion as well. Consistent with this assertion is a study showing that LDL isolated from atherosclerotic lesions possesses properties that resemble those of Ox-LDL formed in vitro [23], indicating that lesion LDL is oxidatively modified and accumulated in vascular walls. Aggregated LDL is much more rapidly taken up by macrophages than native LDL but again the uptake appears to occur via the LDL receptor [24].

Aggregation of LDL in the subendothelial space has been demonstrated [25] and this may be encouraged by the proteoglycans in the artery wall to which LDL binds avidly [26]. On the other hand, it is generally agreed that LDL accesses the artery wall, but VLDL, another apo B-100 carrying lipoproteins also can access the artery wall and directly contribute to atherogenesis. As reviewed below, there is overwhelming evidence for VLDL and especially for VLDL remnants being major atherogenic lipoproteins [27, 28].

Particles resembling VLDL remnants, RLP can be taken up by macrophages to produce foam cells without oxidative modifications [29, 30], stimulate endothelial cells to express a monocyte-specific chemotactic factor [8], increase monocyte adherence to the endothelium [31] and so forth. Further, varying amounts of labeled VLDL apo B-100 have been found in aorta from human and experimental animals after intravenous administration [32–35]. Other possibilities are reported by several investigators that a major part of apo B-100 in atherosclerotic plaques was not originated from LDL. Rapp et al. [36] isolated and characterized immunoreactive apoB-containing lipoprotein particles from human atherosclerotic plaques.

These apoB-100 species were present significant amount in VLDL+IDL fraction, as much as in the LDL fraction. From these observations, VLDL, VLDL remnants or both showed the possibility of entering human atheosclerotic plaques as the origin of apo B-100 in spite of being larger in size than LDL particles.

2.3. Circulating Ox-LDL in Plasma as a Risk for Coronary Artery Disease

LDL circulates in plasma, including a fraction which reenters the circulation from the subendothelial space [37, 38]. The plasma antioxidants provide effective protection against oxidation of LDL [39]. This means that major site of LDL oxidation is the subendothelial space. The transit of LDL across this space may yield a small amount of circulating LDL that is oxidized. Chemical analysis of circulating LDL has been reported to yield a minor fraction, termed LDL that has an increased amount of oxidized lipid [40].

Consistent with these findings, human plasma shows immunoreactivity towards epitopes generated on Ox-LDL [41, 42]. However, the existence of oxidized LDL in the circulation remains controversial on the basis of artifacts that may occur during ex vivo handling of plasma and isolation of LDL. Although the aforementioned data do not address a causal relation between Ox-LDL and atherosclerosis, several studies have shown that epitopes on circulating Ox-LDL can be used to distinguish between patients with and without clinical evidence of atherosclerosis [43, 44].

Using immunologic methods that detect oxidized phosphatidylcholine and their protein adducts [45,46], but not native, acetylated or malondialdehyde-treated LDL, Toshima et al. [47] and Ehara et al. [48,49] reported that acute coronary syndromes are characterized by increased circulating levels of Ox-LDL. Most recently, Tsimikas et al. [50] reported that circulating levels of Ox-LDL are strongly associated with angiographically documented coronary artery disease, and indicated the high association with serum Lp(a) which binds oxidized phospholipids in LDL.

Together, these data indicate that are relatively small amount of LDL containing different types of oxidation specific epitopes can be detected in blood and may reflect atherosclerosis and its different manifestations. What is less clear at present is where these epitopes originate from and which, if any, of the different oxidation-specific epitopes directly relate to and/or are important for disease burden [51].

The concentration of Ox-LDL detected in plasma (less than 0.5% in total LDL) is too low for the induction of Ox- LDL proatherogenic and proinflammatory properties shown from many in vitro studies.

On the other hand, autoantibodies against malondialdehyde- modified lysine residues (anti-oxidized LDL antibodies) have been demonstrated in the serum of both rabbits and humans [22, 52]. Some studies have reported that the titer of these autoantibodies is associated with the burden of and may predict progression of atherosclerosis [53, 54] and myocardial infarction [55, 56]. Higher titers of autoantibodies have also been associated with coronary artery disease [57], peripheral atherosclerosis [58] and higher risk for restenosis following balloon angioplasty [59]. In addition, there is support for a role for anti-oxidized autoantibodies in animal atherosclerosis [60].

But it is worth noting that these results were not observed consistently, because of the co-existence of similar antigens with Ox-LDL in plasma. The presence of autoantibodies in plasma is well known to reflect the results of cellular damage, indicating the possibility that oxidized LDL may play the role for the progression of atherosclerosis in vascular wall macrophages and smooth muscle cells, rather than the role for the initiation of atherosclerosis in endothelial cells. Small, dense LDL has been reported to be more susceptible to be oxidation than large, buoyant LDL [61], but the size of LDL particles, not the susceptibility to oxidation in vitro, seems to be more associated with cardiovascular disease [62].

However, currently an adequate technique to isolate native small, dense LDL from large, buoyant LDL is not available yet. Therefore, it is still difficult to compare the differences in oxidation susceptibility directly between these two LDL sub-types. Small, dense LDL levels in plasma were reported to be highly correlated with the levels of RLP-C [63, 64] which has been speculated as precursor lipoproteins of small, dense LDL. Further, Ando et al. [65] reported that plasma Ox-LDL levels were strongly correlated with RLP-C levels in hemodialysis patients.

2.4. Triglyceride-Rich Lipoprotein Remnants in Plasma as an Risk Factor for the Cardiovascular Diseases, Independent from TG in Plasma

Patients at increased risk of coronary artery disease (CAD) frequently have an atherogenic lipoprotein profile characterized by elevated plasma triglyceride-rich lipoproteins (TRL) levels, a predominance of small, dense LDL and reduced high density lipoprotein (HDL) cholesterol which are highly associated with the characteristics of metabolic syndrome. This profile is often seen in patients with type 2 diabetes mellitus with normal LDL concentration and it is associated with an approximately three-fold increase in risk of atherosclerotic disease [66, 67]. An elevated remnant lipoprotein concentration determined as remnant-like lipoprotein particle-cholesterol (RLP-C) is also a characteristic feature of patients with this atherogenic lipoprotein profile [68] and there is considerable evidence linking increased plasma RLP-C levels with CAD. In this connection, Nakajima et al. [69] reported that plasma RLP-C levels were abnormally high in Japanese patients with coronary heart disease. Similarly, Ikewaki et al. [70] showed that plasma RLP-C significantly increased in postprandial state in patients with coronary artery disease. Further, Leary et al. [71] subsequently found that CAD patients (n =151) from nine centers in the United States and one in Canada had significantly higher median RLP-C levels compared with 302 gender and age-matched control subjects. Devaraj et al. [72] also showed that RLP-C levels were markedly higher in CAD patients compared with healthy control subjects ($P < 0.01$). These results have been supported by other case-control studies reporting that RLP-C levels were significantly high in patients with CAD, patients with resterosis after percutaneous transluminal coronary angioplasty [73,74], in vasospastic angina [75,76], coronary artery stenosis [77], coronary artery endothelial dysfunction [78,79], sudden cardiac death [80,81], intermittent claudication [82], increased intima-media thickness of the carotid artery [83] and in CAD patients with normal cholesterol or triglyceride (TG) levels [84,85].

In a large study, NcNamara et al. [86] measured RLP-C and RLP-TG in fasting plasma samples from 1567 women in the Framingham Heart Study. Multiple logistic regression analysis adjusting for other major risk factors (like age, hypertension, smoking, diabetes, LDL-C, HDL-C beta-blocker use and replacement hormones) revealed that RLP-C was an independent risk factor for cardiovascular disease (CVD) in these women and independent from TG (Table 1). RLP-C has similarly been shown to be an independent risk factor for CAD in Korean patients with type 2 diabetes [87] and in Japanese patients more than 65 years of age [88].

More recently, prospective data have been presented supporting the prognostic value of RLP-C measurement. Three studies have been reported by Kugiyama et al. at Kumamoto University Hospital in Japan, in which CAD patients (men and women [89], postmenoposal women [90], type 2 diabetes patients [91] who had angiographically documented arterial stenosis were investigated. Their lipid and RLP-C levels were measured at baseline and then were followed for 2 to 3 years until the occurrence of a clinical event (recurrent or refractory angina pectoris requiring coronary revascularization, nonfatal myocardial infarction, or cardiac death). In all these three studies, higher RLP-C levels were associated with greater probability of a coronary event and were found to be independent risk factors (other than hitherto known risk factors like age, gender, smoking, hypertension, triglycerides, cholesterol, HDL-C) and were shown to be statistically significant predictor of future coronary events.

Recently high plasma levels of RLP-C have been reported in the metabolic syndrome as a risk for endothelial dysfunction and coronary artery disease [92–95].

3. PREVENTION

3.1. What Is the Most Effective Risk Factor Targeted for the Prevention of Cardiovascular Disease?

Plasma total cholesterol (TC) and triglyceride (TG) levels have been measured as diagnostic markers in order to prevent cardiovascular diseases, because these markers have shown the usefulness as the risk prediction for many years. Therefore, the risk factor for the therapeutic target has been developed to reduce TC and TG historically. However, several new diagnostic markers have emerged recently and provided the additional information for the prevention of cardiovascular diseases.

AS shown in Figure 2, there are many subclasses of lipoproteins among chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).

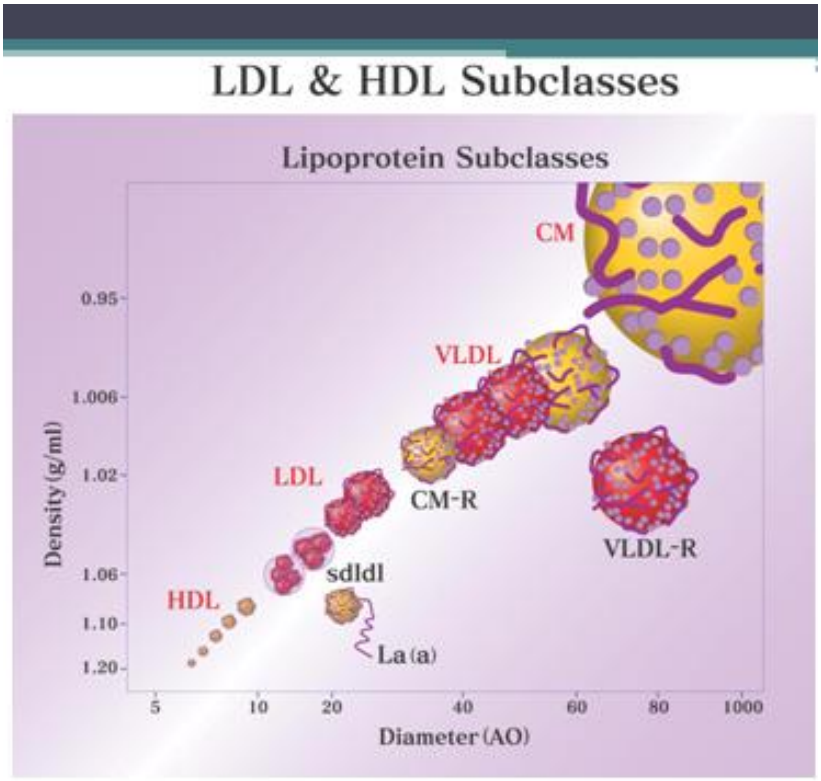

Figure 2. Subclasses of plasma lipoproteins. There are many subclasses of lipoproteins in CM, VLDL, LDL, and HDL, which have different biological and chemical characteristics. Remnant lipoproteins are found in VLDL fraction and Ox-LDL and small dense LDL are found in LDL fraction.

The analytical methods for each subclasses are now under development. Among them, LDL-C including Ox-LDL and small dense LDL, and remnant lipoproteins are the major new diagnostic markers for the prevention of cardiovascular disease. Lowering these risk factors in plasma has been targeted by the various therapeutic drugs and nutritional treatment.

The most effective target for the prevention of cardiovascular disease may be remnant lipoproteins based on the following evidence we have investigated during the last two decades, which is the most common risk factors for cardiovascular diseases, except familial lipid and lipoprotein disorders. LDL or small dense LDL are the final products metabolized from TG-rich lipoproteins which include atherogenic remnants. Most of the atherogenic properties of lipoproteins belong to remnant lipoproteins rather than LDL in plasma.

3.2. Different Role of Plasma LDL and Remnant Lipoproteins at Coronary Atherosclerosis and Cardiovascular Events; from the Studies of Autopsies in Sudden Cardiac Death Cases

We have investigated the risk factors of sudden cardiac death (SCD), especially Pokkuri Death Syndrome (PDS) (sudden cardiac death without coronary artery atherosclerosis observed mostly in Southeastern Asian young males), during the last two decades [96] and found the different roles between LDL and remnant lipoproteins as cardiovascular risk factors in plasma. Based on our autopsy studies, more than two thirds of SCD cases were found to be associated with postprandial remnant hyperlipoproteinemia [97-101]. The occurrence of sudden cardiac death has been observed prevalently at midnight (Figure 3), which is highly correlated with the highest TG and remnant lipoprotein levels in plasma during the day (Figure 4). LDL-C levels do not change during the day as remnant lipoproteins.

If severe spasm of the coronary artery is to be a crucial event prior to cardiac death in PDS cases, we may say that the vasospasm is not very likely to occur in coronary arteries with severe coronary artery atherosclerotic lesions due to reduced elasticity and increased stiffness or hardness of the vascular wall. Caucasians experience more severe coronary atherosclerosis than Japanese or other Southeastern Asians.

Accordingly, this might be one explanation why PDS is uncommon among Caucasians.

In view of this background, PDS could be an interesting disease case to study coronary heart disease (CHD), which is independent of severity of coronary atherosclerosis and plaque ruptures in spite of remnant hyperlipoproteinemia. Significantly younger age of PDS cases compared to the other SCD cases may be one of the reasons why PDS cases were not associated with severe coronary atherosclerosis. The prevalence of severe coronary atherosclerosis is known to be strongly associated with age.

We found that plasma lipid (TC, TG) and lipoprotein (LDL-C, RLP-C, and RLP-TG) levels were significantly elevated in these sudden cardiac death cases as compared with those in control death cases when the severity of coronary atherosclerosis was pathologically graded above (1+), reflecting the clinical feature of severe coronary atherosclerosis (97-99). Most of the coronary arteries in PDS cases were pathologically graded as (−) and (±), indicating no coronary atherosclerosis [97].

Plasma LDL-C in SCD cases was shown to be highly correlated with the severity of coronary artery atherosclerosis [99]. This is in line with the perception (albeit by implication) that LDL-C plays a major role in the progression of coronary atherosclerosis in CHD patients.

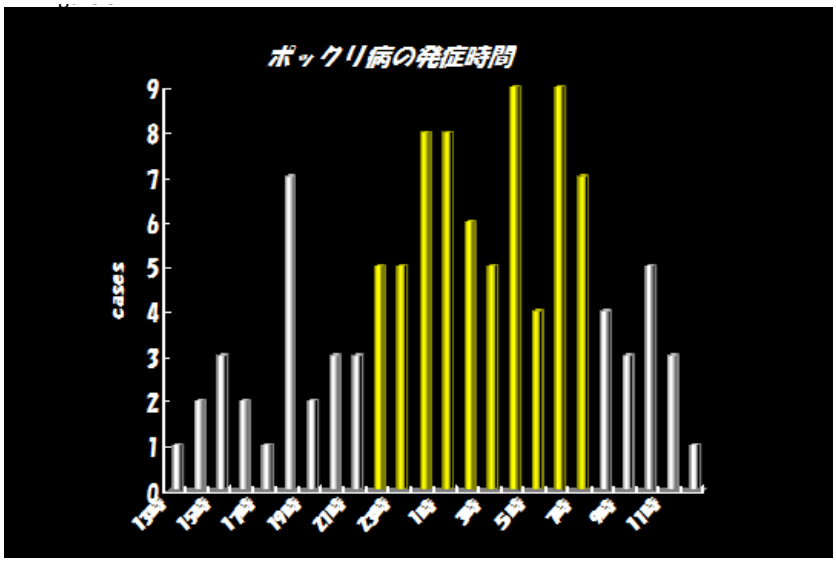

Figure 3. The prevalence of sudden cardiac death occurred during the day time. Sudden cardiac death occurred most frequently at around 2 PM at midnight. (Takeichi et al, *Int. J. Legal Med.* 1997;110: 213-219.).

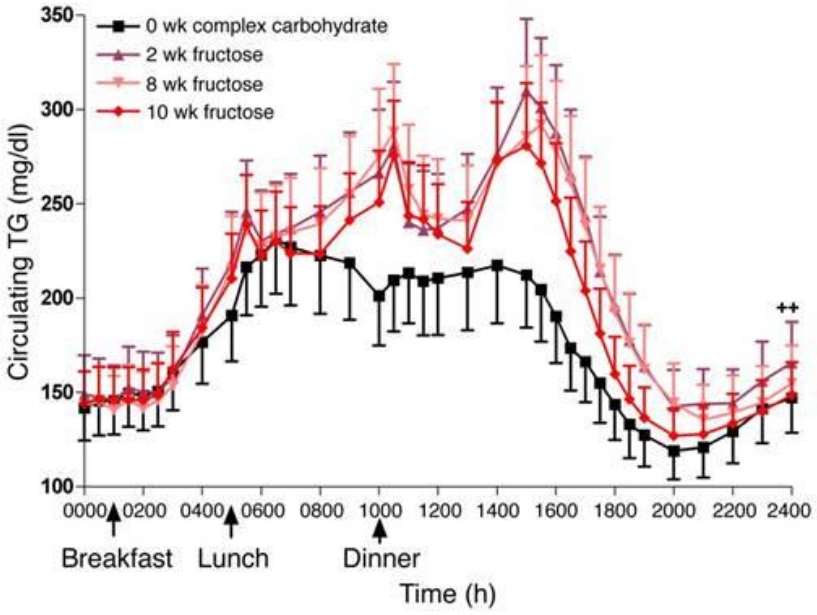

Figure 4. The plasma TG levels were found to be significantly increased during the day associated with food intake. Only in the early morning did the TG levels in all cases returned to the basal levels, and were highest in the middle of the night at regular food intake with additional glucose and fructose beverage (Stanhope et al, J Clin Invest. 2009; 119: 1322-34.).

We found that the incidence of elevated plasma LDL-C was significantly greater in SCD cases with coronary atherosclerosis compared with than in controls and PDS cases. However, plasma LDL-C levels were all within normal range in PDS cases [102].

Hence, LDL-C did not seem to play a significant role at cardiovascular events in PDS, despite being slightly elevated within normal range, rather the data strongly indicated an association between plasma LDL-C and the progression of coronary atherosclerosis in SCD cases. Elevated plasma remnant lipoproteins (RLP) levels were the most striking observation in PDS (RLP-C likelihood ratio; 3.13, RLP-TG; 2.73, LDL-C; 1.52, TC; 1.30, TG; 1.07) for predicting sudden cardiac death in the fasting (gastric content; absent) and postprandial state (gastric content; present) (Table 2).

Despite the high plasma concentration of RLPs in PDS cases, the progression of atherosclerosis at coronary arteries was not observed. It might be valid to say that increased plasma RLPs may initiate the vascular endothelial damage and this is followed by an influx of large amounts of LDL into the vascular wall. Then it follows to form an advanced atherosclerotic lesion with macrophages and smooth muscle cells as Nakajima et al reviewed previously [6]. PDS cases may be in the early stage of atherosclerosis, which can lead cardiovascular events under certain conditions such as with severe stress without strong morphological changes.

Therefore, we proposed that the occurrence of cardiovascular events at coronary arteries and the severity of atherosclerotic lesions in CHD should be considered as separate factors. Therefore, the intervention should be targeted to suppress the cardiovascular events more aggressively than to slow down the progression of atherosclerosis. Takeichi and Fujita did not observe frequent plaque ruptures in coronary arteries at autopsy in Japanese SCD cases [102].

The literature on atherosclerosis has long been dominated by data in Caucasian patients who in most cases had severe atherosclerosis at the time of fatal clinical events.

Hence, fatal clinical events have been believed to occur in relation to the severity of atherosclerosis in coronary arteries. In contrast, fatal clinical events in PDS cases had occurred in the absence of coronary atherosclerosis or plaque rupture. Plasma LDL-C levels were also within normal range associated with no coronary atherosclerosis in PDS cases.

This again puts more weight on RLP as the causative factor of cardiovascular events. Interestingly, we found that RLP-TG (TG concentration in remnant lipoproteins) was not an indicator for predicting the presence or progression of coronary atherosclerosis even in SCD [102]; however, it was significantly associated with fatal clinical events in SCD including PDS.

Table 1. RLP-C is a CHD risk in females at Framingham Heart Study

Variable	
RLP-C > 75th percentile	
Odds ratio (per year)	2.27
95% Confidence limits	1.37-3.77
P Value	0.0015
TG > 2.25mmol/l(200mg/dl)	
Odds ratio (per year)	1.48
95% Confidence limits	0.71-3.10
P Value	0.30

McNamara JR, Schaefer EJ, et al: Atheroschlerosis 2001;154: 229– 36.

Table 2. Plasma lipid and lipoprotein concentrations in the presence ro absence of gastric contents in sudden coronary death and control cases

Plasma lipid and lipoprotein concentrations in the presence or absence of gastric contents in sudden coronary death and control cases

Absence

	Control (n=17)		Pokkuri disease (n=21)		
	Median	25%-75% Tile	Median	25%-75% Tile	P value*
Cholesterol (mg/dL)	181	112-211	166	133-200	NS
Triglyceride (mg/dL)	116	62-126	115	100-159	NS
RLP-C (mg/dL)	6.0	3.9-12.3	12.0	6.0-16.3	< 0.05
RLP-TG (mg/dL)	39	21-48	69	51-94	<0.001
VLDL-C (mg/dL)	13	4-34	18	15-37	NS
LDL-C (mg/dL)	104	62-149	89	82-114	NS
HDL-C (mg/dL)	34	32-43	42	30-68	NS

Presence

	Control (n=34)		Pokkuri disease (n=36)		
	Median	25%-75% Tile	Median	25%-75% Tile	P value*
Cholesterol (mg/dL)	154	137-188	187	158-237	< 0.05
Triglyceride (mg/dL)	117	80-136	157	93-258	< 0.05
RLP-C (mg/dL)	12.1	8.7-14.2	14.0	9.5-27.8	< 0.05
RLP-TG (mg/dL)	49	43-72	86	60-167	< 0.001
VLDL-C (mg/dL)	23	8-35	28	16-45	NS
LDL-C (mg/dL)	98	69-126	122	95-140	NS
HDL-C (mg/dL)	40	26-65	41	35-54	NS

*Mann-Whitney U test NS, non statistically significant (p>0.05)

Takeichi et al. *Atherosclerosis* 1999;142: 309-315.

The bioactive components co-localized with triglycerides in RLP such as oxidized phospholipids or their metabolites [103] may enhance the formation of coronary vascular lesions and may induce severe spasm in coronary arteries. These results also suggested that triglycerides in RLP were not associated with the progression of atherosclerotic plaques, but cholesterol in RLP was strongly associated with the severity of atherosclerosis [99, 102]. Therefore RLP-TG could be an appropriate diagnostic marker for predicting cardiovascular events but not the severity of coronary atherosclerosis, whereas RLP-C could be a marker for predicting both cardiovascular events and the severity of coronary atherosclerosis. LDL-C could be a marker for predicting the severity of coronary atherosclerosis, but not cardiovascular events. Elevated Oxidized LDL seems to be associated with the presence of vulnerable plaque at blood vessels [6], not a causative factor for the formation or initiation of atherosclerosis because of it extremely low concentration in plasma.

4. Treatment for Cardiovascular Disease; Statins, CETP Inhibitor and Probucol as Therapeutic Drugs

4.1. Stains Increase LDL Receptor in Liver and Remove Plasma Remnant Lipoproteins More Effectively than LDL

HMG-CoA reductase inhibitors (statins) are known to decrease cellular cholesterol synthesis and consequently reduce the hepatic production of VLDL and increase expression

of LDL receptor and lower plasma LDL-C levels [3]. Clinical trials have shown that improvements in plasma LDL-C levels are associated with retardation of atherosclerosis and reduction in coronary artery morbidity and mortality [4,5]. The major mechanism of this therapeutic effect has been recognized as the increase of LDL receptor expression in liver to remove elevated LDL-C in plasma by statins. However, recently, remnant lipoproteins have been increasingly implicated in progression of atherosclerosis, with elevated fasting remnant lipoprotein levels shown to predict clinical events independently in coronary artery disease patients [6]. A major target for remnant lipoprotein research has been postprandial dyslipidemia. Postprandial dyslipidemia has been found to be associated with endothelial dysfunction as an early indicator of atherogenesis [104,105]. Elevated remnant lipoprotein levels have also been associated with coronary endothelial dysfunction, with remnants shown to stimulate expression of proathero-thrombotic molecules in endothelial cells [106, 107].

Hence, the prevention and treatment of atherosclerosis merits pharmacotherapy targeted at regulating postprandial dyslipidemia, namely, RLP. Postprandial RLP are the atherogenic lipoproteins that appear and increase in plasma at the initial step of lipoprotein metabolism after food intake and then change to further metabolized lipoproteins, such as LDL. The postprandial state with increased RLP in plasma continues almost throughout the day, except in the early morning, while this is not the case in LDL. Therefore, RLP are atherogenic risks and should be the primary therapeutic target to prevent cardiovascular disease. Increased LDL is not directly associated with the daily food intake like RLP.

Possible mechanisms suggested for abnormal accumulation of lipoproteins postprandially in plasma are defective clearance via receptor-mediated pathways and/or increased competition for high-affinity processes because of increased numbers of intestinally and hepatically derived particles postprandially.

Plasma RLP containing chylomicron and VLDL remnants isolated from postprandial plasma was used to investigate the comparative reactivity to LDL receptor and VLDL receptor cDNA-transfected cells. We studied whether RLP are bound to LDL receptor more efficiently than LDL because RLP is apoE-rich. RLP competed more efficiently with DiI-β-VLDL than LDL in LDL receptor-transfected cells. These results suggest that RLP is more efficiently bound and internalized into LDL receptor than LDL. In VLDL receptor-transfected cells, RLP was more efficiently bound and internalized through VLDL receptor than β-VLDL particles even though we did not show any difference of binding ability between RLP and β-VLDL in LDL receptor-transfected cells. In contrast to TGRLs, LDL was not recognized by the VLDL receptor as Takahashi et al first reported [108].

It seems that VLDL receptor preferentially binds apoE-rich RLP rather than β-VLDL. These findings indicate that plasma RLP are removed by hepatic LDL receptor and muscular VLDL receptor, and the up-regulation of the expression of these lipoprotein receptors may be a therapeutic approach for anti-atherosclerosis. The fact that LDL receptor preferentially binds TGRLs rather than LDL was elucidated by Kita et al. [109].

They concluded that LDL receptor deficiency induced the primary deficiency of the removal of TGRLs (VLDL and IDL), and subsequently the enhanced conversion of VLDL to LDL was a cause of high plasma LDL level in homozygous Watanabe heritable hyperlipidemic (WHHL) rabbits. As statins decrease cellular cholesterol synthesis and consequently reduce the hepatic production of VLDL and increase expression of LDL receptor[110], these properties suggest that statins may be potential agents for regulating the plasma levels of both RLP and LDL-C. Recent studies showed the effects of high-dose, long-

term statin treatment on the metabolism of postprandial lipoproteins in heterozygous FH [101, 112, 113]. Statins may be able to induce half of normal LDL receptor in heterozygous FH that enhances the removal of RLP and LDL.

However, it has remained unknown whether RLP and LDL are removed by only increased LDL receptor expression with statin treatment or whether other lipoprotein receptors are working. It is likely that normal LDL receptor expression in heterozygous FH has already been up-regulated maximally by low-dose statins, and other mechanisms for reducing TGRLs (including RLP) and LDL particles may be working in the case of strong statins. In this study, we found that pitavastatin (NK-104) induced VLDL receptor expression in skeletal muscle cells (L6 cells) at significantly high concentration (approximately 1,000-fold) compared with the effect of NK-104 on LDL receptor expression in HepG2 (114)

The direct comparison between RLP and LDL has shown that RLP have superior binding and internalization reactivity to LDL receptor, which is similar to the reactivity with VLDL receptor. These results suggest that RLP may be more primarily and efficiently catabolized in liver than LDL through increased LDL receptor expression by statin treatment. The removal of TGRLs (including RLP) by LDL receptor may induce decreased plasma LDL-C level because of the removal of precursors of LDL. Additionally, the induction of muscular VLDL receptor expression by strong statins may be one of the therapeutic targets for reducing plasma RLP, but we need to be cautious about the strong statin-induced muscular VLDL receptor expression in terms of rhabdomyolysis.

4.2. CETP Inhibitor

4.2.1. CETP Inhibitors Inhibit the Formation of Remnant Lipoproteins Primarily and then Increase HDL-C as the Result

RLP is known to be formed by catabolizing of TRL (CM, VLDL) with a decrease in TG and an increase in cholesterol, so it is predicted that lipase (LPL, HL) and CETP are involved in the RLP formation [115]. Previous studies have shown that in vitro lipolysis of VLDL by exogenous LPL enhanced CETP-mediated CE transfer from HDL to VLDL [116], and CE in HDL is preferentially transferred to VLDL-1 in the postprandial state of type IIB hyperlipidemia [117]. Moreover, it has been reported that plasma CETP activity was increased coincidently with the increase of postprandial plasma TG [118] and was closely correlated with RLP-C level in patients with nephritic range proteinuria [119]. Although these reports suggest that CETP is involved in the formation of cholesterol-rich remnant particles, we have performed the study to obtain more direct evidence of the involvement of CETP in it [120].

The present study demonstrated that RLP-C is increased by 37 °C incubation of human plasma. As the amounts of RLP-C increase were positively correlated with plasma TG levels (r^2 = 0.555), the RLP-C increase was suggested to indicate the RLP formation from TRL. Also, the 37 °C incubation of plasma promoted the CE transfer from HDL to RLP. As there was a closely relationship between the RLP-C increase and the CE transfer to RLP (r^2 = 0.908, plasma TG levels of >100 mg/dl), the CE transfer from HDL to RLP lead to the RLP-C increase. Furthermore, exogenous r-CETP of physiological concentrations (0.5-1.5 μg/ml) promoted the RLP-C increase dose-dependently, and inhibition of endogenous CETP by JTT-705 (CETP inhibitor, Japan Tabaco) and the monoclonal antibody JHC1 suppressed the RLP-

C increase and the CE transfer to RLP with IC_{50} values similar to those for the inhibition of CETP activity. These all results demonstrate that CETP is essential to the RLP-C increase in this in vitro study.

Guerin et al. have reported that elevated rates of CETP-mediated CE transfer from HDL to TRL are intimately associated with the enhanced formation and accumulation of CE-rich RLP in type IIB hyperlipidemin subjects during the postprandial phase [117]. Therefore, we have examined CE transfers from HDL to RLP and non RLP in TRL fraction ($d < 1.006$ g/ml) and the effects of JTT-705 on the transfers. Although the amounts of CE transfer to RLP and non RLP were not so different, the inhibitory effect of JTT-705 was stronger than that on non RLP. The result suggests that CETP did not only transfer CE to RLP and non RLP, but also CETP promoted the novel RLP formation from non RLP in TRL through the CE-TG exchange, namely, JTT-705 inhibited the increase of RLP-C directly and indirectly.

As the result of examination of CE transfer from HDL to IDL (VLDL remnants), CE transfer to IDL was observed and CE transfer was also inhibited by JTT-705. Although IDL was obtained by the ultracentrifugation, these results were same as that observed in RLP measured using the immunoaffinity gels and, therefore, support that the increase of RLP-C in this study corresponds to the increase of cholesterol in remnant lipoproteins.

On the other hand, it has been reported that plasma LCAT activity was also increased in 2 h or 6 h [118] postprandially, so the effect of LCAT inhibitor on RLP-C increase was examined. As LCAT inhibitor DTNB did not affect on RLP-C increase up to 1 mM, LCAT was supposed to be less involved in the RLP-C increase in the present study. In conclusion, the results of this study demonstrate that CETP promotes the formation of cholesterol-rich RLP through the transfer of CE from HDL to TRL and the inhibition of CETP activity is expected to decrease RLP-C level effectively. Recently, plasma CETP levels were shown be significantly correlated with CAD risk among subjects with high TG levels [121], and it is possible that these subjects with high CETP and high TG levels have high levels of RLP-C. Although HMG-CoA reductase inhibitors have been reported to decrease RLP-C in patients with hypercholesterolemia [122] and Type-2 diabetes [123], CETP inhibitors [124] are also useful for the reduction of RLP-C level.

4.2.2. The Role of CETP and ANGPTL3 for Increasing Plasma HDL-C Levels. ANGPTL3 Inhibit HTGL Activity and Increase HDL-C More Prevalently than CETP Inhibition

We determined serum ANGPTL3 and CETP levels in HALT cases (HDL>90 mg/dl) and compared the abnormal frequencies of these proteins which are known to be associated with increased HDL-C [125]. This study did not focus on the gender differences in these proteins, because the normal range of the standardized parameter “HDL-C” is known to be the same in men and women as seen in Japanese reference range, which is different from USA reference range for HDL-C. To determine the normal range of ANGPTL3 in Japanese population, we developed our own sandwich ELISA of ANGPTL3 for this study. ANGPTL3 determined by other ELISA methods showed either higher serum levels [126] or different clinical significance when compared with our method [127]. Also we recruited cases with very high HDL-C (HALT) at the health check-up centers randomly and expected to find either low CETP [128] or high ANGPTL3 [129-131]. As previously reported [131], these trends were not so clear in moderately high HDL-C cases, however we found significantly high ANGPTL3 levels in cases with HDL-C above 90 mg/dl in this study. Therefore we focused

on the analysis of ANGPTL3 and CETP in HALT cases for finding a convincing difference between the two proteins on HDL metabolism. High HDL-C cases like above 100 mg/dl seem to be difficult to find in Caucasians [132], while such cases are prevalent in Japanese population [133]. One of the causes is suspected to be a high frequency of CETP polymorphism in the Japanese population [134, 135]. However, this study may present a different mechanism for the high frequency of HALT in Japanese population. ANGPTL3 is now known to be a major inhibitor of EL [131]. Therefore, plasma ANGPTL3 concentration may reflect EL activity in plasma via the ANGPTL3-EL pathway [136]. Our preliminary data obtained with the newly developed EL activity assay showed that ANGPTL3 concentration correlated inversely with EL activity (r=−0.27, Pb0.005) and HDLC (r=−0.13, Pb0.05) in post-heparin plasma, but not in pre-heparin plasma, indicating that high ANPTL3 plasma levels are associated to low EL activities and high HDL-C (authors' unpublished observations). Badellino et al. [137] recently developed an ELISA for measurement of human plasma EL. In this study, median EL mass in pre-heparin plasma was 442 ng/ml (interquartile range=324–617). Median postheparin mass was approximately 3-fold higher, 1313 ng/ml (888– 1927). The correlation between pre-heparin EL mass and postheparin EL mass was 0.46 and both assays showed inverse correlation with HDL-C. These results showed similar trend with those of the new EL activity assay method we are now developing. Comparative studies between EL mass and EL activity including the concentration of ANGPTL3 in plasma need to be studied further as LPL and HTGL assays [138]. Recently, CETP inhibitors [124] have been developed and several reports from clinical trials have already been published [139-141], reporting significant anti-atherogenic effects in experimental animals, but not yet in humans. HDL-C levels were significantly increased by CETP inhibitors, but these HDL particles became very large in size like those in CETP deficient cases [142]. Further, CETP inhibitor molecules adhere to and are carried on HDL particles in the blood, which could be considered as an unwanted interaction [140, 141]. Therefore the efficacy of CETP inhibitors is yet to be accepted [143]. The frequency of CETP deficiency or polymorphism seems to be very rare in Caucasians, but quite frequent in Japanese. Therefore, if the ANGPTL3-EL pathway unlike CETP is not physiologically impaired in Caucasians, it could be a better target for raising HDL-C. This study showed approximately 10 fold higher frequency of abnormally increased ANGPTL3 in HALT cases as compared with the frequency of low CETP. For sometime, we have been speculating that the prevalence of CETP polymorphism or deficiency may be a major cause of high HDL-C in Japanese population [144, 145]. The CETP assay kit we used in this study showed comparatively lower serum levels of CETP than the levels assayed by Dai-ichi ELISA kit (the most frequently used assay kit), which also indicated a high correlation between activity and mass of CETP in average Japanese population [146].

Therefore, we have calculated the normal range of CETP independently in cases with normal HDL-C in this study subjects and determined the low cut-off value as mean-2SD. We also compared the abnormal low CETP and high ANGPTL3 frequencies in HALT cases by 10–90 percentile analysis. CETP less than 10% tile was 21.3% and ANGPTL3 above 90% tile was 76.4% in this study subjects, respectively. Another reason for 10% tile of CETP as the lower cut-off value was the frequency of CETP common mutation (approximately 10%) in average Japanese population [134].

Both mean±2SD analysis and 10–90 percentile analyses showed significantly greater frequency of abnormally high ANGPTL3 than those of low CETP. Therefore we could

predict the possibility that the ANGPTL3-EL pathway, including hepatic preprotein convertase [136], may have a major physiological role in HDL metabolism. As Ishida et al. [147] proposed EL as the major pathway for HDL metabolism in mouse, we also suggest that ANGPTL3- EL or HTGL is the major pathway for HDL metabolism in humans regardless of the presence or absence of CETP. Interestingly, there was a high correlation between ANGPTL3 and HDL-C in seven CETP deficient cases in this study.

This means that ANGPTL3-EL or HTGL pathways may independently associate with the increase of HDL-C from that of CEPT. CETP deficiency cases were found to be not always associated with high HDL-C from these cases. We have been interested in finding the mechanism of HDL-C decrease after probucol treatment. Miida et al. [148] recently reported that probucol significantly reduced serum ANGPTL3 levels and increased preβ1-HDL, indicating the possibility of increasing the EL activity by reducing ANGPTL3, the inhibitor of EL. However, as yet, an EL activity assay has not been reported, probably due to the catalytic activity in the human EL being inhibited by ApoC-II in serum.

This makes it difficult to distinguish EL activity from LPL/HTGL activity in the human post-heparin plasma [138]. Therefore, ANGPTL3 concentration may take the place of EL or HTGL activity, similar to ApoC-III for LPL activity [138] and remnant lipoproteins [149]. These drawbacks will continue until a direct plasma EL activity assay has been developed to compare directly with CETP activity. Further studies on the polymorphism of ANGPTL3 are warranted for investigating a more direct relationship between ANGPTL3 and EL activity.

4.3. Probucol, a New Life for Old Drug

Probucol has demonstrable anti-inflammatory actions that contribute to a reduction in experimental atherosclerosis. Also, probucol reduces the adhesion of inflammatory cells *in vivo* as demonstrated by the inhibition of mononuclear cell adhesion and reduced expression of vascular cell adhesion molecule [150] following balloon injury in hypercholesterolemic rabbits. Similarly, in LDL receptor-deficient Watanabe heritable hyperlipidemic (WHHL) rabbits, the fibrous cap in the lesions of animals fed probucol had lower macrophage content, but an increase in vascular smooth muscle cells [151]. Taken together, these studies demonstrate that probucol reduces atherosclerosis and cardiovascular disease while improving the state of inflammation and heightened oxidative stress in the affected blood vessels.

4.3.1. Protective Effects of Probucol on Vascular Endothelial and Smooth Muscle Cells

Endothelial dysfunction is associated with an increased risk of cardiovascular events and a vasoconstrictor response to acetylcholine indicates the presence of endothelial dysfunction[152]. The endothelium-dependent vasomotor response to acetylcholine is significantly attenuated in humans by oral treatment with probucol. In support of this, probucol promotes the growth of endothelial cells and promotes endothelium-dependent arterial relaxation and functional re-endothelialization following aortic balloon injury, as measured by the extent of re-endothelialization, nitric oxide production and nitric oxide-mediated vasodilatation [153]. There are a number of potential mechanisms by which probucol may enhance endothelial function. First, probucol protects against hypochlorite-

mediated, endothelium-dependent relaxation of the aorta in rabbits *in vivo* [154]. Second, the effects of probucol on increasing functional re-endothelialization and inhibiting smooth muscle proliferation [153] are similar to biological processes seen with the increased activity of heme oxygenase-1 (HO-1) [155]. Similarly, pharmacological inhibition of heme oxygenase activity blocks the ability of probucol to promote re-endothelialization and to inhibit intimal hyperplasia following vascular injury *in vivo* [155]. These new findings suggest that probucol's effects on endothelial cell growth and function and on inhibition of smooth muscle proliferation are greatly mediated by HO-1. Increasingly, new evidence points to a key role of HO-1 as anti-oxidant property (156-158). Probucol increases the expression of HO-1 and heme oxygenase activity in balloon-injured rabbit aortas and rabbit aortic smooth muscle cells [155]. HO-1 is a redox-sensitive enzyme and the promoter region of the HO-1 gene contains multiple copies of antioxidant response elements that are critical for enzyme induction [159] and that are tightly regulated by the redox-sensitive transcription factor NrF-E2-related factor-2. Unlike probucol, vitamin E does not induce HO-1 in vascular smooth muscle cells *in vitro* [155], consistent with the notion that 2-electron, but not 1-electron, antioxidants may exhibit part of their anti-atherosclerotic effects via induction of HO-1. Similar antioxidant proteins and enzymes such as metallothionein and SOD are also known to be significantly induced by probucol associated with NrF-2 gene expression [159]

4.3.2. Lipid Metabolism and Cholesterol Efflux by Probucol

Probucol's cholesterol-lowering activity was first discovered in 1964 during screening of phenolic antioxidants. The drug is relatively weak when compared to statins, 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitor. How probucol reduces plasma and LDL cholesterol still remains uncertain. However, early studies in LDL receptor-deficient WHHL rabbits demonstrated that probucol reduced the atheroma burden independent of the drug's cholesterol-lowering effects [160]. In fact, the ability of probucol to inhibit experimental atherosclerosis independent of the drug's lipid-lowering effect was one of the corner stone of the oxidative modification theory of atherosclerosis. Abundant data support the oxidative modification hypothesis of atherosclerosis, according to which oxidation of LDL is an early event in, and contributes to, atherogenesis [161]. Although probucol was designed originally as a phenolic antioxidant, the peroxyl radical scavenging activity of probucol is only ~16% that of the most abundant phenolic antioxidant and form of vitamin E in humans, α-tocopherol. Yet, early animal studies consistently reported that probucol reduced lipoprotein oxidation in plasma *ex vivo* or in the vessel wall [162]. However, the oxidative modification hypothesis of atherosclerosis has been recently challenged by the failure of antioxidants, in particular vitamin E [163], to reduce disease progression and clinical events in patients at risk of, or with established, atherosclerosis. Subsequent studies have dissociated the anti-atherosclerotic effects of probucol from its ability to affect lipoprotein lipid oxidation. First, in rabbits, probucol inhibited atherosclerosis to a greater extent than probucol analogs which had greater anti-oxidant activity [164]. Furthermore, probucol did not alter the proportion of aortic lipids that are oxidized. Thus, while probucol exhibit anti-oxidant activity, this property does not seem to account for its anti-atherosclerotic properties. Previously, it had been thought that the strong antioxidant properties of probucol arose from the two phenol moieties. Recent evidence suggests, however, that the sulfur moiety in probucol is critical for *in vivo* protection from atherosclerosis [155] Thus, probucol and probucol dithiobisphenol, but not the sulfur-free probucol bisphenol, inhibited disease in apolipoprotein E-deficient mice and

hypercholesterolemic rabbits following aortic balloon injury. Paradoxically, probucol lowers HDL cholesterol yet inhibits atherosclerosis. The controversial and anti-atherogenic feature of probucol is most likely attributable to molecular mechanisms: promoting cholesterol efflux, and enhancing reverse cholesterol transport (RCT) by activation of CETP [134, 165] and scavenger receptor class B type 1 (SR-B1)[166]. The apparent reduction of HDL-C by probucol may be due to the remodeled function of HDL: increased preβ1-HDL [148] ("lipid-poor" apoA-1) participating in cellular lipid efflux. These mechanisms could be responsible for probucol-induced regression of xanthoma and its anti-atherogenic effects, though probucol is an effective inhibitor of ABCA1-mediated cholesterol efflux [167]. A recent report by Miida et al [148] suggested the possibility that probucol activates the endothelial lipase by inhibiting angiopoietin-like protein3 (ANGPTL3) and enhance HDL metabolism and cholesterol efflux by increasing preβ1-HDL levels. Contrary to the results of studies using WHHL rabbits, some experimental studies using apolipoprotein E-knockout mice reported that probucol demonstrated pro-atherogenic effects [168]. These contradictory findings in mice with no CETP may rather support the hypothesis regarding cholesterol efflux and the RCT pathway of probucol through CETP. An important role of CETP in the mechanism of cholesterol efflux and RCT is evident in the recent negative clinical trial results of a CETP inhibitor [169] as well as the epidemiological reports [170] of increased coronary heart disease in patients with CETP deficiency and the molecular approach to review of CETP deficiency [171]. Marked hyper-HDL2 cholesterolemia associated with CETP deficiency can be atherogenic regardless of elevated HDL-C, which has been indicated in a long-ignored Japanese report by Matsuzawa et al [172]. The CETP activator instead of the inhibitor might provide a partly rational therapeutic approach to prevent atherosclerosis, after a review of the evolving field of pro-atherogenic HDL, a novel role for human CETP in the defense against an exacerbated production of pro-inflammatory mediators [173], and CETP polymorphism among individuals or ethnics groups [174].

Over the past several decades, probucol has established itself as a potent anti-oxidant with a broad spectrum of other pharmacologic actions; further, it has demonstrated significant therapeutic effects on diverse diseases in humans. Its mechanisms of actions at the molecular level have recently been elucidated and are as diverse as its therapeutic effects. More recently, probucol was demonstrated to significantly reduce CHD risks in patients with heterozygous FH who have very high CHD risks [175]. It is anticipated that probucol will overtake agents like statins, which are reported to lower LDL-C, which is a well known CHD risk factor. There is compelling reason to believe that this old and often misunderstood drug has much more to offer than hitherto known even if it reduces HDL-C levels.

CONCLUSION

Oxidative modification of LDL (Ox-LDL) has been widely believed to play a key role in the initiation and progression of atherosclerosis since Steinberg et al. first proposed this hypothesis in 1989 [2]. This concept has provided strong support for the efficacy of LDL-C lowering drugs. Several atherosclerotic phenomena such as the progression of atherosclerotic lesion have been explained by this hypothesis, but it is equally important to address issues which do not support Ox-LDL per se as the most important risk factor for the initiation of

atherosclerosis. For example, the concentration of Ox-LDL in plasma may be less than 0.5% of total LDL in CHD patients and to which the endothelial cells are exposed. This plasma concentration may not be enough for proatherogenic and proinflammatory activities of Ox-LDL and the initiation of ‘‘response-to-injury’’ in endothelial cells, judging from the results of many in vitro studies.

The most significant role of Ox-LDL in atherogenesis has been explained in the subendothelial space for interactions with macrophages and smooth muscle cells, not in endothelial cells. The discovery that LOX-1 receptor activation by RLP by Shin et al. [8] has opened the window for a new oxidative modification hypothesis that says RLP but not Ox-LDL in plasma is a major ligand for the LOX-1 receptor in endothelial cells, causing endothelial dysfunction and the initiation of atherosclerosis. RLP are already oxidized in plasma and reported the existence of large amount of lysophospahtidylcholine in cylomicron remnants, a constituent of RLP and their proatherogenic and proinflammatory properties. In other words, major lipoproteins oxidized in plasma may be remnant lipoproteins and not LDL, existing as an oxidative modification.

Ox-LDL and RLP revealed common proatherogenic and proinflammatory characteristics, indicating the same biological functions shown at similar concentrations in in vitro studies. This may suggest that the same oxidized phospholipids exist in these lipoprotein particles which mediate their atherogenicity. The isolation method of RLP from plasma developed by Nakajima and co-workers [6] made it possible to verify the oxidative susceptibility of remnant lipoproteins and to compare the proatherogenic and proinfalmmatory properties of RLP with those of Ox-LDL.

Plasma RLP-C concentration has been shown to be more convincingly associated with the increased risk of premature atherosclerosis by many clinical studies than plasma circulating Ox- LDL. Further, endothelial dysfunction is more likely to be caused by RLP than by circulating Ox-LDL per se in plasma, judging from the plasma concentration of these lipoproteins. Taken together, reducing plasma RLP rather than LDL should be the target of hyperlipidemic therapy especially in patients with metabolic syndrome which is highly associated with plasma RLP-C level, but not with LDL-C levels.

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REFERENCES

[1] Ross R, Glomset JA. The pathogenesis of atherosclerosis. *N. Engl. J. Med.* 1976;295:369– 77.

[2] Steinberg D, Parthasarathy S, Crew TE, Khoo JC, Witztum JL. Beyond cholesterol: modification of low-density lipoprotein that increase its atherogenecity. *N. Engl. J. Med.* 1989;320:915–24.

[3] Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. *Science* 1986; 232:34-47.

[4] Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet* 1994; 344:1383-1389.

[5] Sacks FM, Pfeffer MA, Moye LA, et al. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N. Engl. J. Med.* 1996; 335:1001-1009.

[6] Nakajima K, Nakano T, TanakaA. Article title: The oxidative modification hypothesis of atherosclerosis: the comparison of atherogenic effects on oxidized LDL and remnant lipoproteins in plasma. *Clin. Chim. Acta*. 2006; 367:36-47.

[7] Sawamura T, Kume N, Aoyama T, et al. An endothelial receptor for oxidized low-density lipoprotein. *Nature* 1997;386:73–7.

[8] Shin HK, Kim YK, Kim KY, Lee JH, Hong KW. Remnant lipoprotein particles induce apoptosis in endothelial cells by NAD(P)H oxidase-mediated production of superoxide and cytokines via lectin-like oxidized low-density lipoprotein receptor-1 activation: prevention by cilostazol. *Circulation* 2004;109:1022 –8.

[9] Goldstein JL, Ho YK, Basu SK, Brown MS. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Axad. Sci. USA* 1979;76:333– 7.

[10] Henriksen T, Mahoney EM, Steinberg D. Enhanced macrophage degradation of low density lipoprotein previously incubated with cultured endothelial cells: recognition by receptors for acetylated low density lipoproteins. *Proc. Natl. Acad. Sci. USA* 1981;78: 6499–503.

[11] Kodama T, Reddy P, Kishimoto C, Krieger M. Purification and characterization of a bovine acetyl low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA* 1988;85: 9238– 42.

[12] Endemann G, Stanton LW, Madden KS, Bryant CM, White RT, Protter AA. CD36 is a receptor for oxidized low density lipoproteins. *J. Biol. Chem.* 1993;268:11811 –6.

[13] Shimaoka T, Kume N, Minami M, et al. Molecular cloning of a novel scavenger receptor for oxidized low density lipoprotein, SR-PSOX, on macrophages. *J. Biol. Chem.* 2000;275:40663– 6.

[14] Goldstein JL, Brown MS. The low density lipoprotein pathway and its relation to atherosclerosis. *Annu. Rev. Biochem.* 1977;46:897–930.

[15] Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc. Natl. Acad. Sci. USA* 1984;81:3883– 7.

[16] Haberland ME, Fogelman AM, Edwards PA. Specificity of receptormediated recognition of malondialdehyde-modified low density lipoproteins. *Proc. Natl. Acad. Sci. USA* 1982;79:1712– 6.

[17] Haberland ME, Olch CL, Fogelman AM. Role of lysines in mediating interaction of modified low density lipoproteins with the scavenger receptor of human monocyte macrophages. *J. Biol. Chem.* 1984;259:11305– 11.

[18] Esterbauer H, Gebicki J, Puhl H, Jurgens G. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radic. Biol. Med.* 1992;13:341–90.

[19] Boyd HC, Gown AM, Wolfbauer G, Chait A. Direct evidence for a protein recognized by a monoclonal antibody against oxidatively modified LDL in atherosclerotic lesions from a Watanabe heritable hyperlipidemic rabbit. *Am. J. Pathol.* 1989;135:815– 25.

[20] Harberland ME, Fong D, Cheng L. Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. *Science* 1988;241:215–8.

[21] Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witztum JL. Apo E deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. *Arterioscler. Thromb* 1994;14:605– 16.

[22] Palinski W, Rosenfeld ME, Yia-Herttuala S, et al. Low density lipoprotein undergoes oxidative modification in vivo. *Proc. Natl. Acad. Sci. USA* 1989;86:1372– 6.

[23] YIa-Herttuala S, Painski W, Rosenfeld ME, et al. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J. Clin. Invest.* 1989;84: 1086–95.

[24] Khoo JC, Miller E, McLoughlin P, Steinberg D. Enhanced macrophage uptake of low density lipoprotein after self-aggregation. *Arteriosclerosis* 1988;8:348– 58.

[25] Frank JS, Fogelman AM. Ultrastructure of the intima in WHHL and cholesterol-fed rabbit aortas prepared by ultra-rapid freezing and freeze-etching. *J. Lipid. Res.* 1989;30:967– 78.

[26] Camejo G, Hurt-Camejo E, Wiklund O, Bondjers G. Association of apoB lipoproteins with arterial proteoglycans: pathological significance and molecular basis. *Atherosclerosis* 1998;139:205– 22.

[27] Havel J. Role of triglyceride-rich lipoproteins in progression of atherosclerosis. *Circulation* 1989;81:694– 6.

[28] Phillips NR, Waters D, Havel R. Plasma lipoproteins and progression of coronary artery disease evaluated by angiography and clinical events. *Circulation* 1993;88:2762–70.

[29] Tomono S, Kawazu S, Kato N, et al. Uptake of remnant like particles (RLP) in diabetic patients from mouse peritoneal macrophages. *J. Atheroscler. Thromb* 1994;1:98–102.

[30] Kawakami A, Tani M, Chiba T, et al. Pitavastatin inhibits remnant lipoprotein-induced macrophage foam cell formation through apoB-48 receptor-dependent mechanism. *Arterioscler. Thromb Vasc. Biol.* 2005;25:424– 9.

[31] Kawakami A, Tanaka A, Nakajima K, Shimokado K, Yoshida M. Atorvastatin attenuates remnant-induced monocyte adhesion to vascular endothelium under flow conditions. *Circ. Res.* 2002; 91:263–71.

[32] Nicoll A, Duffield R, Lews B. Flux of plasma lipoproteins into human arterial intima. *Atherosclerosis* 1981;9:229– 42.

[33] Shaikh M, Wootton R, Nordestgaard BG, et al. Quantitative studies of transfer in vivo of low density, Sf 12-60 and Sf 60-400 lipoproteins between plasma and arterial intima in humans. *Arterioscler. Thromb* 1991;11:569– 77.

[34] Vasile E, Antohe F, Siminonescu M, Siminonescu N. Transport pathway of beta VLDL by aortic endothelial cells of normal and hypercholesterolemic rabbit. *Atherosclerosis* 1989;75:195– 210.

[35] Nordestgaard BG, Tybjaerg -Hansen A. Influx in vivo of low density, intermediate density and very low density lipoproteins into aortic intimas of genetically hyperlipidemic rabbits. *Arterioscler. Thromb* 1992;1:6–18.

[36] Rapp JH, Lespine AL, Hamilton RL, et al. Triglyceride-rich lipoproteins isolated by selected affinity anti-apolipoprotein B immunosorption from human atherosclerotic plaque. *Arterioscler. Thromb* 1994;14:1767– 74.

[37] Schwenke DC, Carew TE. Initiation of atherosclerotic lesion in cholesterol-fed rabbits: II. Selective retention of LDL vs. selective increases in LDL permeability in susceptible sites of arteries. *Arteriosclerosis* 1989;9:908– 18.

[38] Schwenke DC, Zilversmit DB. The arterial barrier to lipoprotein influx in the hypercholesterolemic rabbit: I. Studies during the first two days after mild aortic injury. *Atherosclerosis* 1989;77:91 –103.

[39] Frei B, Stocker R, Ames BN. Antioxidant defenses and lipid peroxidation in human blood plasma. *Proc. Natl. Acad. Sci. USA* 1988;85:9748– 52.

[40] Sevanian A, Hwang J, Hodis H, Cazzolato G, Avogaro P, Bittolo-Bon G. Contribution of an in vivo oxidized LDL to LDL oxidation and its association with dense LDL subpopulations. *Arterioscler. Thromb Vasc. Biol.* 1996;16:784– 93.

[41] Holvoet P, Perez G, Zhao Z, Brouwers E, Bernar H, Collen D. Malondialdehyde-modified low-density lipoprotein in patients with atherosclerotic disease. *J. Clin. Invest.* 1995;95:2611– 9.

[42] Palinski W, Horkko S, Miller E, et al. Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from apolipoprotein E-deficient mice. Demonstration of epitopes of oxidized low density lipoprotein in human plasma. *J. Clin. Invest.* 1996;98:800– 14.

[43] Holvoet P, Collen D, Van de Werf. Malondialdehyde-modified LDL as a marker of acute coronary syndromes. *JAMA* 1999;281:1718 – 21.

[44] Holvoet P, Vanhaecke J, Janssens S, Van de werf F, Collen D. Oxidized LDL and malondialdehyd-modified LDL in patients with acute coronary syndromes and stable coronary artery disease. *Circulation* 1998;98:1487– 94.

[45] Itabe H, Yamamoto H, Suzuki M, et al. Oxidized phosphatidylecholins that modify proteins. Analysis of monoclonal antibody against oxidized low density lipoproteins. *J. Biol. Chem.* 1996;271:33208– 17.

[46] Itabe H, Yamamoto H, Imanaka T, et al. Sensitive detection of oxidatively modified low density lipoprotein using a monoclonal antibody. *J. Lipid. Res.* 1996;37:45 – 53.

[47] Toshima S, Hasegawa A, Kurabayashi M, et al. Circulating oxidized low density lipoprotein levels. A biochemical risk marker for coronary heart disease. *Arterioscler. Throb Vasc. Biol.* 2000; 20:2243–7.

[48] Ehara S, Ueda M, Naruko T, et al. Elevated levels oxidized low density lipoprotein show a positive relationship with the severity of acute coronary syndromes. *Circulation* 2001;103:1955 –60.

[49] Ehara S, Ueda M, Naruko T, et al. Pathophysiological role of oxidized low-density lipoprotein in plaque instability in coronary artery disease. *J. Diabetes Complications* 2002;16:60– 4.

[50] Tsimikas S, Brilakis ES, Miller ER, et al. Oxidized phospholipids, Lp(a) lipoproteins, and coronary artery disease. *N. Engl. J. Med.* 2005;353:46– 57.

[51] Tsimikas S, Witztum JL. Measuring circulating oxidized low density lipoproteins to evaluate coronary risk. *Circulation* 2001; 103:1930– 2.

[52] Palinski W, Witztum JL. Immune responses to oxidative neoepitopes on LDL and phospholipids modulate the development of atherosclerosis. *J. Intern. Med.* 2000;247:371– 80.

[53] Maggi E, Chiesa R, Melissano G, et al. LDL oxidation in patients with severe carotid atherosclerosis. A study of in vitro and in vivo oxidation markers. *Arterioscler. Thromb.* 1994;14:1892– 9.

[54] Salonen JT, Yia-Herttuala S, Yamamoto R, et al. Autoantibody against oxidized LDL and progression of carotid atherosclerosis. *Lancet* 1992;339:883– 7.

[55] Erkkila AT, Narvanen O, Lehto S, Uusitupa MI, Yia-Herttuala S. Autoantibodies against oxidized low-density lipoprotein and cardiolipin in patients with coronary heart disease. *Arterioscler. Thromb Vasc. Biol.* 2000;20:204–9.

[56] Wu R, Nityanand S, Berglund L, Lithell H, Holm G, Lefvert AK. Antibodies against cardiolipin and oxidatively modified LDL in 50-year-old men predict myocardial infarction. *Arterioscler. Thromb Vasc. Biol.* 1997;17:3159– 63.

[57] Bui MN, Sack MN, Moutsatsos G, et al. Autoantibody titers to oxidized low-density lipoprotein in patients with coronary atherosclerosis. *Am. Heart. J.* 1996;131:663– 7.

[58] Bergmark C, Wu R, de Faire U, Lefvert AK, Swedenborg J. Patients with early-onset peripheral vascular disease have increased levels of autoantibodies against oxidized LDL. *Arterioscler. Thromb. Vasc. Biol.* 1995;15:441– 5.

[59] George J, Harats D, Bakshi E, et al. Anti-oxidized low density lipoprotein antibody determination as a predictor of restenosis following percutaneous transluminal coronary angioplasty. *Immunol. Lett.* 1999;68:263–6.

[60] Pratico D, Tangirala aaaark, Horkko S, Witztum JL, Palinski W, FitzGerald GA. Circulating autoantibodies to oxidized cardiolipin correlate with isoprostane F (2alpha) VI levels and the extent of atherosclerosis in apoE-deficient mice: modulation by vitamin E. *Blood* 2001;97:459–64.

[61] Tribble DL, Rizzo M, Chait A, Lews DM, Blanche PJ, Krauss RM. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small, dense low-density lipoproteins. *Am. J. Med.* 2001;110:103– 10.

[62] Liu ML, Ylitalo K, Nuotio I, Salonen R, Salonen JT, Taskinen MR. Association between carotid intima-media thickness and low-density lipoprotein size and susceptibility of low-density lipoprotein to oxidation in asymptomatic members of familial combined hyperlipidemia families. *Stroke* 2002;33:1255– 60.

[63] Campos E, Kotite L, Blanche P, et al. Properties of triglyceride-rich and cholesterol-rich lipoproteins in the remnant-like particle fraction of human blood plasma. *J. Lipid. Res.* 2002;43:365– 74.

[64] Koba S, Hirano T, Murayama S, et al. Small dense LDL phenotype is associated with postprandial increases of large VLDL and remnantlike particles in patients with acute myocardial infarction. *Atherosclerosis* 2003;170:131– 40.

[65] Ando M, Sanaka T, Nihei H. Eicosapentanoic acid reduces plasma levels of remnant lipoproteins and prevents in vivo peroxidation of LDL in dialysis patients. *J. Am. Soc. Nephrol.* 1999;10:2177– 84.

[66] Gordon T, Kannel WB, Castelli, et al. Lipoproteins, cardiovascular diseases and death. The Framingham Study. *Arch. Intern. Med.* 1981;141:1128– 31.

[67] Austin MA, McKnight B, Edwards KI, et al. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: a 20-year prospective study. *Circulation* 2000;101:2777 – 82.

[68] Twickler TB, Dallinga-Thie GM, Cohn JS, Chapman MJ. Elevated remnant-like particle cholesterol concentration: a characteristic feature of the atherogenic lipoprotein phenotype. *Circulation* 2004;109:1918– 25.

[69] Nakajima K, Saito T, Tamura A, et al. Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gel. *Clin. Chim. Acta.* 1993;223:53– 71.

[70] Ikewaki K, Shige H, Nakajima K, Nakamura H. Postprandial remnant-like particles and coronary artery disease. In: Woodford FP, Davignon J, Sniderman A, editors. Atherosclerosis X. *Elservier Science* B.V. p. 200– 2.

[71] Leary ET, Wang T, Baker DJ, et al. Evaluation of an immunoseparation method for quantitative measurement of remnant-like particlecholesterol in serum and plasma. *Clin. Chem.* 1998;44:2490– 8.

[72] Devaraj S, Vega G, Lange R, Grundy SM, Jialal I. Remnant-like particles-cholesterol levels in patients with dysbetalipoproteinemia and coronary artery disease patients. *Am. J. Med.* 1998;104:445– 50.

[73] Tanaka A, Ejiri N, Fujinuma Y, et al. Remnant-like particles and coronary arteries after PTCA. *Ann. N. Y. Acad. Sci.* 1995;748: 595– 8.

[74] Oi K, Shimokawa H, Hirakawa Y, et al. Postprandial increase in plasma concentrations of remnant-like particles: an independent risk factor for restenosis after percutaneous coronary intervention. *J. Cardiovasc. Pharmacol.* 2004;44:66– 73.

[75] Sakata K, Miho N, Shirotani M, Yoshida H, Takada Y, Takada A. Remnant-like particles cholesterol is a major risk factor for myocardial infarction in vasospastic angina with nearly normal coronary artery. *Atherosclerosis* 1998;136:225– 31.

[76] Miwa K, Makita T, Ishii K, Okuda N, Taniguchi A. High remnant lipoprotein levels in patients with variant angina. *Clin. Cardiol.* 2004;27:338–42.

[77] Masuoka H, Ishikura K, Kamei S, et al. Predictive value of remnant-like particle cholesterol/high-density lipoprotein cholesterol ratio as a new indicator of coronary artery disease. *Am. Heart J.* 1998;136:226– 30.

[78] Kugiyama K, Doi H, Motoyama T, et al. Association of remnant lipoprotein levels with impairment of endothelium-dependent vasomotor function in human coronary arteries. *Circulation* 1998; 97:2519–26.

[79] Inoue T, Saniabadi AR, Matsunaga R, Hoshi K, Yaguchi I, Morooka S. Impaired endothelium-dependent acetylcholine-induced coronary artery relaxation in patients with high serum remnant lipoprotein particles. *Atherosclerosis* 1998;139:363– 7.

[80] Takeichi S, Yukawa N, Nakajima Y, et al. Association of plasma triglyceride-rich lipoprotein remnants with coronary atherosclerosis in cases of sudden cardiac death. *Atherosclerosis* 1999;42:309–15.

[81] Takeichi S, Nakajima Y, Yukawa N, et al. Plasma triglyceride-rich lipoprotein remnants as a risk factor of FPokkuri disease_. *Leg. Med.* 2001;3:84–94.
[82] Wang T, Elam MB, Forbes WP, Zhong J, Nakajima K. Reduction of remnant lipoprotein cholesterol concentrations by cilostazol in patients with intermittent claudication. *Atherosclerosis* 2003; 171:337–42.
[83] Karpe F, Boquist S, Tang R, Bond GM, de Faire U, Hamsten A. Remnant lipoproteins are related to intima-media thickness of the carotid artery independently of LDL cholesterol and plasma triglycerides. *J. Lipid. Res.* 2001;42:17– 21.
[84] Masuoka H, Kamei S, Wagayama H, et al. Association of remnantlike particle cholesterol with coronary artery disease in patients with normal total cholesterol levels. *Am. Heart. J.* 2000;139:305–10.
[85] Masuoka H, Kamei S, Ozaki M, et al. Predictive value of remnantlike particle cholesterol as an indicator of coronary artery stenosis in patients with normal serum triglyceride levels. *Intern. Med.* 2000; 39:540– 6.
[86] McNamara JR, Shah PK, Nakajima K, et al. Remnant-like particle (RLP) cholesterol is an independent cardiovascular disease risk factor in women: results from the Framingham Heart Study. *Atherosclerosis* 2001;154:229– 36.
[87] Song J, Park H, Hong SH, Lee HK, Park YB, Kim JQ. Remnant-like particle cholesterol levels in Korean patients with coronary artery disease and non-insulin dependent diabetes mellitus. *Clin. Chem. Lab. Med.* 2000;38:427– 32.
[88] I noue T, Uchida T, Kamishirado H, et al. Remnant-like lipoprotein particles as risk factors for coronary artery disease in elderly patients. *Horm. Metab. Res.* 2004;36:298–302.
[89] Kugiyama K, Doi H, Takazoe K, et al. Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. *Circulation* 1999;99:2858–60.
[90] Fukushima H, Kugiyama K, Sugiyama S, et al. Comparison of remnant-like lipoprotein particles in postmenopausal women with and without coronary artery disease and in men with coronary artery disease. *Am. J. Cardiol.* 2001;88:1370– 3.
[91] Fukushima H, Sugiyama S, Honda O, et al. Prognostic value of remnant-like lipoprotein particle levels in patients with coronary artery disease and type II diabetes mellitus. *J. Am. Coll. Cardiol.* 2004;43:2219– 24.
[92] Chan DC, Watts GF, Barrett PH, Mamo JCL, Redgrave TG. Markers of triglyceride-rich lipoprotein remnant metabolism in visceral obesity. *Clin. Chem.* 2002;48:278– 83.
[93] Chan DC, Watts GF, Barrett PH, O'neill FH, Redgrave TG. Relationships between cholesterol homeostasis and triglyceride-rich lipoprotein remnant metabolism in the metabolic syndrome. *Clin. Sci.* 2003;104:383– 8.
[94] Nakamura T, Takano H, Umetani K, et al. Remnant lipoproteinemia is a risk factor for endothelial vasomotor dysfunction and coronary artery disease in metabolic syndrome. *Atherosclerosis* 2005; 181:321– 7.
[95] Satoh A, Adachi H, Tsuruta M, et al. High plasma level of remnantlike particle cholesterol in the metabolic syndrome. *Diabetes Care* 2005;28:2414– 8.
[96] Nakajima K, Takeichi S, Nakajima Y, Fujita MQ. Pokkuri Death Syndrome; Sudden Cardiac Death Cases without Coronary Atherosclerosis in South Asian Young Males. *Forensic. Sci. Int.* 2010; 207:6-13.

[97] Takeichi S, Nakajima Y, Yukawa N, et al. Association of plasma triglyceride-rich lipoprotein remnants with "Pokkuri disease". *Legal Med.* 2001; 3: 84- 94.

[98] Takeichi S, Nakajima Y, Osawa M, et al. The possible role of remnant-like particles as a risk factor for sudden cardiac death. *Int. J. Legal Med.* 1997;110:213-219.

[99] Takeichi S, Yukawa N, Nakajima Y, et al. Association of plasma triglyceride-rich lipoprotein remnants with coronary artery atherosclerosis in cases of sudden cardiac death. *Atherosclerosis* 1999;142:309-315.

[100] Nakajima K, Nakajima Y, Takeichi S, Fujita MQ. ApoB-100 carrying lipoprotein, but not apoB-48, is the major subset of proatherogenic remnant-like lipoprotein particles detected in plasma of sudden cardiac death cases. *Atherosclerosis* 2007, 194:473-482.

[101] Nakajima K, Saito T, Tamura A, et al. Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti A-1 immunoaffinity mixed gels. *Clin. Chim. Acta.* 1993; 223: 53-71.

[102] Nakajima, K, Nakajima,Y, Takeichi S, Fujita MQ. Plasma Remnant-Like Lipoprotein Particles or LDL-C as Major Pathologic Factors in Sudden Cardiac Death Cases. *Atherosclerosis* 2008; 198:237-46.

[103] Doi H, Kugiyama K, Ohgushi M, et al. Membrane active lipids in remnant lipoproteins cause impairment of endothelium-dependent vasorelaxation. *Arterioscl. Thromb Vasc. Biol.* 1999;19:1918-2.

[104] Lundman P, Eriksson M, Schenck-Gustafsson K, Karpe F, Tornvall P. Transient triglyceridemia decreases vascular reactivity in young, healthy men without risk factors for coronary heart disease. *Circulation* 1997; 96:3266-3268.

[105] Vogel RA, Corretti MC, Plotnick GD. Effect of a single high-fat meal on endothelial function in healthy subjects. *Am. J. Cardiol.* 1997; 79:350-354.

[106] Kugiyama K, Doi H, Motoyama T, et al. Association of remnant lipoprotein levels with impairment of endothelium-dependent vasomotor function in human coronary arteries. *Circulation* 1998; 97:2519-2526.

[107] Doi H, Kugiyama K, Oka H, et al. Remnant lipoproteins induce proatherothrombogenic molecules in endothelial cells through a redox-sensitive mechanism. *Circulation* 2000; 102:670-676.

[108] Takahashi S, Kawarabayasi Y, Nakai T, Sakai J, Yamamoto T. Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc. Natl. Acad. Sci. USA* 1992; 89:9252-9256.

[109] Kita T, Brown MS, Bilheimer DW, Goldstein JL. Delayed clearance of very low density and intermediate density lipoproteins with enhanced conversion to low density lipoprotein in WHHL rabbits. *Proceedings of the National Academy of Sciences of the United States of America* 1982; 79:5693-5697.

[110] Aguilar-Salinas CA, Barrett H, Schonfeld G. Metabolic modes of action of the statins in the hyperlipoproteinemias. *Atherosclerosis* 1998; 141:203-207.

[111] Dane-Stewart CA, Watts GF, Mamo JC, Dimmitt SB, Barrett PH, Redgrave TG. Elevated apolipoprotein B-48 and remnant-like particle-cholesterol in heterozygous familial hypercholesterolaemia. *Eur. J. Clin. Invest.* 2001; 31:113-117.

[112] de Sauvage Nolting PR, Twickler MB, Dallinga-Thie GM, Buirma RJ, Hutten BA, Kastelein JJ. Elevated remnant-like particles in heterozygous familial hypercholesterolemia and response to statin therapy. *Circulation* 2002; 106:788-792.

[113] Watts GF, Cummings MH, Umpleby M, et al. Simvastatin decreases the hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in heterozygous familial hypercholesterolaemia: pathophysiological and therapeutic implications. *Eur. J. Clin. Invest.* 1995; 25:559-567.

[114] Imagawa M, Takahashi S, Zenimaru Y, Kimura T, Suzuki J, Miyamori I, Iwasaki T, Hattori H, Yamamoto TT, Nakano T, Nakajima K. Comparative reactivity of remnant-like lipoprotein particles (RLP) and low-density lipoprotein (LDL) to LDL receptor and VLDL receptor: Effect of a high-dose statin on VLDL receptor expression. Clin. Chim. Acta. 2011 Nov 7. [Epub ahead of print.

[115] Lagrost, L. 1994. Regulation of cholesteryl ester transfer protein (CETP) activity: review of in vitro and in vivo studies. *Biochem. Biophys. Acta.* 1215: 209-236.

[116] Boekholdt SM,.Kuivenhoven J-A, Wareham NJ, et al. Plasma levels of cholesteryl ester transfer protein and the risk of future coronary artery disease in apparently healthy men and women. *Circulation.* 2004;110: 1418-23.

[117] Guerin, MP. Egger C, Soudant WL, et al. Cholesteryl ester flux from HDL to VLDL-1 is preferentially enhanced in type IIB hyperlipidemia in the postprandial state. *J. Lipid. Res.* 2002;. 43: 1652-1660.

[118] Fielding CJ, Havel RJ, Todd KM, Yeo KE, Schloetter MC, Weinberg V, Frost PH. Effects of dietary cholesterol and fat saturation on plasma lipoproteins in an ethnically diverse population of healthy young men. *J. Clin. Invest.* 1995*;* 95: 611-618.

[119] Deighan, CJ, Caslake MJ, McConnell M, Boulton-Jones JM, Packard CJ. The atherogenic lipoprotein phenotype: small dense LDL and lipoprotein remnants in nephritic range proteinuria. *Atherosclerosis.* 2001; 157: 211-220.

[120] Okamoto H, Miyai A, Sasase T, er al. Cholesteryl ester transfer protein promotes the formation of cholesterol-rich remnant like lipoprotein particles in human plasma. *Clin. Chim. Acta.* 2007; 375:92-98.

[121] Boekholdt SM, Kuivenhoven JA, Wareham NJ,et al. Plasma levels of cholesteryl ester transfer protein and the risk of future coronary artery disease in apparently healthy men and women. *Circulation* 2004; 110: 1418-1423.

[122] Sakabe K, Fukuda N, Wakayama K, Nada T, Shinohara H, Tamura Y. Time course differences for statin-induced pleiotropic effects in hypercholesterolemic patients. *Int. J. Cardiol.* 2004; 94: 111-117.

[123] Sone H, Takahashi A, Shimano H. et al. HMG-CoA reductase inhibitor decreases small dense low-density lipoprotein and remnant-like particle cholesterol in patients with type-2 diabetes. *Life Sci.* 71: 2403-2412.

[124] Okamoto H, Yonemori F, Wakitani K, Minowa T, Maeda K, Shinkai H. A cholesteryl ester transfer protein inhibitor attenuated atherosclerosis in rabbits. *Nature.* 2000; 406: 203-207.

[125] Moon HD, Nakajima K, Kamiyama K, TakanashiK, Sakurabayashi I, Nagamine T. Higher frequency of abnormal serum angiopoietin-like protein 3 than abnormal cholesteryl ester transfer protein in Japanese hyperalphalipoproteinemic subjects. *Clinica. Chimica. Acta.* 2008; 398: 99–104.

[126] Hatsuda S, Shoji T, Shinohara K, et al. Association between plasma angiopoietinlike protein 3 and arterial wall thickness in healthy subject. *J. Vasc. Res.* 2007;44:61–6.

[127] Stejskal D, Karpisek M, Humenanska V, Solichova P, Stejskal P. Angiopoietin-like protein 3: development, analytical characterization, and clinical testing of a new ELISA. *Gen. Physiol. Biophys.* 2007;26:230–3.

[128] Inazu A, Koizumi J, Mabuchi H. Cholesteryl ester transfer protein and atherosclerosis. *Curr. Opin. Lipidol.* 2000;11:389–96.

[129] Koishi R, Ando Y, Ono M, et al. Angptl3 regulates lipid metabolism in mice. *Nat. Genet.* 2002 Feb;30:151–7.

[130] Shimizugawa T, Ono M, Shimamura M, et al. ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase. *J. Biol. Chem.* 2002;277:33742–8.

[131] Shimamura M, Matsuda M, Yasumo H, et al. Angiopoietin-like protein3 regulates plasma HDL cholesterol through suppression of endothelial lipase. *Arterioscler. Thromb Vasc. Biol.* 2007;27:366–72.

[132] Jeppesen J. Triglycerides, high-density lipoprotein cholesterol, and risk of ischemic heart disease: a view from the Copenhagen male study. *Metab. Syndr. Relat. Disord.* 2003;1:33–53.

[133] Kamiyama K, Fukuchi S, Tanaka K, et al. Serum glucose and lipid levels of Urawa inhabitants at health checkup. *Saitama. Med. J.* 2000;35:434–40 (in Japanese).

[134] Ishigami M, Yamashita S, Sakai N, et al. High-density lipoproteins from probucol-treated patients have increased capacity to promote cholesterol efflux from mouse peritoneal macrophages loaded with acetylated low-density lipoproteins. *Eur. J. Clin. Invest.* 1997; 27(4):285-92.

[135] Hirano K, Ikegami C, Tsujii K, et al. Probucol enhances the expression of human hepatic scavenger receptor class B type I, possibly through a species-specific mechanism. *Arterioscler. Thromb Vasc. Biol.* 2005; 25(11):2422-7.

[136] Jin W, Wang X, Millar JS, et al. Hepatic proprotein convertases modulate HDL metabolism. *Cell Metab.* 2007; 6:129–36.

[137] Badellino KO,Wolfe ML, Reilly MP, Rader DJ. Endothelial lipase concentrations are increased in metabolic syndrome and associated with coronary atherosclerosis. *PLoS Med.* 2006;3: 22.

[138] Imamura S, Kobayashi J, Nakajima K, et al. A novel method for measuring human lipoprotein lipase and hepatic lipase activities in postheparin plasma. *J. Lipid. Res.* 2008;49:1431–7.

[139] de Grooth GJ, Kuivenhoven JA, Stalenhoef AF, et al. Efficacy and safety of a novel cholesteryl ester transfer protein inhibitor, JTT-705, in humans: a randomized phase II dose-response study. *Circulation* 2002;105:2159–65.

[140] Schaefer EJ, Asztalos BF. Increasing high-density lipoprotein cholesterol, inhibition of cholesteryl ester transfer protein, and heart disease risk reduction. *Am. J. Cardiol.* 2007;100:25–31.

[141] Nissen SE, Tardif JC, Nicholls SJ, et al. Effect of torcetrapib on the progression of coronary atherosclerosis. *N. Engl. J. Med.* 2007;356:1304–16.

[142] Okazaki M, Usui S, Nakamura3 M, Yamashita S. Evaluation of an HPLC method for LDL-cholesterol determination in patients with various lipoprotein abnormalities in comparison with beta-quantification. *Clin. Chim. Acta.* 2008;395:62–7.

[143] Sirtori CR, Mombelli G. Viability of developing CETP inhibitors. *Cardiovasc. Ther.* 2008;26:135–46.

[144] Akita H, Chiba H, Tsuchihashi K, et al. Cholesteryl ester transfer protein gene: two common mutations and their effect on plasma high-density lipoprotein cholesterol content. *J. Clin. Endocrinol. Metab.* 1994;79:1615–8.

[145] Nagano M, Yamashita S, Hirano K, et al. Molecular mechanisms of cholesteryl ester transfer protein deficiency in Japanese. *J. Atheroscler. Thromb* 2004;11:110–21.

[146] Ikewaki K, Mabuchi H, Teramoto T, et al. Association of cholesteryl ester transfer protein activity and TaqIB polymorphism with lipoprotein variations in Japanese subjects. *Metabolism* 2003; 52:1564–70.

[147] Ishida T, Choi S, Kundu RK, et al. Endothelial lipase is a major determinant of HDL level. *J. Clin. Invest.* 2003;111:347–55.

[148] Miida T, Seino U, Miyazaki O, et al. Probucol markedly reduces HDL phospholipids and increased prebeta1-HDL without delayed conversion into alpha-migrating HDL: Putative role of angiopoietin-like protein 3 in probucol-induced HDL remodeling. *Atherosclerosis* 2008 ;200:32.

[149] Nakajima K, Saito T, Tamura A, et al. Cholesterol in remnant-like lipoproteins in human serum using monoclonal anti apo B-100 and anti apo A-I immunoaffinity mixed gel. *Clin. Chim. Acta.* 1993;223:53–71.

[150] Fruebis J, Gonzalez V, Silvestre M, Palinski W. Effect of probucol treatment on gene expression of VCAM-1, MCP-1, and M-CSF in the aortic wall of LDL receptor-deficient rabbits during early atherogenesis. *Arterioscler. Thromb Vasc. Biol.* 1997;17: 1289–302.

[151] Brasen JH, Koenig K, Bach H *et al*. Comparison of the effects of alpha-tocopherol, ubiquinone-10 and probucol at therapeutic doses on a therosclerosis in WHHL rabbits. *Atherosclerosis* 2002; 163: 249–59.

[152] Ludmer PL, Selwyn AP, Shook TL *et al*. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N. Engl. J. Med.* 1986; 315: 1046–51.

[153] Lau AK, Leichtweis SB, Hume P *et al*. Probucol promotes functional reendothelialization in balloon-injured rabbit aortas. *Circulation* 2003; 107: 2031–36.

[154] Witting PK, Wu BJ, Raftery M, Southwell-Keely P, Stocker R. Probucol protects against hypochlorite-induced endothelial dysfunction. Identification of a novel pathway of probucol oxidation to a biologically active intermediate. *J. Biol. Chem.* 2005; 280:15612–8.

[155] Wu BJ, Kathir K, Witting PK *et al.* Antioxidants protect from atherosclerosis by a heme oxygenase-1 pathway that is independent of free radical scavenging. *J. Exp. Med.* 2006; 203:1117–27.

[156] Ishikawa K, Sugawara D, Wang X *et al*. Heme oxygenase-1 inhibits atherosclerotic lesion formation in ldl-receptor knockout mice. *Circ. Res.* 2001; 88: 506–12.

[157] Juan SH, Lee TS, Tseng KW *et al*. Adenovirus-mediated heme oxygenase-gene transfer inhibits the development of atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2001; 104: 1519–25.

[158] Deng YM, Wu BJ, Witting PK, Stocker R. Probucol protects against smooth muscle cell proliferation by upregulating heme oxygenase-1. *Circulation* 2004; 110: 1855–60.

[159] Alam J, Stewart D, Touchard C *et al*. Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene. *J. Biol. Chem.* 1999; 274: 26071-8.

[160] Kita T, Nagano Y, Yokode M *et al*. Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA* 1987; 84 (16): 5928–31.

[161] Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* 1989; 320:915–24.

[162] Sasahara M, Raines EW, Chait A *et al*. Inhibition of hypercholesterolemia-induced atherosclerosis in the nonhuman primate by probucol. I. Is the extent of atherosclerosis related to resistance of LDL to oxidation? *J. Clin. Invest.* 1994; 94: 155–64.

[163] Stocker R, Keaney JF, Jr. Role of oxidative modifications in atherosclerosis. *Physiol. Rev.* 2004; 84(4):1381-478.

[164] Witting PK, Pettersson K, Ostlund-Lindqvist A-M *et al*. Dissociation of atherogenesis from aortic accumulation of lipid hydro(per)oxides in Watanabe heritable hyperlipidemic rabbits. *J. Clin. Invest.* 1999; 104: 213–20.

[165] Franceschini G, Sirtori M, Vaccarino V, Gianfranceschi G, Rezzonico L, Chiesa G, Sirtori CR. Mechanisms of HDL reduction after probucol. Changes in HDL subfractions and increased reverse cholesteryl ester transfer. *Arteriosclerosis* 1989;9:462-9.

[166] Hirano K, Ikegami C, Tsujii K, et al. Probucol enhances the expression of human hepatic scavenger receptor class B type I, possibly through a species-specific mechanism. *Arterioscler. Thromb Vasc. Biol* 2005; 25(11):2422-7.

[167] Favari E, Zanotti I, Zimetti F, Ronda N, Bernini F, Rothblat GH. Probucol inhibits ABCA1-mediated cellular lipid efflux. *Arterioscler. Thromb Vasc. Biol.* 2004; 24(12):2345-50.

[168] Moghadasian MH. Dietary phytosterols reduce probucol-induced atherogenesis in apo E-KO mice. Atherosclerosis 2006; 188(1):28-34.

[169] Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. *N. Engl. J. Med.* 2007; 357(21):2109-22.

[170] Hirano K, Yamashita S, Nakajima N, et al. Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. Marked hyperalphalipoproteinemia caused by CETP gene mutation is not associated with longevity. *Arterioscler. Thromb Vasc. Biol.* 1997; 17(6):1053-59.

[171] Nagano M, Yamashita S, Hirano K, et al. Molecular mechanisms of cholesteryl ester transfer protein deficiency in Japanese. *J. Atheroscler. Thromb* 2004; 11(3):110-21.

[172] Matsuzawa Y, Yamashita S, Kameda K, Kubo M, Tarui S, Hara I. Marked hyper-HDL2-cholesterolemia associated with premature corneal opacity. A case report. *Atherosclerosis* 1984; 53(2): 207-12.

[173] Cazita PM, Barbeiro DF, Moretti AI, Quintão EC, Soriano FG. Human cholesteryl ester transfer protein expression enhances the mouse survival rate in an experimental systemic inflammation model: a novel role for CETP.Shock. 2008; 30:590-5.

[174] Thompson JF, Reynolds JM, Williams SP, Wood LS, Paciga SA, Lloyd DB. Frequency and function of CETP variants among individuals of Asian ancestry. *Atherosclerosis* 2008 in press.

[175] Yamashita S, Bujo H, Arai H, et al. Long-term probucol treatment prevents secondary cardiovascular events: a cohort study of patients with heterozygous familial hypercholesterolemia in Japan. *J. Atheroscler. Thromb.* 2008;15:292–303.