Apolipoprotein CIII Links Hyperlipidemia With Vascular Endothelial Cell Dysfunction

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Background—Apolipoprotein CIII (apoCIII) is a component of some triglyceride-rich very-low-density and low-density lipoprotein and is elevated in dyslipidemia with insulin resistance and the metabolic syndrome. We previously reported that apoCIII directly activates proinflammatory and atherogenic signaling in vascular endothelial cells through protein kinase C-β (PKCβ). Because PKCβ impairs the response of vascular endothelial cells to insulin, we tested the hypothesis that apoCIII affects insulin signaling in vascular endothelial cells and its function in vitro and in vivo.

Methods and Results—ApoCIII inhibited insulin-induced tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1), decreasing phosphatidylinositol 3-kinase (PI3K)/Akt activation in human umbilical vein endothelial cells. These effects of apoCIII led to reduced endothelial nitric oxide synthase (eNOS) activation and NO release into the media. ApoCIII activated PKCβ in human umbilical vein endothelial cells, resulting in IRS-1 dysfunction via serine phosphorylation. ApoCIII also activated mitogen-activated protein kinase through PKCβ. The impaired insulin signaling was restored by PKCβ inhibitor or MEK1 inhibitor. ApoCIII-rich very-low-density lipoprotein and apoCIII impaired insulin signaling in the aorta of C57BL/6J mice and in human umbilical vein endothelial cells, which was recovered by PKCβ inhibitor. They also inhibited endothelium-dependent relaxation of the aortas of C57BL/6J mice. In summary, apoCIII in very-low-density lipoprotein impaired insulin stimulation of NO production by vascular endothelium and endothelial dysfunction in vivo. This adverse effect of apoCIII was mediated by its activation of PKCβ, which inhibits the IRS-1/PI3K/Akt/eNOS pathway.

Conclusion—Our results suggest that apoCIII is a crucial link between dyslipidemia and insulin resistance in vascular endothelial cells with consequential deleterious effects on their atheroprotective functions. (Circulation. 2008;118:731-742.)

Key Words: apolipoproteins  ■  endothelium  ■  hyperlipoproteinemia  ■  insulin  ■  nitric oxide synthase

Endothelial dysfunction is regarded as a causal factor in the initiation and development of cardiovascular disease, including hypertension and atherosclerosis.12,13 It is characterized by the reduced bioavailability of the signaling molecule nitric oxide.
oxide (NO), which has potent vasodilatory and antiatherosclerotic properties. Vascular endothelium is a target tissue of insulin, and insulin resistance exists at the level of vascular endothelial cells. Insulin promotes bioavailability of NO by activating the signaling pathway involving the insulin receptor (IR), IR substrate-1 (IRS-1), phosphatidylinositol 3-kinase (PI3K), and Akt, which leads to the activation of endothelial NO synthase (eNOS). Insulin resistance and subsequent hyperinsulinaemia also stimulate secretion of a potent vasoconstrictor endothelin-1 (ET-1) through mitogen-activated protein (MAP) kinase independently of PI3K-dependent signaling, which also contributes to endothelial dysfunction.

Endothelial dysfunction is often seen in dyslipidemia and the metabolic syndrome, and the apoCIII level is high in these morbid conditions. ApoCIII is produced mainly in the liver, and its promoter has a negative insulin response element, which may account for the link between the impaired insulin action in hepatocytes and high apoCIII level. In the periphery, it is possible that reverse causation may be operative, involving apoCIII and insulin action, because protein kinase C-β (PKCβ) inhibits insulin action in endothelial cells and apoCIII activates PKCβ in the same cells. Thus, we tested the hypothesis that apoCIII itself impairs insulin signaling in endothelial cells, which results in diminished NO production. This hypothesis would add to the mechanisms by which apoCIII contributes to atherosclerosis and link dyslipidemia with vascular endothelial dysfunction.

Methods

Cell Culture and Reagents

Human umbilical vein endothelial cells (HUVECs) were purchased from Clonetics Corp (San Diego, Calif) and cultured as previously described. HUVECs, at passage 2 to 3 were used for the assays. Apolipoproteins were purified from human plasma with high-performance liquid chromatography and immunoaffinity column chromatography (Academy Biomedical Co, Inc, Houston, Tex). Endothelin levels in apolipoproteins, measured with a Limulus amoebocyte lysate chromogenic test (Associates of Cape Cod, East Falmouth, Mass), were <0.03 EU/mL. Free fatty acid (FFA) levels in apolipoproteins determined enzymatically were <20 nmol/L, which is much lower than reported to inhibit insulin signaling in endothelial cells. Antiapolipoprotein antibodies were purchased from Academy Biomedical Company, Inc, PDX8025, wortmannin, and PKCβ-specific inhibitor [3-(1-(3-imidazol-1-ylpropyl)-1H-indol-3-yl)-4-anilino-1H-pyrrrole-2,5-dione] were purchased from Calbiochem (San Diego, Calif). Insulin was purchased from Wako (Tokyo, Japan).

Lipoprotein Preparation

Blood was drawn in tubes containing EDTA from 10 healthy volunteers after 12 hours of fasting. The subjects were not taking cardiovascular medications, antioxidants, or estrogen. VLDL (d<1.006) with apoCIII (VLDL CIII+) or without apoCIII (VLDL CIII-) was isolated from plasma as described previously. The protocol of this study complied with the guidelines for the conduct of research involving human subjects by the Committee on Human Research at Tokyo Medical and Dental University. Apolipoprotein levels in VLDL preparations were determined by ELISA as described previously. Triglyceride levels in VLDL preparations were determined enzymatically. Endotoxin levels in VLDL preparations were <0.03 EU/mL.

Immunoblotting

After being cultured for 24 hours in the serum-deprived medium without albumin, HUVECs were incubated with apoCIII or VLDL CIII+ and then stimulated with insulin. Total lysate and the membrane fraction of the cell lysate were prepared as described previously. Lysate was assayed with immunoblotting using anti-phospho-Akt antibody, anti-Akt antibody, anti-phospho-PI3K antibody, anti-PI3K antibody, anti-phospho-IRS-1 (Tyr989) antibody, anti-IRS-1 antibody, anti-phospho-eNOS antibody, anti-eNOS antibody, anti-phospho-IRβ antibody, anti-IRβ antibody (Santa Cruz Biotechnology, Inc, Santa Cruz, Calif), anti-phospho-IRS-1 (Ser473) antibody (Cell Signaling Technology, Inc, Danvers, Mass), anti-phospho-ERK1/2 antibody, anti-ERK1/2 antibody, anti-phospho-JNK antibody, and anti-JNK antibody (New England Bios, Beverly, Mass). Activation of PKC was assessed by detecting the membrane-bound protein that translocated from cytosol fraction using antibodies to anti-PKCβI antibody and anti-PKCβII antibody (Santa Cruz).

Quantification of NO and ET-1

NO levels in the culture media or plasma were measured with the Nitric Oxide Colorimetric Assay Kit (Biomol, Plymouth Meeting, Pa) following the manufacturer’s instructions. ET-1 levels in the culture media were measured with an ELISA kit (R&D Systems, Minneapolis, Minn).

In Vivo ApoCIII Stimulation

Please see the Materials and Methods section of the online Data Supplement.

Isometric Tension Measurements

Please see supplementary Materials and Methods section.

Statistical Analysis

Results are given as mean±SD. Data were analyzed with an unpaired t test or 2-way ANOVA, with values of P<0.05 considered significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

ApoCIII Inhibits Insulin-Stimulated IRS-1 Tyrosine Phosphorylation in HUVECs

On insulin stimulation, IRS-1 is tyrosine phosphorylated by the IR to activate PI3K/Akt in vascular endothelial cells. We found that exposing HUVECs to apoCIII before insulin treatment blocked this established effect of insulin in a concentration-dependent manner at as early as 30 minutes of exposure (Figure 1A and 1B). Therefore, subsequent experiments used apoCIII at 100 μg/mL for 30 minutes unless otherwise indicated. ApoCIII did not affect the expression of IRβ or insulin-induced IRβ phosphorylation in HUVECs, suggesting that apoCIII affects insulin signaling downstream of IR (Figure 1C). Anti-apoCIII antibody, but not isotype-matched IgG, completely restored insulin-induced IRS-1 tyrosine phosphorylation (supplemental Figure 1A).
ApoCIII Inhibits Insulin-Stimulated PI3K/Akt and eNOS Activation in HUVECs
PI3K and Akt are sequentially activated downstream of IRS-1, which in turn activates effector molecules in endothelial cells such as eNOS. ApoCIII attenuated insulin-induced phosphorylation of PI3K/Akt in a concentration-dependent manner (Figure 2A and 2B). ApoCIII attenuated insulin-stimulated eNOS activation (Figure 2C) and NO release into the culture media in a concentration-dependent manner (Figure 2D). Wortmannin, a PI3K inhibitor, abolished insulin-induced eNOS activation and NO release into the culture media. ApoCIII did not affect the expression of eNOS in HUVECs.

ApoCIII Activates PKC in HUVECs
Various isoforms of PKC mediate inflammation and other cellular events in atherosclerosis. Activation of PKC in endothelial cells and vascular tissue inhibits insulin-induced activation of eNOS, leading to impaired vasodilation.20 We recently showed that apoCIII in VLDL activates PKC in monocytes and PKC in vascular endothelial cells, in which selective inhibition of either PKC isoform abolished apoCIII-induced upregulation of adhesion molecules.10,11 Moreover, we previously showed that remnant lipoproteins that are rich in apoCIII activate PKCα and PKCβ in human monocyctic cells and PKCα and PKCδ in rat aortic smooth muscle cells.22,23 We therefore tested the hypothesis that apoCIII inhibits insulin responses in vascular endothelial cells by activating PKC. ApoCIII increased membrane-bound PKCII (Figure 3A), indicating its activation. ApoCIII minimally affected PKC-I activation. Anti-apoCIII antibody, but not isotype-matched IgG, completely abolished apoCIII-induced PKCII activation (supplemental Figure IB). A PKC-specific inhibitor attenuated the inhibitory effect of apoCIII on eNOS activation and NO release into the culture media (Figure 3B and 3C). These results demonstrate the central role of PKCII in impaired insulin signaling induced by apoCIII.

ApoCIII Activates MAP Kinase in HUVECs
Insulin also activates the small GTP binding protein Ras, which then initiates a phosphorylation cascade responsible for growth, mitogenesis, and ET-1 production involving the sequential activation of Raf, MEK, and ERK.17 We examined whether apoCIII affects insulin-induced ERK activation in endothelial cells. ApoCIII induced phosphorylation of ERK and augmented insulin-induced ERK activation (Figure 4A). A PKC-specific inhibitor partially reversed apoCIII-induced
ERK activation (Figure 4B). Andreozzi et al\(^2\) reported that endothelial dysfunction is mediated through activation of JNK or ERK. Recent studies reported that ERK and PKC isoforms induce Ser\(^{616}\) phosphorylation of IRS-1.\(^{25}\) It is known that serine phosphorylation of IRS-1 inhibits its ability to be tyrosine phosphorylated by IR and to bind and activate PI3K. We tested the possibility that impaired insulin-stimulated IRS-1 function is associated with increased serine phosphorylation induced by PKC/H9252 or ERK. As shown in Figure 4C, apoCIII induced Ser\(^{616}\) phosphorylation of IRS-1. ApoCIII-induced Ser\(^{616}\) phosphorylation of IRS-1 was blocked by PKCβ or ERK. As shown in Figure 4C, apoCIII induced Ser\(^{616}\) phosphorylation of IRS-1. ApoCIII-induced Ser\(^{616}\) phosphorylation of IRS-1 was blocked by PKCβ-specific inhibitor and partially reversed by PD98059, an MEK1-specific inhibitor. PKCβ-specific inhibitor completely and PD98059 partially restored Tyr\(^{989}\) phosphorylation of IRS-1 (Figure 4D). PD98059 also partially reversed the inhibitory effects of apoCIII on NO release (Figure 4E), suggesting the involvement of ERK in this process. In contrast, apoCIII did not activate JNK or p38 MAP kinase in endothelial cells, and apoCIII-induced Ser\(^{616}\) phosphorylation of IRS-1 was not affected by their inhibitors (data not shown). Finally, we confirmed that apoCIII increased the ET-1 level in the culture media, which was abolished by PKCβ-specific inhibitor and PD98059 (Figure 4F). These results indicate that apoCIII may cause or augment an imbalance between vasodilator and vasoconstrictor actions of insulin through pathway-specific insulin resistance.\(^{13,17}\)

**ApoCIII Inhibits Insulin Signaling in the Aortas of C57BL/6J Mice**

We next tested whether apoCIII would affect an insulin-stimulated eNOS pathway in vivo. Human apoCIII level in the plasma was 78±18 µg/mL after 30 minutes of apoCIII (500 µg per body) injection into C57BL/6J mice (mouse apoCIII level in the plasma was 38±12 and 33±14 µg/mL before and after apoCIII injection, respectively). Short-time stimulation with apoCIII did not significantly change
fasting plasma triglycerides, FFA, and insulin levels (triglycerides, 52±18 and 61±19 mg/dL; FFA, 0.89±0.12 and 0.96±0.20 mmol/L; insulin, 178±18 and 167±21 pmol/L before and after 30 minutes, respectively). However, apoCIII activated PKCβII and ERK in the aortas of C57BL/6J mice at 500 µg per body (Figure 5A and 5B). ApoCIII stimulated Ser115 phosphorylation of IRS-1 (orthologous to Ser116 in human IRS-1), which was attenuated by a PKCβ-specific inhibitor (Figure 5C). ApoCIII reduced the insulin effect on tyrosine phosphorylation of IRS-1 and eNOS in the aorta (Figure 5D and 5E). PKCβ-specific inhibitor reversed these inhibitory effects of apoCIII. It also restored the NO level in the plasma reduced by apoCIII (Figure 5F).

VLDL CIII+ Inhibits Insulin Signaling in the Aortas of C57BL/6J Mice
ApoCIII is a component of some VLDL and other lipoproteins in blood. We tested whether VLDL CIII+ or VLDL CIII− affects the insulin-stimulated NOS pathway in vivo. Triglycerides in VLDL CIII+ and VLDL CIII− were 10.3±2.8 and 3.3±0.8 µg/µg apoB, respectively. ApoCIII in VLDL CIII+ was 0.80±0.14 µg/µg apoB. Lipid and insulin after 30 minutes of VLDL injection were as follows: triglycerides, 75±18 and 125±38 mg/dL; FFA, 1.51±0.25 and 1.63±0.35 mmol/L; and insulin, 190±34 and 184±24 pmol/L. VLDL CIII− and VLDL CIII+, respectively. There were no significant differences in plasma FFA and insulin levels between the 2 groups.
Figure 4. Effect of apoCIII on ERK activation in HUVECs. A, HUVECs were incubated with apoCIII and then stimulated with insulin (100 nmol/L for 30 minutes). Representative blots are shown. *P<0.05 vs apoCIII(-)/insulin(+), #P<0.05 vs apoCIII(-)/insulin(+). B, HUVECs were incubated with apoCIII. In experiments using PKCβ-specific inhibitor, it was added to the media at 10 nmol/L 30 minutes before apoCIII incubation. Representative blots are shown. *P<0.05 vs apoCIII(+)/PKCβ-specific inhibitor(-). C, HUVECs were incubated with apoCIII. In experiments using PKCβ-specific inhibitor (10 nmol/L) or PD98059 (50 nmol/L), it was added to the media 30 minutes before apoCIII incubation. Representative blots are shown. *P<0.05 vs apoCIII(-)/inhibitor(-). D, F, HUVECs were incubated with apoCIII and then stimulated with insulin (100 nmol/L for 30 minutes). In experiments using PKCβ-specific inhibitor (10 nmol/L) or PD98059 (50 nmol/L), it was added to the media 30 minutes before apoCIII incubation. Representative blots are shown. *P<0.05 vs apoCIII(-)/inhibitor(-); #P<0.05 vs apoCIII(-)/inhibitor(-). E, HUVECs were incubated with apoCIII for 30 minutes and then stimulated with insulin (100 nmol/L for 30 minutes). In experiments using PD98059, it was added to the media at 50 nmol/L 30 minutes before apoCIII incubation. Representative blots are shown. p Indicates phosphorylated. *P<0.01 vs apoCIII(-)/insulin(+)/PD98059(-), #P<0.05 vs apoCIII(+)/insulin(+)/PD98059(-).
This article was retracted in February 2012.

Kawasaki et al ApoCIII Induces Endothelial Dysfunction

Human apoCIII level in plasma was $72\pm15 \mu g/mL$ after 30 minutes of VLDL CIII+ injection (mouse apoCIII level in the plasma was $35\pm18$ and $31\pm15 \mu g/mL$ before and after VLDL CIII+ injection, respectively). Human VLDL CIII+ stimulated PKCβII activation and Ser612 phosphorylation of IRS-1 in the aorta of C57BL/6J mice. In contrast, VLDL CIII− had minimal effect on these processes (Figure 6A and 6B). PKCβ-specific inhibitor partially inhibited Ser612 phosphorylation of IRS-1 by VLDL CIII+ (Figure 6C) and restored insulin-stimulated Tyr989 phosphorylation of IRS-1 in the aortas (Figure 6D) and NO release into the plasma (Figure 6E). VLDL CIII− had minimal effect on insulin-induced NO release.

We confirmed the effect of VLDL CIII+ in vitro. Pretreatment of VLDL CIII+ with an anti-apoCIII function-blocking antibody attenuated PKCβII activation by VLDL CIII+ in HUVECs (supplemental Figure IC). VLDL CIII+ also is rich in apoC1 and apoE (0.23±0.08 and $0.35\pm0.05 \mu g/\mu g$ apoB, respectively) compared with VLDL CIII−. However, anti-apoC1 and anti-apoE antibodies had minimal effects on PKCβII activation (supplemental Figure IC). Both apoC1 and apoE did not activate PKCβII of HUVECs or inhibit insulin-induced NO release into the culture media at $100 \mu g/mL$ (supplemental Figure IIA and IIB). They also did not activate PKCβII of the aorta of C57BL/6J mice or inhibit NO release into the plasma (supplemental Figure IIC and IID). Thus, acute administration of apoCIII-rich VLDL impaired insulin signaling in the aortas of C57BL/6J mice, suggesting the pivotal role of apoCIII itself and PKCβII in this process.
ApoCIII Inhibits Endothelium-Dependent Relaxation in the Aortas of C57BL/6J Mice

We finally determined whether apoCIII induces endothelial dysfunction in vivo using isometric tension measurements. In preliminary experiments, carbachol-induced relaxation of the aortic ring of C57BL/6J mice was abolished by the NOS inhibitor \( L-N^\text{G}-\text{nitro arginine} \) or removal of its endothelium, suggesting that the measurement reflects vascular endothelial cell– and NO-dependent relaxation (supplemental Figure III).

ApoCIII stimulation significantly impaired endothelium-dependent relaxation of the isolated aortic rings of C57BL/6J mice, which was recovered by PKC\( \beta \)-specific inhibitor (Figure 7A). VLDL CIII+ also impaired endothelium-dependent relaxation. In contrast, VLDL CIII− had minimal effect on it (Figure 7B).

**Figure 6.** Effect of VLDLCIII+ on insulin signaling in the aortas of C57BL/6J mice. A, B, C57BL/6J mice were injected intravenously with VLDL CIII+ or VLDL CIII− (500 \( \mu \text{g} \) apoB per body). After 30 minutes, the aortas and blood were collected. Representative blots are shown. *\( p<0.05 \) vs VLDL CIII−. C, C57BL/6J mice were injected intravenously with VLDL CIII+ (500 \( \mu \text{g} \) apoB per body). After 30 minutes, the aortas were collected. In experiments using PKC\( \beta \)-specific inhibitor, it was injected intravenously at 10 mg/kg body weight 30 minutes before apoCIII injection. Representative blots are shown. *\( p<0.05 \) vs apoCIII(+)/PKC\( \beta \)-specific inhibitor(−). D, E, C57BL/6J mice were injected intravenously with VLDL CIII+ or VLDL CIII− (500 \( \mu \text{g} \) apoB per body). In experiments using PKC\( \beta \)-specific inhibitor, it was injected intravenously at 10 mg/kg body weight 30 minutes before apoCIII injection. After 30 minutes, animals were stimulated with intraperitoneal insulin (0.75 mU/g body weight for 10 minutes). Then, the aortas and blood were collected. Representative blots are shown. *\( p<0.05 \) vs VLDL CIII+(−)/insulin(+)/PKC\( \beta \)-specific inhibitor(−); #\( p<0.05 \) vs VLDL CIII+(−)/insulin(+)/PKC\( \beta \)-specific inhibitor(−).

**Discussion**

The present study reports for the first time that apoCIII and apoCIII-rich VLDL cause insulin resistance in vascular en-
PKCβ in vascular endothelial cells and induces vascular cell adhesion molecule-1 upregulation. Selective inhibition of PKCβ abolished this process. Moreover, remnant lipoproteins that are rich in apoCIII activate PKCs in human monocyteic U937 cells and rat aortic smooth muscle cells. In the present study, apoCIII activated PKCβII in HUVECs. ApoCIII-induced serine phosphorylation of IRS-1 was dependent on PKCβ. Indeed, a PKCβ-specific inhibitor reversed the inhibitory effect of apoCIII on this insulin signaling. Several PKC isozymes, including PKCβII, negatively regulate IRS-1 function by serine phosphorylation. These results indicate a central role of PKCβII in impaired insulin signaling induced by apoCIII. Although the mechanism(s) by which apoCIII activates PKCs in endothelial cells or monocytes remain to be fully elucidated, we recently reported that apoCIII activates PKCs through a pertussis toxin–sensitive G protein and phospholipase C in THP-1 cells, suggesting that a distinct pathway or receptor may be involved in this process. Further studies are needed to elucidate the specific apoCIII signaling pathways.

This study also identified PKCβ as the key molecule that regulates the MAP kinase and eNOS pathways. Insulin activates the small GTP binding protein Ras, which then initiates a phosphorylation cascade involving Raf, MEK, and ERK. We previously reported that apoCIII-rich remnant lipoproteins stimulate the proliferation of vascular smooth muscle cells through PKC-mediated MAP kinase activation. Recent reports suggest that there is crosstalk between the eNOS and MAP kinase pathways. Andreozzi et al reported that interleukin-6 impairs the vasodilator effects of insulin that are mediated by the eNOS pathway in endothelial cells through activation of JNK and ERK. These reports prompted us to examine whether apoCIII also would activate MAP kinase in HUVECs through its involvement in the eNOS pathway. We found that apoCIII itself activated ERK and augmented insulin-stimulated MAP kinase activation. The inhibition of this pathway partially reversed the effects of apoCIII on Ser616 phosphorylation of IRS-1, suggesting that MAP kinase contributes to the impairment of the eNOS pathway. Activated MAP kinase also stimulates secretion of ET-1 from endothelial cells. In accordance, we found that apoCIII increased ET-1 release. Thus, apoCIII may cause or augment an imbalance between vasodilator and vasoconstrictor actions of insulin through its distinctive signaling pathway (Figure 8).

We evaluated the results obtained in cultured endothelial cells in an in vivo model. Administration of apoCIII impaired insulin signaling in the aortas of C57BL/6J mice. Moreover, apoCIII impaired endothelium-dependent relaxation of aortic rings of C57BL/6J mice, suggesting that apoCIII induces endothelial dysfunction. ApoCIII-rich VLDL, but not apoCIII-deficient VLDL, exerted similar inhibitory effects, although these results reflect overall effects of apoCIII or apoCIII-rich VLDL on whole aorta, not on vascular endothelium alone. What should be noted here is that apoCIII-rich VLDL contains other apolipoproteins (eg, apoCl, E) and lipid moieties that are different from apoCIII-deficient VLDL.
Thus, it is possible that apoCIII may not be the only component that accounts for the effects of apoCIII-rich VLDL. Moreover, apoCIII impairs catabolism of triglyceride-rich lipoproteins in vivo, which may modify other lipid parameters that affect insulin sensitivity of endothelial cells. However, considering the results of our experiments using apoCIII alone in vitro and in vivo and those using an anti-apoCIII function blocking antibody in vitro, we conclude that apoCIII in hypertriglyceridemic VLDL directly impairs insulin signaling in vascular endothelial cells, at least in part. Previous clinical studies have shown an acute and direct effect of hypertriglyceridemia on vascular endothelium. Acute hypertriglyceridemia induced by an oral fat load caused endothelial dysfunction in subjects with dyslipidemia and increased circulatory cellular adhesion molecules in healthy subjects. Further study is needed to clarify the involvement of apoCIII in this phenomenon.

ApoCIII impaired insulin signaling in vascular endothelial cells and caused endothelial dysfunction at ≈100 μg/mL in vitro and in vivo. This concentration corresponds to the middle of the population range. However, it is in the upper part of the range for apoCIII in VLDL because apoCIII resides on not only apoB lipoproteins but also high-density lipoprotein. Indeed, epidemiological studies have shown a gradient of risk for apoCIII in apoB lipoproteins starting from a lower level, eg, 2 mg/dL, the first quartile. Thus, we believe that apoCIII affects endothelial cells at clinically relevant concentrations in the upper part of the population distribution, supporting the clinical relevance of the present study.

Studies using apoCIII transgenic mice provide a complex, as-yet unresolved, picture of apoCIII and insulin action. Human apoCIII transgenic mice had hypertriglyceridemia and high serum FFA levels but normal glucose tolerance and normal adipocyte responses to glucose. Another study reported that apoCIII transgenic mice showed impaired insulin secretion, which is consistent with apoCIII causing apoptosis in cultured pancreatic β cells. We also examined the effects of apoCIII on insulin response in other insulin-sensitive cells. ApoCIII had minimal effects on insulin signaling in 3T3L1 cells and HepG2 cells (data not shown). These findings suggest that the apoCIII effect on insulin signaling may be cell-type specific.

Endothelial dysfunction associated with hypertriglyceridemia has been understood from the traditional view that FFA and other lipid moieties in triglyceride-rich lipoproteins impair insulin action and/or endothelial dysfunction. However, our findings may add a new mechanism in which triglyceride-rich lipoproteins are carriers of a causal factor for it, ie, apoCIII, although this apoCIII mechanism is not exclusive of the lipid hypothesis. Our observations provide novel insights into a role for apoCIII as a key molecule that could link dyslipidemia with endothelial dysfunction in the metabolic syndrome.

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CLINICAL PERSPECTIVE

Endothelial dysfunction contributes to cardiovascular diseases, including hypertension, atherosclerosis, and coronary artery disease. It is characterized by the reduced bioavailability of nitric oxide (NO), which has potent vasodilatory and antiatherosclerotic properties. Insulin activates endothelial NO synthase (eNOS) in endothelial cells and stimulates the production of NO, and insulin resistance in vascular endothelium leads to its dysfunction. Insulin resistance and endothelial dysfunction are often seen in diabetes, obesity, and dyslipidemia, major risk factors for cardiovascular disease. The plasma apolipoprotein (apo) CIII level is high in these conditions. We recently showed that apoCIII activates vascular endothelial cells through protein kinase C-β (PKCβ). Because PKCβ inhibits insulin signaling in endothelial cells, the present study tested the effect of apoCIII on endothelial insulin signaling. We showed that apoCIII in very-low-density lipoprotein inhibited insulin activation of the eNOS pathway and the production of NO in vascular endothelial cells. ApoCIII also impaired endothelium-dependent relaxation of the mice aortas in vivo. This adverse effect of apoCIII was mediated by its activation of PKCβII, which inhibits the function of insulin receptor substrate 1. Insulin resistance and endothelial dysfunction associated with hypertriglyceridemia have been understood from the traditional view that free fatty acids and other lipid moieties in triglyceride-rich lipoproteins impair insulin signaling. However, our findings may add a new mechanism in which triglyceride-rich lipoproteins are carriers of a causal factor apoCIII that impairs insulin signaling in vascular endothelial cells and suggest that apoCIII could link dyslipidemia with endothelial dysfunction.
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The corresponding author, Dr Akio Kawakami, reported to the editors that the authors of this manuscript have raised concerns related to the accuracy of the data presented in this article. In the process of following up the findings reported in this study, they were unable to reproduce some experiments due to Dr Akio Kawakami’s negligence in keeping proper original records. This information was reported to the editors by Dr Kawakami directly. The authors apologize to the readers of *Circulation* for any inconvenience caused by this retraction.