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Remnant Lipoproteins are a Stronger Risk Factor for Cardiovascular Events than LDL-C – From the Studies of Autopsies in Sudden Cardiac Death Cases

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1. Introduction

Plasma LDL-C level is the most well established risk factor for coronary heart disease (CHD) (1). Accordingly, the numerous studies have shown that the LDL-C lowering drugs, statins significantly reduced plasma LDL-C together with approximately 30% reduction in cardiovascular events (2). Therefore, it has been generally believed that the cardiovascular events are directly associated with the elevated LDL-C or its modified oxidized LDL (3). In this manuscript, we have reviewed the patho-physiological role of LDL-C and remnant lipoproteins at cardiovascular events in Japanese sudden cardiac death (SCD) cases (Table 1), especially in SCD cases with nearly normal coronary arteries (coronary atherosclerosis grade (-) and (±), namely Pokkuri Death Syndrome (PDS). As the formation and physiological role of LDL in liver and plasma has been well established, those of remnant lipoproteins (RLP) have also been established recently as a risk for CHD (4-6). As shown in Figure 1, TG-rich lipoprotein (TRL) remnants are formed in the circulation when apoB-48 containing chylomicrons (CM) of intestinal origin or apoB-100 containing VLDL of hepatic origin are converted by lipoprotein lipase (and to a lesser extent by hepatic lipase) into smaller and denser particles of LDL. Compared with their nascent precursors, TRL remnants are depleted of triglycerides, phospholipids, apoA-I and apoA-IV in the case of CM and are enriched in cholesteryl esters, apoCs and apoE (6). They can thus be identified, separated, or quantified in plasma on the basis of their density, charge, size, specific lipid components, apolipoprotein composition and apolipoprotein immunospecificity. This should mean that we have now two identified cardiovascular risk factors, LDL and RLP (CM remnants and VLDL remnants) (Figure 1), in SCD and PDS cases and attempted to understand the differences in their contributions to CHD.

Recent evidences have suggested that elevated plasma levels of remnant lipoprotein – cholesterol (RLP-C) and reduced lipoprotein lipase (LPL) activity relate to the promotion of coronary artery events associated with spasm (7-9), which has been often observed as a

major risk of sudden cardiac death (10). Likewise, we previously reported a significant association between sudden cardiac death and plasma levels of RLP-C and RLP-triglyceride (RLP-TG), especially in cases of Pokkuri death (11). Pokkuri death in Japanese refers the cases who "die suddenly and unexpectedly", and had not taken any medications prior to death. PDS has been categorized as one type of SCD syndrome, but not having coronary atherosclerosis and without cardiac hyperplasia. Most of such cases have been observed in Asian young males, and as yet, no report of PDS is seen in Caucasians.

| | Control (n=76) | | | SCD (n=165) | | |
| | mean±SD | | | mean±SD | | |
	Non-athero (n=49)	Athero (n=27)	P value	Non-athero (n=48)	Athero (n=117)	P value
Age in years	42.7±16.5	51.3±14.5	< 0.01	37.5±13.1	54.5±10.5	< 0.0001
Male/Female	41/8	24/3	NS	43/5	90/27	NS
Heart weight (g)	358±86	387±80	NS	356±82	414±91	< 0.001
Body weight (kg)	61.7±11.7	62.9±9.5	NS	65.2±11.9	63.9±12.7	NS
Body height (cm)	165±8.2	164±9.1	NS	168±9.6	164±8.5	NS
BMI	22.6±4.8	23.5±3.3	NS	22.9±3.5	23.8±3.7	NS
Postmortem period (h)	8.7±2.9	8.6±3.5	NS	8.9±3.3	8.2±3.4	NS

SCD cases without atherosclerosis (Non-athero; n=48) are categorized as PDS in this manuscript.

Table 1. Demography data of sudden cardiac death and control death cases with and without coronary artery atherosclerosis

Both SCD with coronary atherosclerosis and PDS without coronary atherosclerosis showed abnormally high plasma RLP-C and RLP-TG level, namely postprandial remnant hyperlipoproteinemia in postmortem plasma (11-14) (Table 2). RLP isolated from postmortem plasma by an immunoaffinity gel separation method (15) showed atherogenic and inflammatory effects (16, 17) similar to the RLP isolated from plasma of living subjects (6). In particular, Shimokawa and colleagues (18) reported that RLP isolated from plasma of SCD cases induced strong spasm in in-vivo setting by up-regulating the Rho-kinase pathway in healthy porcine coronary arteries, which might mimic the etiological phenomenon of PDS. But LDL (or Ox-LDL) did not enhance the formation of coronary vascular lesions in regions where coronary spasm could be induced in the same experimental model (19).

Further, Takeichi et al. (11-13) suggested RLP as one of the major risk factors in SCD and PDS. Although LDL-C levels were also elevated in parallel with the majority of SCD cases who have severe coronary atherosclerosis, the role of LDL-C in fatal clinical events was not fully understood in these cases. Therefore, the relationship between plasma levels of RLP-C, RLP-TG, LDL-C and the incidence of cardiovascular events has been studied in SCD cases with and without coronary atherosclerosis as well as in control death cases (Table2). In particular, we were interested in PDS cases among SCD cases as a disease model of coronary artery events which were neither associated with the severity of coronary artery atherosclerosis nor plaque ruptures (20).
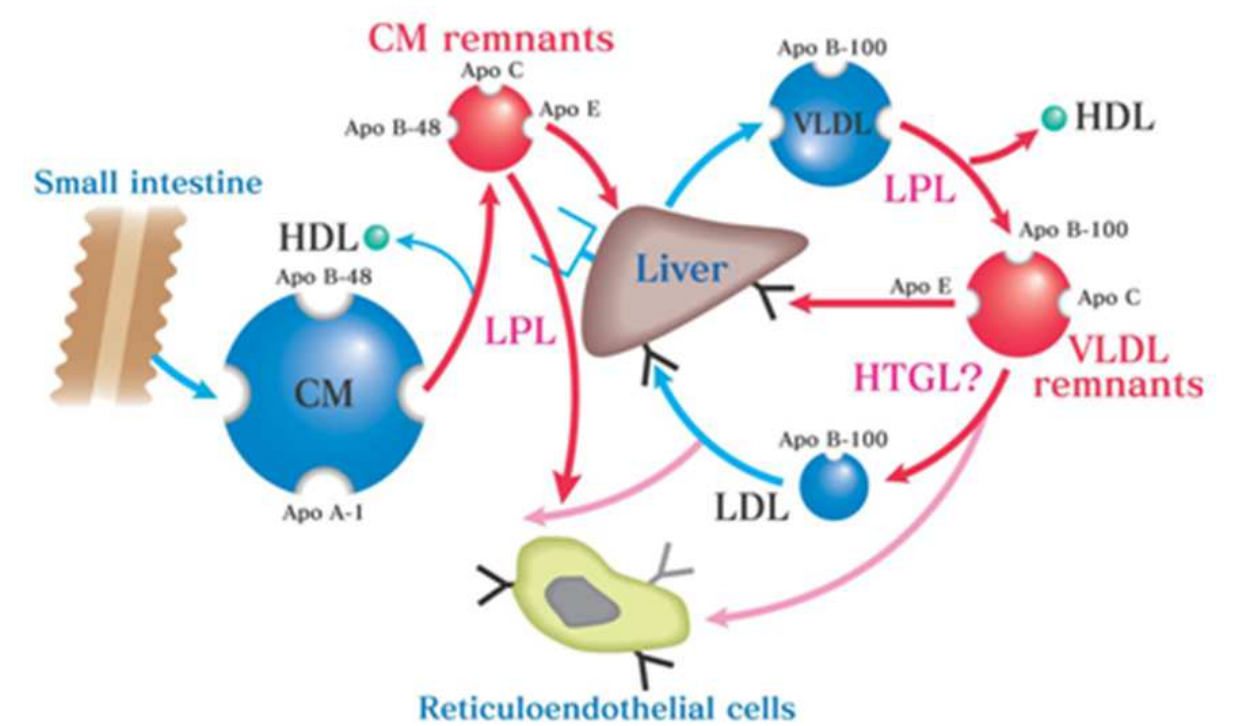
Fig. 1. Metabolic map of lipoproteins. After fat intake, the intestine secretes chylomicrons (CM), the triglycerides of which are lipolyzed by lipoprotein lipase (LPL). The LPL reaction constitutes the initial process in the formation of triglyceride-rich lipoprotein (TRL) remnants (CM remnants and VLDL remnants) . The VLDL secretion process is partly regulated by the rate of FFA influx to the liver. VLDL triglycerides are lipolyzed by endothelial-bound lipoprotein lipase and VLDL remnant particles are formed. The final TRL remnant composition is modulated by the cholesterol ester transfer protein (CETP) reaction with HDL, hepatic lipase (HL), and the exchange of soluble apolipoproteins such as C-I, C-II, C-III and E. The great majority of the remnants are removed from plasma by receptor-mediated processes and the principal receptors are the LDL receptor and the LDL-receptor-related protein (LRP) in liver. It is probable that the CM remnants use both of these routes, whereas the VLDL remnants are more likely to use only the LDL receptor.

	Control (n = 76)		SCD(n = 165)		
	median	25-75% tile	median	25-75% tile	P value[a]
Total cholesterol (mg/dl)	177	134-209	211	175-243	< 0.0001
Triglyceride (mg/dl)	116	81-157	148	100-230	< 0.001
VLDL-C (mg/dl)	20.3	6.0-35.6	27.0	16.1-47.6	< 0.001
LDL-C (mg/dl)	92	67-132	134	99-167	< 0.0001
HDL-C (mg/dl)	42	31-60	41	33-53	NS
RLP-C (mg/dl)	9.1	5.4-13.8	16.4	10.0-26.9	< 0.001
RLP-TG (mg/dl)	49	33-78	81	51-132	< 0.001

Table 2(a). Plasma lipid and lipoprotein levels in total cases of sudden cardiac death (SCD) and control death.

	Control (n = 49)		Pokkuri (n = 48)		
	median	25-75% tile	median	25-75% tile	P value[a]
Total cholesterol (mg/dl)	165	121-205	182	149-221	< 0.01
Triglyceride (mg/dl)	114	75-129	120	96-216	< 0.005
VLDL-C (mg/dl)	20.3	5.1-35.2	27.0	16.2-43.7	NS
LDL-C (mg/dl)	89	67-130	118	83-140	< 0.001
HDL-C (mg/dl)	39	30-59	41	34-56	NS
RLP-C (mg/dl)	7.1	5.1-10.1	14.3	9.5-26.4	< 0.001
RLP-TG (mg/dl)	48	33-67	78	50-117	< 0.001

Table 2(b). Plasma lipid and lipoprotein levels in cases of Pokkuri death and control death (without coronary artery atherosclerosis).

	Control (n = 27)		SCD(n = 117)		
	median	25-75% tile	median	25-75% tile	P value[a]
Total cholesterol (mg/dl)	203	171-216	219	185-248	< 0.05
Triglyceride (mg/dl)	120	82-188	154	106-232	< 0.05
VLDL-C (mg/dl)	21.5	7.0-39.0	26.9	15.8-49.4	NS
LDL-C (mg/dl)	95	69-136	148	103-179	< 0.01
HDL-C (mg/dl)	45	34-63	41	33-51	NS
RLP-C (mg/dl)	12.5	10.1-18.5	17.2	11.4-46.8	< 0.05
RLP-TG (mg/dl)	55	38-84	83	51-142	< 0.05

Table 2(c). Plasma lipid and lipoprotein levels in cases of sudden cardiac death (SCD) and control death(with coronary artery atherosclerosis).

We have established the cut-off values and the likelihood ratios of total cholesterol (TC), TG, RLP-C, RLP-TG and LDL-C in plasma of SCD and control death cases with and without coronary atherosclerosis after adjusting for the postmortem conditions (Table 3) (21). The cut-off values and likelihood ratios of these major plasma lipids and lipoproteins were calculated by using an ROC analysis model for predicting the risk of fatal clinical events with and without coronary atherosclerosis. In approximately two-third of sudden cardiac death cases, we found postprandial remnant hyperlipoproteinemia, especially rich in very low density lipoprotein (VLDL) remnants (14) and significantly elevated LDL-C. Both lipoproteins were significantly elevated in SCD, but LDL-C in PDS cases was within the normal range, together with significantly elevated remnant lipoproteins in fatal clinical events. Therefore, we found that PDS cases were a good disease model to distinguish the role of LDL and RLP as cardiovascular risk factors.

	Cut-off (mg/dl)	Sensitivity (%)	Specificity (%)	PPV Positive predictive value	NPV Negative predictive value	Likelihood ratio
(1) Total (SCD : Control)						
RLP-C	12.8	67.9	68.2	84.4	45.7	2.12
RLP-TG	53.3	72.9	60.8	82.5	46.9	1.86
LDL-C	92.5	79.4	52.7	81.0	50.2	1.68
Total Cholesterol	181	69.3	49.3	77.6	38.7	1.37
Triglycerides	117	66.3	49.3	76.8	36.6	1.31
(2) Non-athero (Pokkuri: Control)						
RLP-C	10.1	70.2	77.5	75.4	72.7	3.13
RLP-TG	67	66.7	75.6	72.8	62.9	2.73
LDL-C	106	58.3	61.7	59.9	60.2	1.52
Total Cholesterol	165	63.8	51.1	56.1	59.0	1.30
Triglycerides	113	53.3	50.0	51.1	52.2	1.07
(3) Athero (SCD : Control)						
RLP-C	13.4	65.3	69.2	90.2	31.5	2.12
RLP-TG	79.4	52.5	72.4	89.2	26.0	1.90
LDL-C	102	79.7	57.1	89.0	39.4	1.86
Total Cholesterol	217	55.2	78.6	91.8	28.8	2.58
Triglycerides	131	64.9	64.3	88.7	29.7	1.82

Number of cases are as follows: (1) total SCD; SCD (n=165) and Control (n=76) : (2) Non-athero; Pokkuri (Non-athero SCD) (n=48) and Non-athero control (n=49): (3) Athero; Athero SCD (n=117) and Athero-control (n=27).

Table 3. Cut-off value of RLP-C and RLP-TG from ROC analysis in predicting sudden cardiac death

2. Role of plasma LDL and remnant lipoproteins at coronary atherosclerosis and cardiovascular events

Based on our autopsy studies, more than two thirds of SCD cases were found to be associated with postprandial remnant hyperlipoproteinemia [11-15]. If severe spasm of the coronary artery is to be a crucial event prior to cardiac death in PDS, 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 (Table1). 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 coronary atherosclerosis was pathologically graded above (1+), reflecting the clinical feature of severe coronary atherosclerosis (11-13). Most of the coronary arteries in PDS cases were pathologically graded as (−) and (±), indicating no coronary atherosclerosis [11]. Plasma LDL-C in SCD cases was shown to be correlated with the severity of coronary artery atherosclerosis [13]. 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. 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 (22). Hence, LDL-C did not seem to play a significant role at cardiovascular events in PDS, despite being 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 (Table 3). 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, 23). 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, the authors 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 [24].

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 of 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 [22]; however, it was significantly associated with fatal clinical events in SCD including PDS (Table 2). The bioactive components co-localized with triglycerides in RLP such as oxidized phospholipids or their metabolites [25] 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 [13, 22]. Therefore RLP-TG could

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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 low concentration in plasma.

3. Postprandial remnant hyperlipoproteinemia as a risk for sudden cardiac death

Several clinical studies have shown that elevated plasma TG levels greatly increase the risk of sudden cardiac death. Results from the Paris Prospective Study (26) and The Apolipoprotein Related Mortality Risk Study (AMORIS) in Sweden (27) demonstrated that increased TG was a strong risk factor for fatal myocardial infarction. However, plasma TG levels can alter very easily within a short time. Therefore it has been difficult to identify the clinical events of elevated TG in the long term prospective studies until recently (28-30).

If the lipid and lipoprotein levels in postmortem plasma correctly reflected the antemortem levels, these data could probably provide the same values with the results obtained from the prospective studies, which require long-term observation for evaluation. The plasma levels of lipids and lipoproteins in sudden death cases may reflect the feature at the moment of fatal clinical events followed by certain inevitable postmortem alterations, but still may reflect the physiological conditions when the cardiac events had occurred. Therefore, we analyzed postmortem plasma under well-controlled conditions to clarify the cause of sudden cardiac death. Plasma RLP-C and RLP-TG levels vary greatly within a short time as the TG levels, compared with other stable plasma markers such as HDL-C and LDL-C. Hence, we believe that the cross-sectional study of RLPs at the moment of sudden death is a superior analytical method than a prospective study of RLP (31) to identify potential risks of CHD. During the investigations of sudden death cases, we found that the postmortem alterations of lipids and lipoproteins in plasma were unexpectedly slight (21) compared with proteins or other bio-markers. Moreover, these plasma lipoprotein levels were very similar to those determined in living patients from the studies in our laboratory.

Remnant lipoproteins are known to increase postprandially as chylomicron (CM) remnants, but very low density lipoprotein (VLDL) remnants also increase at the same time. The remarkable close correlation between the increment in the concentration of TG-rich lipoprotein (TRL) apoB-48 (CM) and apoB-100 (VLDL) after a fat meal indicates that reduced efficiency of CM particle clearance is closely coupled to the accumulation of VLDL particles as proposed by Karpe et al (32). Delayed clearance of CM particles, as evidently occurs in many hypertriglyceridemic states, may thus contribute to the elevation of apoB-100 in TRL. More than two thirds of the SCD including PDS case observed in this study showed stomach full, indicating the strong association with postprandial remnant hyperlipoproteinemia. Significant remnant hyperlipoproteinemia was observed in the plasma of SCD cases compared with the control death cases.

The postprandial increase of apoB-48-carrying CM and CM remnants after fat load is known to correlate well with the increase of RLP-C and RLP-TG (33). These data suggested the

possibility that increased RLP in the postprandial state may be mainly composed of CM remnants. However, unexpectedly, we found no significant differences of apoB-48 levels in plasma or RLP apoB-48, but found significant differences of RLP aoB-100 levels between SCD and control death cases (14). As previously reported by Schneeman et al. (34), postprandial responses (after fat load) of apoB-48 and apoB-100 were highly correlated with those of TRL triglycerides. Although the increase in apoB-48 represented a 3.5-fold difference in concentration as compared with a 1.6-fold increase in apoB-100, apoB-100 accounted for about 80% of the increase in lipoprotein particles in TRL. Our results on plasma evaluation in SCD cases were very similar to the results reported by Schneeman et al (34). RLP apoB-100 levels were significantly elevated in SCD cases in the postprandial state (when RLP-C and RLP-TG were significantly elevated), however, plasma apoB-48 or RLP apoB-48 was not significantly elevated (14). These results strongly suggested that the major subset of RLP associated with fatal clinical events was apoB-100 carrying particles, but not apoB-48 particles.

The absolute amount of apoB-100 in RLP is much greater than that of apoB-48 in RLP. Hence, VLDL remnants, endogenous lipoprotein remnants, generated in the liver, may be more closely associated with the risk of sudden cardiac death than exogenous CM remnants, irrespective of the severity of coronary atherosclerosis. Furthermore, we often found SCD cases that had consumed alcohol on a full stomach. It is known that alcohol increases fatty acids in the liver and enhances VLDL production, and inhibit LPL activity (35). Alcohol intake with a fatty meal is known to greatly enhance TG increase in the postprandial state. The intake of alcohol together with a fatty meal may easily enhance the production of apoB-100 carrying VLDL in the liver, and increase VLDL remnants by inhibiting LPL activity and increase the potential risk of coronary artery in SCD cases.

4. Comparative reactivity of LDL and remnant lipoproteins to LDL receptor in liver

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 [2, 36]. The major mechanism of statin therapeutic effect has been recognized as the increase of LDL receptor in liver to remove an elevated LDL-C in plasma. However, remnant lipoproteins have been also implicated in progression of atherosclerosis [37-40], with elevated remnant lipoprotein levels shown to independently predict clinical events in coronary artery disease (CAD) patients (4). The direct comparison of reactivity between remnant lipoproteins and LDL to LDL receptor in liver has not been studied enough until recently.

Major target for remnant lipoprotein research has been focused on postprandial dyslipidemia. Postprandial dyslipidemia has been found to be associated with endothelial dysfunction [42, 43] an early indicator of atherogenesis. Previous studies have shown that normolipidemic patients with coronary disease have elevated postprandial levels of triglyceride-rich lipoproteins (TRLs) and their remnants compared with healthy control subjects [44-49]. Elevated remnant lipoprotein levels have also been associated with coronary endothelial dysfunction [50], with remnants shown to stimulate expression of proatherothrombotic molecules in endothelial cells [51]. Therefore, the prevention and treatment of atherosclerosis merits pharmacotherapy targeted at regulating postprandial dyslipidemia, namely remnant lipoproteins beyond LDL-C [52]. Postprandial remnant

lipoproteins are the atherogenic lipoproteins which appear and increase postprandially into plasma at the initial step of lipoprotein metabolism after food intake and then change to further metabolized lipoproteins, such as LDL particles (Figure 1). The postprandial state with increased remnant lipoproteins in plasma continues almost whole day except in the early morning (6), while not the case in LDL. Therefore, if postprandial lipoproteins are atherogenic risks as Zilversmit proposed (53), those should be the primary therapeutic target to prevent cardiovascular disease. Increased LDL is not directly associated with the daily food intake as remnant lipoproteins.

Possible mechanisms suggested for abnormal accumulation of lipoproteins postprandially in plasma are defective clearance in liver via receptor-mediated pathways and/or increased competition for high-affinity processes because of increased numbers of intestinally and hepatically derived particles in the postprandial state [32]. HMG-CoA reductase inhibitors decrease cellular cholesterol synthesis and consequently reduce the hepatic production of very-low-density lipoproteins (VLDL) and increase the expression of LDL-receptors in liver [54]. These properties of statins suggest that they are potential agents for regulating the plasma levels of remnant lipoproteins as well as LDL-C.

Atorvastatin is an HMG-CoA reductase inhibitor found to be effective in lowering fasting LDL-C and triglyceride levels [55]. Favorable effects of atorvastatin on postprandial lipoprotein metabolism have been reported in healthy normolipidemic human subjects [56-58]. Recently, atorvastatin and rozuvastatin are reported to decrease small dense LDL-C significantly, which is highly correlated with remnant lipoproteins in plasma, possibly as a precursor of sdLDL (59).

We investigated whether RLP bound to LDL receptor more efficiently than LDL itself via apoE-ligand which is rich in RLP (6). RLP competed more efficiently with β-VLDL than LDL in LDL receptor transfected cells (to be published in Clin Chim Acta 2012 by Takahashi et al). These results suggested that RLP which is mainly apoE-rich VLDL more efficiently binds and internalizes into LDL receptor transfected cells than LDL. Similar results were observed in VLDL receptor transfected cells, although VLDL receptor is not present in liver (60). Takahashi et al found that pitabastatin (NK-104) induced VLDL receptor in skeletal muscle cells with significantly higher concentration (more than 10 folds) compared to HepG2, in which NK-104 enabled to induce LDL receptor.

In FH of a LDL receptor deficiency, statins have a dual mechanism of action involving an increase in the catabolism of LDL via up-regulation of LDL-receptors and a decrease in the hepatic secretion of apolipoprotein (apo) B-100. The net effect is a decrease in the concentration of apoB-containing lipoproteins. As CM remnants are also apo E-rich and mainly cleared via the LDL-receptor [61, 62], an increase in receptor activity and reduced competition from apoB-100-containing lipoproteins was hypothesized to increase the removal rate of remnant lipoproteins from circulation. A recent study investigating the effects of high-dose, long-term statin treatment on the metabolism of postprandial lipoproteins in heterozygous FH, reported a decrease in the fasting and postprandial RLP-C as well as LDL-C [63]. Statins can induce LDL receptor in heterozygous FH which enhance the removal of RLP and LDL simultaneously.

However, it has not been known which, RLP or LDL, is removed earlier or primarily from plasma by increased LDL receptor with statin treatment. Takahashi et al suggested the

possibility that remnant lipoproteins are removed more primarily from plasma by statins and prevent cardiovascular disease, while LDL are more likely reduced as a consequence of reduction of the precursor lipoproteins (to be published in Clin Chim Acta 2012 by Takahashi et al).

Moreover, VLDL receptor which does not affect the removal of remnant lipoproteins in liver may affect on rhabdomyolysis in skeletal muscle cells, in case when VLDL receptors are significantly induced by statins in those cells. When plasma concentration of statins increased abnormally high, VLDL receptor could be induced in the skeletal muscle cells. Then, RLP binds and internalizes into skeletal muscle cells with significantly increased concentration and may cause the rhabdomyolysis in skeletal muscle cells by the cytotoxic effect of remnant lipoproteins (6, 64, 65).

The direct comparison between LDL and RLP has shown that RLP with its apoE-rich ligand has superior binding and internalization reactivity to LDL receptor than LDL in liver, which is a similar reactivity with VLDL receptor. These results suggest that RLP may be more primarily and efficiently metabolized in liver than LDL through increased LDL receptor when treated with statins.

5. Possible molecular mechanism of remnant lipoproteins associated with coronary artery vasospasm

Followings are the hypothesis of molecular mechanism on the initiation and progression of atherosclerosis associated with fatal cardiovascular events we have proposed from our studies on sudden cardiac death during last two decades (Figure 2).

Elevated plasma RLP first cause the initiation of vascular dysfunction at endothelial cell and smooth muscle cells through LOX-1 receptor and activate Rho-kinase pathway in vascular smooth muscle cells to induce coronary artery spasm as vascular smooth muscle hyperconstriction. However, LDL has no such biological properties to initiate the vascular dysfunction. Although Ox-LDL, derived from LDL modified, has very similar biological properties with remnant lipoproteins, the plasma concentration of Ox-LDL is significantly low and can not influence to the following phenomenon like remnant lipoproteins shown by in vitro studies (6).

5.1 Remnant lipoproteins and impaired endothelialium-dependent vasorelaxation

Endothelial activation or dysfunction is known to be an early event in the development of atherosclerosis which is not necessarily associated with strong morphological changes. Kugiyama et al (66) and Inoue et al (67) first found that plasma RLP-C levels, but not LDL-C levels, showed significant and independent correlation with impaired endothelial function reflected as impaired endothelium-dependent vasomotor function (vasorelaxation) in large and resistance coronary arteries in humans. These observations indicated the possibility that high plasma concentration of remnant lipoproteins impair endothelial cell function in human coronary arteries.

Flow-mediated vasodilation (FMD) of the brachial artery during reactive hyperemia has been used as a noninvasive method to assess endothelial function. Kugiyama et al (68) and Funada et al (69) examined FMD by high resolution ultrasound technique before and at the
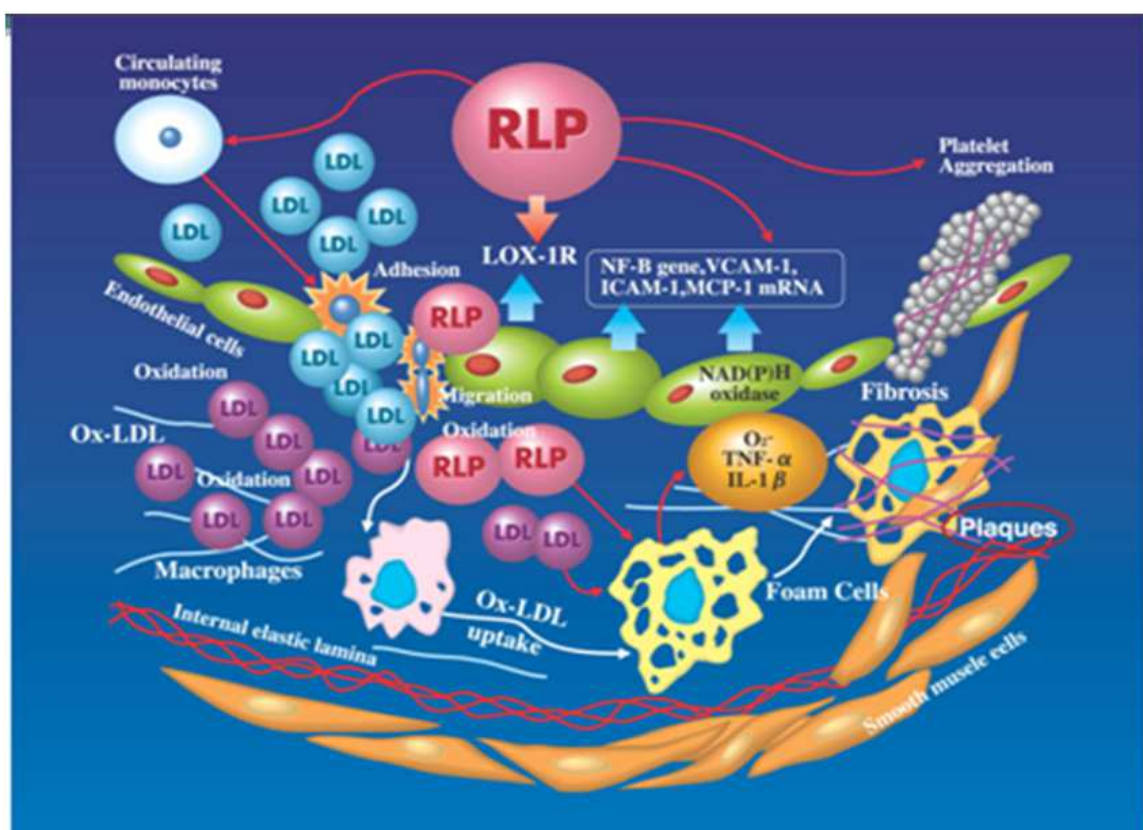
Fig. 2. Effect of RLP and Ox-LDL on athegenesis. The endothelial cell dysfunction is initiated by RLP in plasma followed by the induction of LOX-1 receptor and the associated pathway of various cytokines and enzymes. Ox-LDL promotes the progression of atherosclerosis in subendothelial space after a large efflux of LDL from plasma and form atherosclerotic plaques.

end of a 4 week treatment with oral administration of alpha-tocopherol acetate (300 IU/day). Alpha-tocopherol improved the impairment of endothelium-dependent vasodilation in patients with high RLP-C, but not in patients with low RLP-C. Similarly, RLP and their extracted lipids impaired endothelium-dependent vasorelaxation (EDR) of isolated rabbit aorta at the same concentration of serum RLP-C as found in patients with coronary artery disease (70). In contrast, non-RLP in the VLDL fraction had no effect on EDR. This in vitro study further showed that co-incubation of N-acetylcysteine and reduced glutathione (GSH), antioxidants, that were added to incubation mixture in isolated rabbit aorta containing RLP, almost completely reversed the impaired EDR, suggesting that reactive oxygen species contained in RLP or those generated by RLP played a significant role in the impairment of EDR. Further, Doi et al (51) showed that RLP isolated from patients undergoing treatment with alpha-tocopherol lost their inhibitory action on vasorelaxation of isolated rabbit aorta in response to Ach, whereas RLP from patients receiving placebo had inhibitory action on vasorelaxation. These results suggested that high RLP-C level being oxidized in plasma increased the oxidative stress and contribute to endothelial vasomotor dysfunction in patients with high plasma concentration of RLP-C. Ohara et al (17) reported that remnant lipoproteins isolated from SCD cases suppressed nitric oxide (NO) synthetase activity and attenuate endothelium-dependent vasorelaxation.

Probucol is known to inhibit the oxidative modification of LDL (71), lowering serum cholesterol levels. Ox-LDL has been shown to impair endothelium-dependent vasorelaxation and antioxidants, including probucol, suppressing the impaired EDR (72) as RLP described above.

5.2 Both Ox-LDL and remnant lipoproteins activate LOX-1 receptor in endothelial cells

A scavenger receptor independent pathway for acetyl LDL and oxidized LDL in cultured endothelial cells, has long been known; however, it has been difficult to isolate. Recently, Sawamura and his colleagues (73-76) discovered and characterized lectin-like oxidized LDL receptor-1 (LOX-1) as a vascular endothelial receptor for Ox-LDL. Endothelial dysfunction or activation invoked by oxidatively modified LDL has been implicated in the pathogenesis of atherosclerosis by enhanced intimal thickening and lipid deposition in the arteries. Ox-LDL and its lipid constituents, mainly composed of oxidized products of phospholipids such as lysophosphatidylcholine, impair endothelial production of NO, and induce the endothelial expression of leukocyte adhesion molecules and smooth muscle growth factors, which can contribute to atherogenesis via LOX-1 receptor. Vascular endothelial cells in culture and in vivo internalize and degrade Ox-LDL through a putative receptor-mediated pathway that does not involve macrophage scavenger receptor. The treatment of HUVECs with RLP increased LOX-1 expression in a dose dependent manner (Figure 3) and was completely inhibited by LOX-1- antisense, but not by LOX-1-sence. Monoclonal antibody to LOX-1 reported by Shin et al (77) and antisence LOX-1 oligodeoxynucleotide reported by Park et al (78) significantly reduced RLP-mediated production of superoxide (NADPH oxidase dependent), TNF-alpha, and interleukin-beta, NF-κB activation, DNA fragmentation (cell death: apoptosis). Further Shin et al (77) have emphasized the importance of RLP in increasing the expression of LOX-1 receptor protein in NADPH oxidase dependent superoxide production; the expression of adhesion molecules such as ICAM-1, VCAM -1 and MCP-1 stimulated by RLP is dependent on the activation of LOX-1 receptors. These findings strongly suggest that LOX-1 may play the role of a receptor of RLP as well as Ox-LDL in endothelial cells. Endothelial cell injury caused by RLP via LOX-1 receptor activation evidently can initiate atherosclerosis. Cilostazol, a platelet aggregation inhibitor and vasodilator (79), is known to reduce plasma RLP-C levels in patients with peripheral artery disease (80) and has showed significant protective effect against RLP-induced endothelial dysfunction by suppressing these variables both in-vitro and in-vivo with its antioxidative activity (81).

5.3 Remnant lipoproteins activate LOX-1 receptor in smooth muscle cell

Coronary vasospasm has been considered to occur at vascular smooth muscle cells (VSMCs) and the migration of VSMCs from media to intima and subsequent proliferation play key roles in atherogenesis. A previous report has demonstrated that RLPs induce VSMC proliferation [82]; however, receptors for RLPs in VSMCs have not yet been well characterized until recently reported by Aramaki et al (83), although LRP in the liver, apoB-48-R in macrophages, and VLDL receptor in heart, skeletal muscle, adipose tissue, brain and macrophages [84, 85] have been shown to act as a receptor for RLPs. LOX-1 expression is dynamically inducible by various proatherogenic stimuli, including tumor necrosis factor-α(TNF-α), heparin-binding epidermal growth factor-like growth factor (HB-EGF), and Ox-
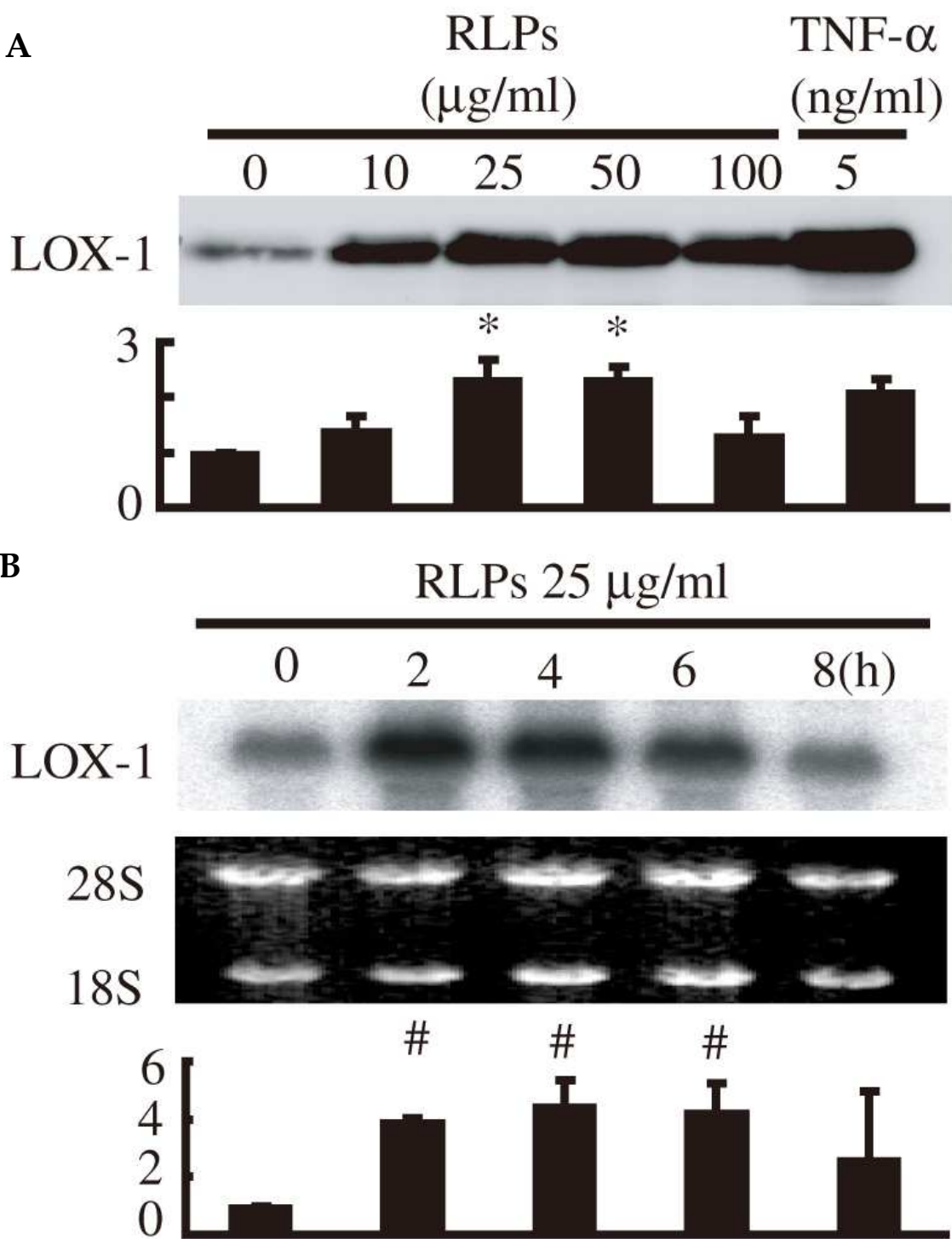
A

RLPs (µg/ml)   **TNF-α (ng/ml)**

0   10   25   50   100   5

LOX-1

3

*   *

0

B

RLPs 25 µg/ml

0   2   4   6   8(h)

LOX-1

28S

18S

#   #   #

6
4
2
0

Fig. 3. RLPs, but not nascent VLDL (n-VLDL), induce LOX-1 expression in BVSMCs. (A)
After BVSMCs were treated with the indicated concentrations of RLPs for 16 h, total cell
lysates were subjected to immunoblotting for LOX-1. TNF-α served as a positive control. (B)
After treatment with 25µg/ml of RLPs for the indicated time periods, total cellular RNA
was subjected to Northern blot analyses. Bands for 28S and 18S ribosomal RNAs were
visualized by ethidium bromide staining to control the amount of RNA loaded. (*) $p < 0.001$
vs. 0g/ml of RLPs, (#) $p < 0.05$ vs. 0 h incubation (cited from Ref 83).

LDL. Furthermore, LOX-1 is highly expressed by macrophages and VSMCs accumulate in the intima of advanced atherosclerotic lesions, as well as endothelial cells covering early atherosclerotic lesions in vivo, indicating that LOX-1 appears to play important roles at various stages of atherogenesis.. Aramaki et al (83) recently provided direct evidence, by cDNA and short interference RNAs (siRNAs) transfection, that LOX-1 acts as a receptor for RLP (Figure 1) and whereby induce VSMC migration, depending upon HB-EGF shedding and the downstream signal transduction cascades. The direct evidences that LOX-1 serves as a receptor for RLPs in vascular smooth muscle cells (VSMCs) were shown by use of two cell lines which stably express human or bovine LOX-1 and siRNA directed to LOX-1. In addition, involvement of metalloproteinase activation, HB-EGF shedding, EGFR transactivation, and activation of ERK, p38 MAPK and PI3K were also observed in RLP induced migration of BVSMCs. Competition studies in cells stably expressing LOX-1 indicated binding site(s) on the LOX-1 molecule for RLPs and oxidized LDL appear to be identical or overlapped, suggesting the C-terminal cysteine-rich C-type lectin-like domain was shown to be the responsible binding site(s) for RLPs [86]. These studies suggested the importance of LOX-1 in RLP-induced atherogenesis, as well as that induced by oxidized LDL. RLPs induced cell migration and LOX-1 expression by RLP-LOX-1 interactions, thus making a positive-feed back loop to further enhance the RLP-induced vascular dysfunction, as already showed in oxidized LDL-induced vascular dysfunction. In accordance with a previous report [77, 78], RLP-induced LOX-1 expression and cell migration depend upon HB-EGF shedding and subsequent EGFR transactivation demonstrated. Furthermore, the involvement of ERK, p38 MAPK and Akt as signal transducrion cascades located downstream to the EGFR transactivation were shown. JNK was not activated by RLPs or not involved in RLP-induced LOX-1 expression or cell migration (84).

These results suggested that RLP induced LOX-1 expression and enhance the activation of smooth muscle cells.

5.4 Remnant lipoproteins activate Rho-kinase in smooth muscle cells and induce vasospasm

Coronary vasospasm has been postulated to play an important role in SCD, although a direct demonstration for the hypothesis is still lacking. Shimokawa and his colleagues demonstrated the close relation between RLP and coronary vasospasm that is mediated by upregulated Rho-kinase pathway (18). The expression and the activity of Rho-kinase are enhanced at the inflammatory coronary lesions in the porcine model with interleukin-1 (19, 88).

RLP isolated from the plasma of SCD cases exert a potent upregulating effect on Rho-kinase in hcVSMC (18). In organ chamber experiments, serotonin caused hyperconstriction of vascular smooth muscle cells (VSMC) from RLP-treated segment, which was significantly inhibited by hydoxyfasudil (a selective Rho-kinase inhibitor). In cultured human coronary VSMC, the treatment with RLP significantly enhanced the expression and the activity of Rho-kinase. These results indicated that RLP isolated from the plasma of sudden cardiac death cases upregulated Rho-kinase in coronary VSMC (promoted inflammation) and markedly enhanced coronary vasospasmic activity.

Further, Oi et al (18) performed in vivo study on the formation of coronary vascular lesion by RLP, using healthy pigs in which they treated pig coronary arteries with RLP (an

equivalent concentration of plasma RLP) isolated from the plasma of SCD cases. After 1 week, intracoronary serotonin caused hyperconstriction in the segment treated with RLP but not in the non-RLP VLDL treated segment (Figure 4). Likewise, RLP treated with hydroxyfasudil, a selective Rho-kinase inhibitor, dose dependently inhibited the coronary spasm in pigs.
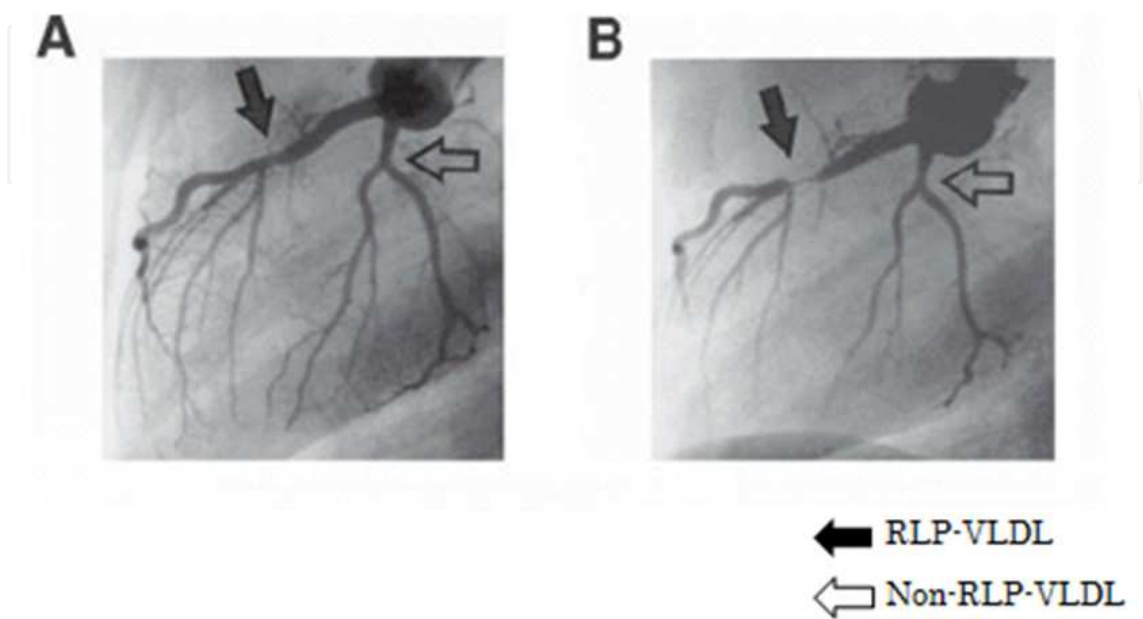

Fig. 4. RLP (RLP in VLDL fraction; RLP-VLDL) from patients with SCD markedly enhance coronary vasospastic activity in pigs. Coronary angiograms before (A) and after intracoronary serotonin (B). Black arrows indicate RLP site at coronary artery; white arrows, non-RLP site. RLP induced significant hyperconstriction at treated coronary site after 1 week, while hydroxyfasudil completely inhibited serotonin (5HT)-induced coronary hyperconstriction at RLP site (cited from Ref 18). These results were explained by the induction of Rho-kinase α and Rho-kinase β, of which mRNA expression was enhanced by the treatment with RLP but not that with non-RLP.

It has been recently reported that sphingosine 1-phosphate (S1P) and sphingosylphosphorylcholine, present in serum lipoproteins, behave as a lipid mediator and cause vasoconstriction through upregulation of Rho/Rho-kinase pathway (89). The possible role of S1P and sphingosylphosphorylcholine in the RLP fraction remains to be elucidated.

These results suggested the importance of intervention to suppress the cardiovascular events more aggressively by such as inhibiting Rho-kinase activation than to slow down the progression of atherosclerosis.

6. Conclusion remarks

Sudden and unexplained cardiac death has been known for many years in Southeast Asian countries, including Japan. These deaths were named differently in each country such as Pokkuri Death Syndrome in Japan, "Lai Tai" in Thailand, "Bangungut " in the Philippines, "Dream Disease" in Hawaii, and "Sudden Unexpected Nocturnal Death Syndrome" among South Asian immigrants in the USA. However, the clinical and pathological features of these

sudden death cases are surprisingly similar with no coronary atherosclerosis and mainly occur among young males during sleep in the midnight, together with an excessive food and alcohol intake.

We have proposed a hypothesis that could explain a possible cause of PDS based on the postprandial increase of remnant lipoproteins in plasma and narrowed circumferences of coronary arteries in PDS cases. The elevated plasma RLP initiates the vascular dysfunction at endothelial cells in narrowed coronary arteries as an early event in the development of atherosclerosis and induces severe coronary spasm under stress or genetic disorder, possibly for example, through activating LOX-1 receptor and Rho-kinase pathway, at smooth muscle cells to cause cardiac arrest. LDL or low concentration of Ox-LDL could not explain these phenomena as RLP. Taken together, we have proposed that the severity of coronary atherosclerosis and the occurrence of cardiovascular events in CHD cases could be considered as separate factors, judging from the physiological role of LDL and RLP in plasma. Therefore, the intervention should be more targeted to suppress the plasma remnant lipoproteins to prevent cardiovascular events more aggressively rather than to slow down the progression of atherosclerosis by LDL.

7. Acknowledgment

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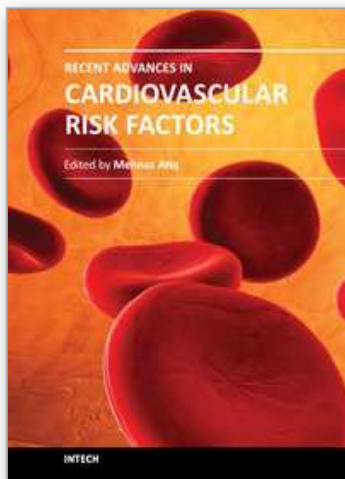
Recent Advances in Cardiovascular Risk Factors

Edited by Prof. Mehnaz Atiq

Among the non-communicable diseases, cardiovascular disorders are the leading cause of morbidity and mortality in both the developed and the developing countries. The spectrum of risk factors is wide and their understanding is imperative to prevent the first and recurrent episodes of myocardial infarction, stroke or peripheral vascular disease which may prove fatal or disabling. This book has tried to present an update on risk factors incorporating new research which has thrown more light on the existing knowledge. It has also tried to highlight regional diversity addressing such issues. It will hopefully be resourceful to the cardiologists, general practitioners, family physicians, researchers, graduate students committed to cardiovascular risk prevention.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Katsuyuki Nakajima and Masaki Q. Fujita (2012). Remnant Lipoproteins are a Stronger Risk Factor for Cardiovascular Events than LDL-C - From the Studies of Autopsies in Sudden Cardiac Death Cases, Recent Advances in Cardiovascular Risk Factors, Prof. Mehnaz Atiq (Ed.), ISBN: 978-953-51-0321-9, InTech, Available from: http://www.intechopen.com/books/recent-advances-in-cardiovascular-risk-factors/remnant-lipoproteins-are-a-stronger-risk-factor-for-cardiovascular-event-than-ldl-c

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