

# Remnant-like lipoprotein particles impair endothelial function: direct and indirect effects on nitric oxide synthase

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**Abstract** Remnant-like lipoprotein particles (RLPs) have been implicated as potentially atherogenic lipoproteins. Endothelial dysfunction is known to be an early event in atherosclerosis and an important contributor to the pathogenesis of coronary artery disease. Moreover, there is considerable evidence linking increased RLP cholesterol levels with endothelial dysfunction, reflected by impaired endothelial vasodilatation and abnormal endothelial secretion. The underlying mechanisms by which RLPs may contribute to endothelial dysfunction are complex and have not been completely elucidated. Because the expression and activation of endothelial nitric oxide synthase (eNOS) are vital to endothelial function, and recent data have implied an association between RLPs and eNOS, this manuscript proposes the hypothesis that RLPs could impair endothelial function via direct and indirect effects on eNOS: RLPs may affect the autophosphorylation of focal adhesion kinase and its downstream phosphatidylinositol kinase/Akt (protein kinase B) signaling pathway, resulting in eNOS inactivation through induction of intracellular oxidative stress in endothelial cells; and RLPs could affect the expression or activation of eNOS indirectly by stimulating secretion of various inflammatory factors from multiple origins. **■** The practical applications of this manuscript provide new insights for the future investigation of RLPs.—Zheng, X. Y. and L. Liu. Remnant-like lipoprotein particles impair endothelial function: direct and indirect effects on nitric oxide synthase. *J. Lipid Res.* 2007. 48: 1673–1680.

**Supplementary key words** endothelial dysfunction • atherosclerosis • inflammation

Remnant-like lipoprotein particles (RLPs), also known as remnant lipoproteins or remnant-like particles, are derived from VLDLs and chylomicrons, which are the major carriers of plasma triglycerides. The actions of lipoprotein lipase and cholesteryl ester transfer protein on VLDL and chylomicrons produce RLPs with decreased triglyceride

and increased cholesteryl ester and apolipoprotein E (apoE). Compared with the nascent triglyceride-rich lipoproteins, RLPs are smaller particles, with a higher density and more cholesteryl ester, which seems to give them more potential atherogenic properties.

RLPs have been separated on the basis of their size, density, charge, specific lipid components, or apolipoprotein composition. The analytical “gold standard” for measuring triglyceride-rich lipoproteins and remnants is density gradient ultracentrifugation; however, this technique is labor-intensive and involves a 24 h analysis to fractionate the plasma lipoproteins. Moreover, only a few samples can be processed at the same time in each ultracentrifuge. Tsai et al. (1) used NMR spectroscopy to perform numerous postprandial analyses of triglyceride-rich lipoproteins in a large interventional study, and observed that chylomicrons and chylomicron remnants/VLDL fraction measurements obtained by NMR had a high degree of correlation with results produced by ultracentrifugation. NMR is a more rapid procedure and uses substantially less sample volume than traditional ultracentrifugation. And NMR is a physical procedure, so that plasma samples can be preserved for biochemical assays of stable analytes. However, NMR is a relatively expensive method, with a special analyzer, and is not widely available in clinical laboratories at present.

Recently, a novel immunoseparation method for RLPs has been developed (2). This method uses two monoclonal antibodies, to human apoB-100 and apoA-I, respectively, to remove most of the apoB-100-containing lipoproteins

Abbreviations: ACh, acetylcholine; apoE, apolipoprotein E; CAD, coronary artery disease; CRP, C-reactive protein; EDR, endothelium-dependent vasorelaxation; eNOS, endothelial nitric oxide synthase; FAK, focal adhesion kinase; FMD, flow-mediated endothelium-dependent dilatation; HUVEC, human umbilical vein endothelial cell; ICAM-1, intercellular adhesion molecule-1; IL-1, interleukin-1; NO, nitric oxide; Ox-LDL, oxidized LDL; RLP, remnant-like lipoprotein particle; RLP-C, RLP-cholesterol; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; VCAM-1, vascular cell adhesion molecule-1.

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Manuscript received 23 January 2007 and in revised form 10 April 2007 and in re-revised form 24 April 2007 and in re-re-revised form 11 May 2007.

Published, JLR Papers in Press, May 13, 2007.  
DOI 10.1194/jlr.R700001JLR200

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This article is available online at <http://www.jlr.org>

(namely, LDLs and nascent VLDLs) and apoA-I-containing lipoproteins (namely chylomicrons and HDLs), leaving behind an unbound fraction including VLDL remnants containing apoB-100 and chylomicron remnants containing apoB-48, both of which are enriched in apoE. Characterization of the unbound lipoproteins of  $d < 1.006$  g/ml has shown that they represent RLPs. The reason that the anti-apoB-100 antibody does not recognize the apoB-100 of apoE-enriched VLDL remnants is not entirely clear, although the amino acid sequence of the apoB-100 epitope region has been found to be homologous to an amphipathic helical region of apoE, suggesting that apoE can compete for binding of the antibody to its epitope on apoB-100 (3). Cholesterol in RLPs (RLP-C) is measured by a sensitive enzyme assay. In comparison with the ultracentrifugation, NMR, and nondenaturing polyacrylamide gel electrophoresis methods, the RLP-C assay has higher specificity, sensitivity, and throughput, as well as lower complexity, which makes it applicable for routine analysis in clinical laboratories.

There is considerable evidence linking increased plasma RLP-C levels with coronary artery disease (CAD). A growing body of studies suggests that RLP-C levels are markedly higher in CAD patients compared with healthy control subjects (4, 5). The most dramatic demonstration of the atherogenic potential of RLPs is observed in type III hyperlipoproteinemia (familial dysbetalipoproteinemia), where xanthoma formation and atherosclerosis can be extensive (6). RLP-C levels have been found to be significantly elevated in patients with this disease. The molecular defect in type III hyperlipoproteinemia is the presence of a mutant form of apoE, which is defective in binding to both apoB and apoE receptors, resulting in an impaired clearance of RLPs (7). ApoE knockout mice develop extensive atherosclerosis and have become one of the most widely used animal models for exploring atherogenesis *in vivo* (8). It is thought that RLPs that accumulate in plasma of these mice are atherogenic and induce foam cell formation. These studies strongly demonstrate the potential adverse effects of RLPs on atherosclerosis.

Atherosclerosis is thought to begin with the trapping of cholesterol-rich lipoproteins within the intima of arterial vessels. In the past, chylomicrons were not normally considered to be directly involved in atherogenesis, because of their larger size and inability to efficiently penetrate arterial tissue. However, there is accumulating evidence that suggests that once chylomicrons are hydrolyzed to their remnants form, the triglyceride-depleted chylomicron remnants penetrate arterial tissue and, moreover, become preferentially trapped within the subendothelial space as concentrated foci. Ongoing studies demonstrate that significant chylomicron remnant accumulation can occur in a number of primary and secondary lipid disorders and in normolipidemic subjects with CAD (9). Moreover, chylomicron remnants have been shown to accumulate in both the rabbit and rat aorta *in vivo* (10–12), and apoE-containing lipoproteins have been found in the human aortic intima (13). These studies provide evidence that the arterial retention of RLPs may pose a significant athero-

genic risk. As relatively smaller remnants particles and considering the close relationship between VLDL and LDL, remnants derived from VLDL could be more atherogenic than chylomicron remnants.

The endothelium, formed by the monolayer of endothelial cells, is not a passive blood-compatible lining for the containment of blood cells and plasma, but rather a metabolically active tissue that subserves a wide range of functions relating to vascular homeostasis. Endothelial cells play an important role in the control of vasomotor tone, permeability, coagulation, growth of vascular smooth muscle cells, and inflammatory responses by releasing or expressing various factors (14, 15). A balanced release of these bioactive factors facilitates vascular homeostasis. Endothelial dysfunction disrupts this balance, thereby predisposing the vessel wall to vasoconstriction, leukocyte adherence, platelet activation, mitogenesis, pro-oxidation, thrombosis, impaired fibrinolytic function, vascular inflammation, and finally, atherosclerosis (16).

Assessment of endothelial cell function refers to a measure of endothelial cell response to stimulation, for example, by vasoactive substances released by or those that interact with the vascular endothelium. Endothelium-dependent vasorelaxation (EDR) can be assessed in the coronary and peripheral circulations in humans (17). Quantitative coronary angiography has been used to measure coronary vasomotor responses, and more recently, ultrasound has been used as a noninvasive measurement of endothelial function, especially when examining brachial arteries. At present, brachial artery ultrasound is widely used to assess vasomotor function. Importantly, EDR assessed by this technique in peripheral artery closely correlates with coronary EDR (18).

Because endothelial dysfunction is known to be an early event in atherosclerosis and an important contributor to the pathogenesis of CAD (19, 20), the present article focuses on the association between RLPs and endothelial dysfunction, and the potential underlying mechanisms by which RLPs may contribute to endothelial dysfunction. In the following segment, endothelial dysfunction will be discussed with two aspects: 1) abnormal endothelial cell secretion and 2) impaired EDR.

#### ABNORMAL ENDOTHELIAL CELL SECRETION

In the inflammatory and proliferative responses of the endothelium, some adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and E-selectin, are expressed by the activated endothelial cells in the atherosclerotic lesions (21), and chemokines such as monocyte chemoattractant protein-1 and cytokines such as interleukin-1 (IL-1) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) are also secreted by the endothelial cells (22).

There is increasing evidence that RLPs can induce various inflammatory factors derived from endothelial cells. Upon incubation of human umbilical vein endothelial cells (HUVECs) with RLPs (50  $\mu$ g/ml), adherent mono-

cytes significantly increased by 3.3-fold, with increased cell surface expression of VCAM-1, ICAM-1, E-selectin, and monocyte chemoattractant protein-1 (23). Doi et al. (24) also found that RLPs (0.1 mg cholesterol/ml) upregulated endothelial expression of ICAM-1 and VCAM-1. Shin et al. (25) reported that RLPs (50  $\mu$ g/ml) significantly increased superoxide formation in HUVECs, associated with increased production of TNF- $\alpha$  and IL-1 $\beta$ , and cell death.

In addition, RLPs effect endothelial expression of plasminogen activator inhibitor-1. In studies incubating human aortic endothelial cells with RLPs, Sawka et al. (26) demonstrated that RLPs from patients with type III hyperlipoproteinemia induced endothelial cell plasminogen activator inhibitor-1 expression, which may contribute to a prothrombotic state. Likewise, RLPs upregulate endothelial expression of tissue factor, which is essential for thrombotic events, in the same range of RLP concentrations as in peripheral plasma in patients with CAD (24).

Evidence is accumulating that these RLP-stimulated effects are dependent on the activation of lectin-like oxidized LDL receptor-1 (LOX-1), a vascular endothelial receptor for oxidized LDL (Ox-LDL). Ox-LDL has been widely believed to play a key role in the initiation and progression of atherosclerosis since Steinberg et al. (27) proposed the Ox-LDL hypothesis as the major cause of atherosclerosis. Monoclonal antibodies to LOX-1 and anti-sense LOX-1 oligodeoxynucleotide significantly reduced these RLP-mediated effects (23, 25). LOX-1 protein was demonstrated to mediate apoptotic cell death in endothelial cells (28). Shin et al. (25) have emphasized the importance of RLPs in increasing the expression of LOX-1 receptor protein in NADPH oxidase-dependent superoxide production associated with DNA fragmentation and apoptotic cell death in HUVECs. Isolated RLPs were found to be oxidized or to be susceptible to oxidation in plasma. A new oxidative modification hypothesis has been proposed that postulates that RLPs, not LDL, are the major oxidized lipoproteins in plasma, based on observations of the plasma concentration of these oxidized lipoproteins. Many studies have shown that the concentration of Ox-LDL in plasma is less than 0.5% of total LDL in CAD patients. The most significant role of Ox-LDL in atherogenesis may be played in the subendothelial space with interactions with macrophages and smooth muscle cells, not in endothelial cells. It is conjectured that RLPs, not Ox-LDL in plasma, are the major ligand for the LOX-1 receptor in endothelial cells, causing endothelial dysfunction and the initiation of atherosclerosis (29).

## IMPAIRMENT OF EDR

There is accumulating evidence that suggests that RLP-C levels have an inverse and independent correlation with EDR.

### Impairment of acetylcholine-induced vasodilatation

It is known that acetylcholine (ACh)-induced vasodilatation is mediated by nitric oxide (NO) released from

the endothelium (30), which diffuses to the underlying smooth muscle cells to cause relaxation. The arterial response to ACh is determined by the balance between endothelium-derived NO and the direct constrictor action of ACh on smooth muscle. Hence, impaired ACh-induced relaxation reflects endothelial dysfunction. In this connection, Inoue et al. (31) reported that ACh induced coronary artery spasm in patients with elevated serum RLP-C levels, an effect that was not observed in the coronary arteries of patients with low or normal serum RLP-C levels. Kugiyama et al. (32) also reported that compared with LDL-cholesterol, age, and smoking history, RLP-C levels had the most significant correlation with abnormal epicardial coronary vasomotor responses to ACh infusion, reflected by impaired dilation or constriction of the epicardial coronary arteries. Further, in a subgroup of 53 consecutive subjects, constrictor responses of epicardial coronary diameters to intracoronary infusion at baseline of  $N^G$ -monomethyl-L-arginine, an inhibitor of NO synthase, reflecting the presence of coronary NO bioactivity, had an inverse and independent correlation with RLP-C levels, shown by use of multivariate analysis (32). It is suggested that the decrease in coronary NO bioactivity may be responsible in part for the inhibitory effects of RLPs.

Consistent with these findings, it has been demonstrated that elevated RLP-C levels were correlated with the impairment of ACh-induced coronary artery response in patients with high insulin resistance but without angiographically significant CAD (33). Furthermore, Funada et al. (34) reported that the brachial artery response after the administration of ACh (50  $\mu$ g) was significantly less in patients with high RLP-C levels than in patients with low RLP-C levels, indicating that RLP-C is an independent lipid factor that regulates peripheral vascular endothelial functions even in normolipidemic patients.

### Impairment of flow-mediated endothelium-dependent dilatation

Measurement of flow-mediated brachial artery vasoactivity using high-resolution ultrasonography has been reported as an accurate and sensitive method for detecting endothelial dysfunction. The forearm blood flow is occluded for 5 min using a blood pressure cuff maintained at a standard pressure. When the pressure is released, reactive hyperemia occurs. This results in shear stress-induced NO release and subsequent vasodilatation. Using this technique, Nakamura et al. (35) reported that elevated fasting RLP-C level was a significant and independent risk factor for impaired flow-mediated endothelium-dependent dilatation (FMD) and angiographically proven CAD in 210 patients with metabolic syndrome. It is worth noting that the postprandial state occurs for more than 12 h daily in most individuals, so the postprandial triglyceride-rich lipoproteins and remnants may play a more important role in atherogenesis than the fasting profiles (36). Plotnick, Corretti, and Vogel (37) observed that a single high-fat meal transiently reduced endothelial function for up to 4 h in healthy, normocholesterolemic subjects, whereas there was no significant change in FMD after a low-fat

meal. The change in FMD after low-fat and high-fat meals was inversely correlated with the 2 h postprandial change in triglyceride levels. Several studies have also demonstrated the relationship between postprandial RLPs and endothelial dysfunction, determined as a marked impairment of FMD (36, 38). These findings suggest that repeated increases in postprandial RLPs in the circulation might impair EDR even in individuals with normal fasting lipids levels.

#### Animal experiments ex vivo

Several animal experiments ex vivo have been done to explore the effects of remnants on EDR and the involved mechanisms. Doi et al. (39) found that RLPs obtained from hyperlipidemic patients who complained of chest pain attenuated ACh-induced EDR in isolated rabbit aorta. A similar inhibition of EDR was subsequently reported by Ohara et al. (40) using RLPs in postmortem blood from subjects who had died suddenly of CAD. Grieve et al. (41) found that after perfusion of the rat aorta with chylomicron remnants, relaxation of the vessels to carbachol was significantly attenuated, and that oxidized chylomicrons had a more marked effect on endothelial function than native chylomicron remnants by interfering with the L-arginine-NO pathway. Moreover, organ chamber experiments showed that EDR impairment was restored by addition of reduced glutathione or N-acetylcysteine, antioxidants, into the incubation buffer containing isolated rabbit aortas and RLPs (42). It has been suggested that RLP-induced impairment of the endothelium appears not to be due to an apolipoprotein receptor-mediated event (39) but rather to lipid fractions in RLPs; i.e., oxidative damage by peroxidized phospholipids in RLPs presumably causes dysfunction of the endothelium (42).

#### Possible mechanisms

*Direct effect on endothelial nitric oxide synthase.* As mentioned above, a decrease in the NO released from the endothelium has been thought to be the major mechanism for inhibition of EDR by RLPs. Further studies made by Ohara et al. (40) demonstrated that RLPs inhibited EDR of rabbit aorta and concentration-dependently inhibited NO production by endothelial cells using DAF-2; however, endothelial nitric oxide synthase (eNOS) did not decrease after incubation with RLPs. These results suggest that RLPs do not affect eNOS synthesis, but rather, depress the activity of the enzyme.

It is known that flow-induced regulation of eNOS depends on integrin signaling and Src activation and the downstream kinase cascade (Src = >PI3K = >Akt = >eNOS) (43–45). In addition, there is evidence demonstrating that focal adhesion kinase (FAK) plays a critical role in flow-induced dilation and eNOS activation via the phosphatidylinositol kinase/Akt (protein kinase B) (PI3K/Akt) pathway (46). Kawakami et al. (47) have reported that RLPs can induce FAK activation in monocytic U937 cells. RLPs could attenuate endothelial vasomotor function through increasing intracellular oxidant level (42).

Accordingly, it can be hypothesized that RLPs could affect the autophosphorylation of FAK and its downstream PI3K/Akt, followed by eNOS inactivation via inducing intracellular oxidative stress in endothelial cells. Further studies are needed to test this hypothesis.

*Indirect effect on eNOS via inflammatory factors.* Increasing evidence has established a fundamental role for inflammation in mediating all stages of atherosclerosis, from its origins to its ultimate complications (48). Ceriello et al. (49) showed that postprandial hypertriglyceridemia had a damaging effect on endothelial function, accompanied by increases in TNF- $\alpha$  and IL-6 in diabetic patients. Twickler et al. (50) also reported that plasma levels of IL-6 and TNF- $\alpha$  increased during the postprandial period and were related to the elevated levels of RLP-C in patients with the adult-onset growth hormone deficiency syndrome, indicating a pronounced postprandial inflammatory response associated with the postprandial RLP-C response.

These inflammatory factors possibly have three different origins. 1) *Monocytes/macrophages.* Saraswathi and Hasty (51) found that after incubation of mouse peritoneal macrophages with VLDL (100  $\mu$ g/ml), TNF- $\alpha$  and IL-1 $\beta$  were upregulated by at least 2-fold in a dose-dependent manner. It can be assumed that RLPs also have a direct effect on monocytes/macrophages proinflammatory processes. 2) *Adipocytes.* Adipose tissue is an important determinant of a low-level, chronic inflammatory state, as reflected by levels of IL-6, TNF- $\alpha$ , and C-reactive protein (CRP) (52). Zhao et al. (53, 54) also observed that the secretion of IL-6 and TNF- $\alpha$  by adipocytes isolated from hypercholesterolemic rabbits was significantly higher than that from control rabbits. Hence, it can be presumed that RLPs may play a potential role in inducing these proinflammatory adipokines through RLP hydrolysis and the subsequent accumulation of intracellular free fatty acids and triglycerides. 3) *Endothelial cells.* Data from Shin et al. (25) showed that RLPs significantly increased production of TNF- $\alpha$  and IL-1 $\beta$  in HUVECs. Additionally, Venugopal, Devaraj, and Jialal (55) demonstrated that human aortic endothelial cells secreted appreciable amounts of CRP when incubated with IL-1 and/or IL-6. CRP, a hepatic acute-phase protein, is largely regulated by circulating levels of IL-6.

It is known that elevated levels of circulating inflammatory markers such as CRP and TNF- $\alpha$  correlate inversely with endothelial vasoreactivity (56–58). Because the expression and activation of eNOS are vital to endothelial function, recently, key studies have emphasized the impact of inflammatory cytokines on eNOS. In vitro studies have demonstrated that TNF- $\alpha$  can decrease eNOS protein expression (59, 60), although studies of the effect of TNF- $\alpha$  on eNOS activity are scarce. Picchi et al. (61) showed that TNF- $\alpha$  levels were higher in Zucker obese fatty rats than that in lean rats, whereas eNOS protein levels were reduced, accompanied by impaired EDR in the Zucker obese fatty rats versus lean rats, indicating that TNF- $\alpha$  may play a pivotal role in endothelial dysfunction in the prediabetic metabolic syndrome. Seidel, Billert, and Kurpisz (62) also demonstrated that both TNF- $\alpha$  and IL-1 $\beta$  exerted

a substantial and statistically significant negative effect on eNOS mRNA level in cultured human coronary artery endothelial cells. In addition, accumulating evidence suggests that CRP could suppress NO production and inhibit eNOS activity (63, 64). Moreover, Verma et al. (65) demonstrated that CRP could cause a decrease in eNOS mRNA expression by endothelial progenitor cells that exerted negative effects on endothelial progenitor cell differentiation, survival, function, etc. Therefore, there may be an indirect mechanism by which RLPs impair endothelial vasodilatation by stimulating secretion of inflammatory factors such as CRP, IL-6, and TNF- $\alpha$  from multiple origins. This hypothesis needs to be confirmed by future research.

## THERAPEUTIC STRATEGIES

### Drug therapy

It has been shown that some drugs mediate their vascular protective benefits, at least in part, through attenuating RLP-induced endothelial dysfunction. Statins and fibrates, two different lipid-lowering drugs, have been demonstrated to have pleiotropic effects on atherosclerosis. Treatment with atorvastatin (10 mg/day) or bezafibrate (400 mg/day) for 4 weeks exerted beneficial effects on FMD, levels of RLP-C and triglyceride, and proinflammatory markers in patients with metabolic syndrome. The reduction of RLP-C levels after treatment with these two drugs had a strong association with the improvement of FMD (35).

Some antioxidants have also been shown to improve endothelial function. Doi et al. (42) reported that RLPs isolated from the plasma of patients treated with  $\alpha$ -tocopherol (300 mg/day), an antioxidant, lost their inhibitory action on vasorelaxation in response to ACh, which was associated with a lower level of phospholipid hydroperoxides. Furthermore, treatment with  $\alpha$ -tocopherol significantly decreased plasma levels of soluble forms of ICAM-1 and VCAM-1 in patients with high RLP levels (24). ProbucoI has been recognized to have antioxidant properties as well as lipid-lowering effects that could contribute to prevention of atherosclerosis. Evidence suggests that long-term treatment with probucoI improves endothelial function in patients with CAD (66). Hence, probucoI may also have a protective role in RLP-induced endothelial dysfunction, although there is not enough data to confirm this hypothesis.

Cilostazol, a platelet aggregation inhibitor and vasodilator, has been demonstrated to reduce plasma RLP-C levels in patients with peripheral artery disease (67) and showed significant protective effects against RLP-induced endothelial dysfunction by suppressing expression of adhesion molecules and chemokines with its antioxidative activity (23, 25).

Pioglitazone, a peroxisome proliferator-activated receptor- $\gamma$  agonist, lowers total postprandial triglyceride, as well as chylomicron- and chylomicron-remnant retinyl palmitate levels to normal (68). Moreover, pioglitazone treatment can improve FMD and reduce CRP concentrations in patients with type 2 diabetes (69, 70). It has been demon-

strated that troglitazone, another peroxisome proliferator-activated receptor- $\gamma$  agonist, upregulates eNOS protein and its mRNA levels in cultured vascular endothelial cells (71).

### Diet therapy

Several studies have shown that dietary intake can modulate serum lipid metabolism. Higashi et al. (72) reported that soy protein isolate reduced RLP-C, triglycerides, and the plasma level of vitamin E. In comparison with triglyceride, diglyceride intake significantly lowers the postprandial increase of RLP-C (73). The ingestion of 4% phytosterol-containing diglyceride also improves serum lipid metabolism in pediatric patients with familial hypercholesterolemia, reflected by a decrease in total cholesterol, LDL-cholesterol, and RLP-C (74).

A high-carbohydrate diet induces an increase in the number of circulating triglyceride-rich particles and their remnants. Abbasi et al. (75) reported that a low-fat, high-carbohydrate diet (60% carbohydrate) had higher fasting plasma triglycerides and RLP-C compared with a 40% carbohydrate diet, and these changes persisted throughout the day in response to breakfast and lunch. Oral glucose loading causes an acute, transient decrease of FMD in healthy subjects (76). However, impaired FMD and higher plasma P-selectin are found after a high-saturated-fat diet but not a low-fat, high-carbohydrate diet in healthy subjects (77). The effect of a high-carbohydrate diet on the interaction between RLPs and endothelial function in healthy subjects needs to be clarified by more research. The harmful effect of a high-carbohydrate diet on FMD in patients with type 2 diabetes is well known.

Data from Higashi et al. (78) suggested that a linoleic acid-enriched diet was associated with decreased postprandial RLP-C levels compared with an oleic acid-enriched diet. Eicosapentanoic acid administration is also an effective and safe treatment to decrease plasma RLPs and prevent *in vivo* peroxidation of LDL in dialysis patients (79). However, higher n-6 (but not n-3) PUFA intake increases fasting triglycerides, RLP-C concentrations, and VLDL size and decreases LDL size in apoA-5-1131C carriers, suggesting that n-6 PUFA-rich diets are related to a more atherogenic lipid profile in these subjects (80). Docosahexaenoic acid supplementation restores FMD in hyperlipidemic children (81), whereas high saturated fat causes deterioration in FMD compared with high monounsaturated fat, polyunsaturated fat, or high-carbohydrate diets in healthy subjects. Inflammatory responses may also be increased on a high-saturated-fat diet (77). Trans monounsaturated fatty acids and saturated fatty acids have similar effects on postprandial FMD (82).

When fed a high-cholesterol diet, mice showed a 2-fold elevation of plasma cholesterol levels, with marked increases in VLDL and LDL cholesterol on gel filtration chromatography (83). Rabbits fed a high-cholesterol (1.5%) diet showed a significant reduction in FMD (84).

Above all, a balanced calorie-restricted diet, lower in carbohydrate, cholesterol, and saturated fat and higher in unsaturated fat, may be more beneficial in reducing

cardiovascular disease risk. In certain individuals, such as apoA-5-1131C carriers and patients with type 2 diabetes, precise modifications in diet may be required.

## CONCLUSION

In summary, RLPs can induce endothelial dysfunction, reflected by impaired endothelial vasodilatation and secretion, which may initiate atherosclerosis. One major mechanism for inhibition of EDR by RLPs is decreasing NO production from endothelium. On the basis of some key studies, we propose the hypothesis that RLPs can affect the autophosphorylation of FAK and its downstream PI3K/Akt, followed by eNOS inactivation via induction of intracellular oxidative stress in endothelial cells.

Alternatively, evidence indicates a pronounced postprandial inflammatory response associated with the postprandial RLP-C response, and the adverse impact of inflammatory factors on eNOS. In addition, several studies have demonstrated that different types of cells, such as monocytes/macrophages, adipocytes, and endothelial cells, can release various inflammatory factors under certain stimulation. Therefore, there may be an indirect mechanism by which RLPs could impair endothelial function by stimulating secretion of inflammatory factors, such as CRP, IL-6, and TNF- $\alpha$ , from multiple origins. Further studies are needed to test these hypotheses.

This work was supported by the National Natural Science Foundation of China (Project 30500209) and the Program for New Century Excellent Talents in University (Project NCET-06-0684).

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