C-terminal interactions of apolipoprotein E4 respond to the postprandial state

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Abstract **Increased triglyceride-rich lipoproteins (TGRLs) in the postprandial state are associated with atherosclerosis. We investigated whether the postprandial state induced structural changes at the apolipoprotein E4 (apoE4) C terminus, its principal lipid binding domain, using electron paramagnetic resonance (EPR) spectroscopy of a site-directed spin label attached to the cysteine of apoE4-W264C. Spin coupling between labels located in the C termini was followed after mixing with preprandial and postprandial human plasma samples. Our results indicate that postprandial plasma triggers a reorganization of the protein such that the dipolar broadening is diminished, indicating a reduction in C-terminal interaction. The loss of spectral broadening was directly correlated with an increase in postprandial plasma triglycerides and was reduced with delipidated plasma. The spin-labeled apoE4 displayed a lipid preference of VLDL > LDL > HDL in the preprandial and postprandial states. The apoE4 shift to VLDL during the postprandial state was accompanied by a loss in spectral broadening of the protein. These findings suggest that apoE4 associated with LDL maintains self-association via its C terminus and that this association is diminished in VLDL-associated protein. Lipolyzed TGRL reflected a depletion of the C-terminal interaction of apoE4. Addition of palmitate to VLDL gave a similar response as lipolyzed TGRL, suggesting that lipolysis products play a major role in reorganizing apoE4 during the postprandial state.**—Tetali, S. D., M. S. Budamagunta, J. C. Voss, and J. C. Rutledge. **C-terminal interactions of apolipoprotein E4 respond to the postprandial state.** ***J. Lipid Res.*** **2006.** 47: **1358–1365.**

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Chronic vascular inflammation is increasingly recognized as playing a central role in atherosclerosis (1, 2). Recent studies have focused on repetitive injury of the vascular wall during the postprandial state (3, 4), where endothelial cells lining the vascular wall are repeatedly exposed to a wide variety of lipoproteins, cytokines, and hemostatic, inflammatory, and anti-inflammatory factors. Little is known about how these postprandial factors influence lipid metabolism, and specifically the behavior of apolipoproteins in the preprandial and postprandial state.

Apolipoprotein E (apoE) is a 34 kDa glycoprotein unique among apolipoproteins because of its many functions, including the assembly, processing, and removal of plasma lipoproteins (5). It is a component of VLDL, intermediate-density lipoprotein, chylomicrons, HDL (6), and LDL (7). ApoE serves as a ligand for LDL receptors and, through its interaction with these receptors, participates in the transport of cholesterol and other lipids among various cells of the body (8). In humans, there are three common isoforms of apoE, apoE2, apoE3, and apoE4, with apoE3 being the most common isoform. ApoE4 differs from apoE3 by having an arginine at position 112, rather than the cysteine residue in apoE3 (9). The *epsilon 4* allele is present in ~25% of the population (10, 11) and linked to an increased risk for the development of atherosclerosis and Alzheimer's disease (12–14).

ApoE contains a 22 kDa N-terminal domain (residues 1–191) and a 10 kDa C-terminal domain (residues 222–299) spaced by a protease-sensitive loop (15). The N-terminal domain contains the LDL receptor-binding region (residues 136–150 in helix 4), and the C-terminal domain has a high affinity for lipid and is responsible for lipoprotein lipid binding (16). Among the human isoforms, apoE4 shows a unique domain interaction in which the arginine at position 112 induces an interaction of arginine-61 in the N-terminal domain with glutamate-255 in the C-terminal domain, a feature thought to be responsible for the preferential association of apoE4 with VLDL (17, 18). Because of the preferential association of apoE4 with VLDL (10, 13) and the increased uptake of apoE4-VLDL by hepatic lipoprotein receptors, LDL receptor expression is downregulated (13). Thus, the *epsilon 4* allele results in slightly increased plasma LDL cholesterol levels (by 10 mg/dl).

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The literature strongly suggests that exchangeable apolipoproteins undergo conformational rearrangements upon association with lipids (19, 20), which in turn affect their functionality. In addition, it is likely that apoE adopts distinct conformational and oligomeric states when associated with different classes of lipid particles (21, 22). Because the postprandial state offers a dramatic change in the levels and ratios of lipoprotein particles, we hypothesized that these changes can alter the structure of apoE. Here, we present the first report on the effects of the preprandial and postprandial states on apolipoprotein structure. Rearrangements of apoE4 induced by the postprandial state, and specifically triglyceride-rich lipoprotein (TGRL) lipolysis products, are detected using electron paramagnetic resonance (EPR) spectroscopy. This work provides a basis to address isoform-dependent differences of apoE behavior during postprandial lipid metabolism, a critical factor in vascular disease risk. In addition, we demonstrate a new approach that allows for real-time observation of apolipoprotein structure in plasma samples.

MATERIALS AND METHODS

Materials

Streptokinase was purchased from ICN Pharmaceuticals, Vacutainers and venipuncture supplies were purchased from Fisher Scientific International, Inc. Glass capillaries were obtained from VitroCom, Inc. Fatty acid-free BSA, the TGRL assay kit, lipoprotein lipase, guanidine thiocyanate, and triolein were from Sigma-Aldrich Co. 1,2-Dimyristoyl-*sn*-glycero-3 phosphocholine (DMPC) was from Avanti Polar Lipids, and palmitic acid was from Nu-Chek Prep, Inc. Titan gel electrophoresis supplies were purchased from Helena Laboratories.

Human plasma samples

Healthy human subjects were recruited from the University of California Davis campus. The study was approved by the Human Subjects Research Committee of the University of California Davis. The study protocol was explained to each participant, and written informed consent was obtained. The volunteers were given a moderately high-fat meal (40% calories from fat), and blood was obtained by venipuncture into streptokinase-containing (1,500 units) tubes before (preprandial, 0 h) and after ingestion of the test meal (3.5 and 6 h), as described previously (23). The meal consisted of one regular size bagel, two tablespoons of cream cheese, one scrambled egg, two teaspoons of margarine, 8 ounces of whole milk, and 85 g of cantaloupe.

Plasma from the blood samples was separated by centrifugation at 1,200 *g* for 15 min, which pellets the blood cells leaving the plasma as a supernatant. Assays for LDL-cholesterol, HDL-cholesterol, total cholesterol, triglycerides, apoE content, and nonesterified fatty acids were performed at the Clinical Nutrition Research Unit at the University of California Davis and the University of California Davis Medical Center.

TGRL was isolated from postprandial plasma by ultracentrifugation at 285,000 *g* for 4 h and subsequently assayed for triglyceride concentration using the TG-Assay Kit (Sigma-Aldrich). Lipolysis products of TGRL were obtained by incubating TGRL with lipoprotein lipase (3 U/ml) at 37°C for 30 min. Palmitate stock was prepared according to Listenberger, Ory, and Schaffer (24).

Delipidation of plasma was performed according to Cham and Knowles (25). One volume of plasma containing streptokinase (15 U/ml) was added to 2 volumes of a mixture of butanol-di-isopropyl ether (40:60, v/v). The vials were tightly closed and fastened on a mechanical rotator providing end-over-end rotation at ~30 rpm for 30 min. After extraction, the mixture was centrifuged at low speed (2,000 rpm) for 2 min to separate aqueous and organic phases. The aqueous phase containing the delipidated proteins was removed by careful suction with needle and syringe. Traces of butanol that remained in the aqueous solution were removed by washing with 2 volumes of di-isopropyl ether. The delipidation procedure removed triglycerides and cholesterol completely and did not alter the pH.

Site-directed spin-labeling of apoE

ApoE4 with a single cysteine moiety substituted at amino acid position 264 (264C) was covalently modified with a methanethiosulfonate spin label (21). The labeled apoE4 was added to preprandial and postprandial plasma samples, incubated at 37°C for 1 h, and subsequently subjected to EPR studies. The spin-labeled apoE4 was kindly provided by Danny M. Hatters in Dr. Karl H. Weisgraber's laboratory (Gladstone Institutes of Cardiovascular and Neurological Disease, University of California, San Francisco).

Preparation of lipid-bound apoE4

For examination of apoE4 bound to either DMPC or lipid emulsions, spin-labeled protein (1 mg/ml final concentration) was combined with DMPC (at a final concentration of 5 mg/ml) as described previously (21). For the emulsion-bound apoE (1 mg/ml final protein concentration), unfractionated emulsions of egg yolk phosphatidylcholine and triolein (at a final phospholipid concentration of 14.5 mM) were combined with spin-labeled protein as described previously (21).

Separation of lipoproteins by Titan gel electrophoresis

Preprandial and postprandial plasma samples were incubated with apoE4 (264C) at 37°C for 1 h and applied (2 μl/lane) on Titan-agarose (precasted) gels. Lipoproteins of the plasma samples were separated by the Titan gel lipoprotein electrophoresis system according to the manufacturer's instructions (Helena Laboratories). ApoE4 alone, preincubated with Tris-buffered saline, also was run on the gel to determine the migration position of free (unbound) apoE4 (0.5 mg/ml) in the plasma treatments used for the site directed spin labeling (SDSL)-EPR study. After staining and destaining procedures, the electrophoretically resolved lipoprotein bands and a gel slice corresponding to apoE4 alone were excised and transferred to a 1.5 ml microfuge tube. The protein-only lane from the point of sample application to the end of the gel was excised into several 5 mm slices by laying the slices onto a graph sheet and transferred to 1.5 ml microfuge tubes. To each gel sample of the above, 30 μl of gel solubilization buffer (4.5 M guanidinium isocyanate) was added and incubated at 65°C for 3 min. To determine the amount of added apoE4 associated with each fraction, the solubilized samples then were thoroughly mixed and scanned by EPR as described below.

EPR spectroscopy

EPR measurements were performed in a JEOL X-band spectrometer fitted with a loop-gap resonator as described previously (26). Briefly, a 3.5 μl aliquot of purified, spin-labeled apoE4 (264C) at a concentration of 1 mg/ml protein in TBS was mixed with an equal volume of sample containing plasma, TGRL, or

VLDL with or without 0.5 M palmitate, loaded into a one-sided sealed glass capillary, incubated at 37°C for 1 h, and scanned by EPR. For all samples, appropriate vehicle controls were used. The spectra were obtained by a single 2 min scan over 100 G at a microwave power of 2 mW and a modulation amplitude of 1 G at room temperature (20–22°C) or at 37°C. To assess the signal content in guanidine-extracted gel samples, the signal-averaged spectra from six 20 G scans (20 s each) over the central ($m_I = 0$) line were recorded.

RESULTS

Selection of apoE4 W264C as a marker of lipid binding

Because lipid binding is initiated by the C-terminal domain of apoE (27), a sulfhydryl-specific nitroxide spin label was targeted to this region of the protein (**Fig. 1**). Human apoE4 lacks an endogenous cysteine; thus, a single-cysteine replacement at amino acid position 264 of purified human apoE4 results in a highly efficient and specific incorporation of methanethiosulfonate spin label at the cysteine-substituted site. As recently demonstrated (21), a magnetic dipolar interaction between spin labels directed to position W264C reflects the oligomeric state of the protein in the absence of lipid. Fan et al. (28) also showed that single amino acid mutations in the region of residues 253–289 did not affect the oligomerization of the protein. The apoE C-terminal domain single mutant W264R did not show any difference from its wild type in any properties, including oligomerization, secondary structure adoption, or lipid binding activity (28).

In the lipid-free tetramers of apoE4 (264C), the spin label at position 264 falls along the same face of a projected α-helix, showing substantial self-interaction. This was attributed to a dipolar interaction between the same sites (in the tetramer) and immobilization of the spin-labeled side chains caused by the quaternary contact and thus generated a broad spectrum (**Fig. 2**). The dipolar broadening is maintained upon lipidation by DMPC, which is assumed to mimic the nascent HDL conformation of apoE. In contrast, the dipolar broadening is relieved when apoE4 is bound to phospholipid-triolein emulsion particles (Fig. 2), indicating that the spin labels are >20 Å distal from one another in this state. These triglyceride-rich emulsions more closely resemble large TGRLs (i.e., very low density lipoproteins and chylomicrons) (29, 30).

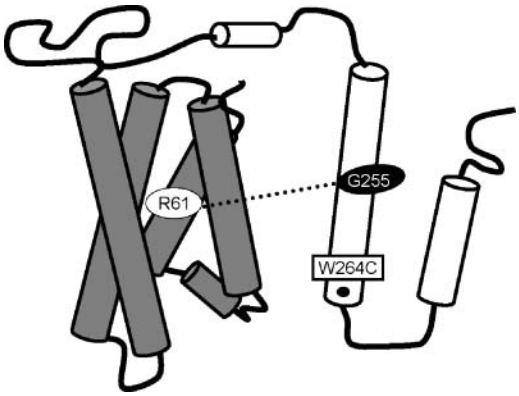

Fig. 1. Schematic representation of apolipoprotein E4 (apoE4) structure. The C-terminal tryptophan at amino acid position 264 was mutated to cysteine and subsequently labeled with nitroxyl spin label. The dotted line represents a salt bridge, between arginine-61 (R61) and glutamate-255 (G255), of apoE4, showing domain interaction.

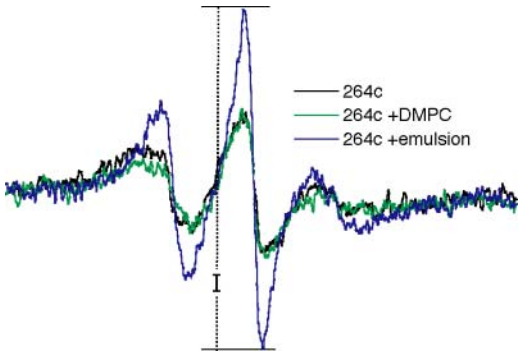

Fig. 2. Effect of lipid binding on the electron paramagnetic resonance (EPR) spectra of spin-labeled apoE4. Comparison of lipid-free, 1,2-dimyristoyl-*sn*-glycero-3 phosphocholine (DMPC)-complexed, and emulsion-complexed apoE4. Each spectrum represents the same number of spins from a final spin-labeled apoE4 concentration of 1 mg/ml. The peak height measurement of the $m_I = 0$ spectral intensity is indicated by the dashed line.

ApoE4 binds to human VLDL > LDL in the postprandial state

To investigate how human preprandial and postprandial plasma affect structure at the C terminus of apoE4, plasma samples from human volunteers were tested on exogenously added human apoE4 containing a site-directed spin label at position 264C. In these specimens, apoE content in volunteer plasma ranged from 0.04 to 0.017 mg/ml, with a mean value of 0.023 ± 0.007 mg/ml. To these samples, spin-labeled apoE4 (W264C) was added to give a final concentration of 0.5 mg/ml (0.0147 M).

To test the extent and binding preference of spin-labeled apoE4 to lipoprotein species in human plasma, we evaluated the localization of apoE4 (264C) to lipoprotein fractions after apoE4 (264C) was added to plasma and the sample was separated by agarose gel electrophoresis (**Fig. 3A**). Plasma samples were collected from volunteers after fasting (preprandial) and 3.5 or 6 h after the test meal (postprandial). As shown in Fig. 3B, when apoE4 (W264C) was incubated with preprandial plasma, apoE4 (264C) was distributed in both LDL and VLDL fractions (20% and 30%, respectively). However, in the postprandial 3.5 h sample, much more apoE4 was found in the VLDL fraction than in the LDL fraction (40% and 10%, respectively). ApoE4 (264C) was not associated with chylomicrons and was detected at very low levels from HDL fractions. This result is consistent with earlier studies showing that apoE4 associates primarily with VLDL and, to a lesser extent, with HDL (31, 32). At 6 h postprandially, apoE4 (264C) showed a distribution similar to the preprandial sample (Fig. 3B). These results demonstrate that

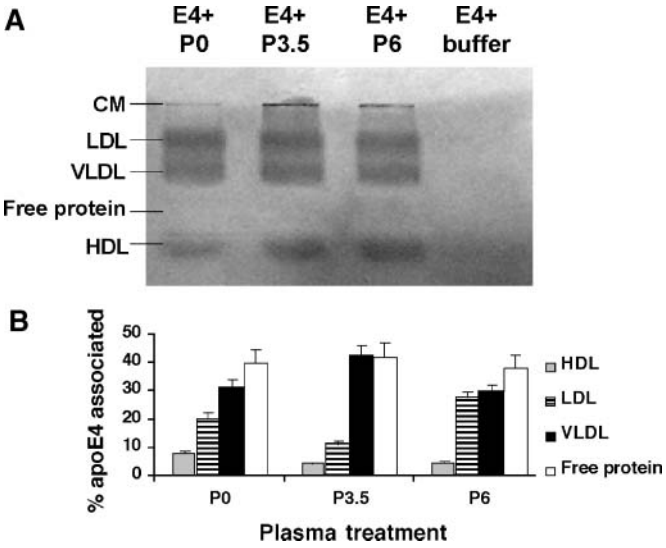

Fig. 3. Binding of spin-labeled apoE4 to plasma lipids. A: Lipoprotein separation of preprandial and postprandial plasma samples treated with apoE4 (264C) by Titan gel electrophoresis. Healthy human subjects were recruited, and blood was obtained by venipuncture preprandially (fasting; 0 h) and postprandially (3.5 and 6 h) after ingesting a 40% fat meal. ApoE4 (264C) at a final concentration of 0.5 mg/ml was incubated with preprandial or postprandial plasma at 37°C for 1 h. After incubation, samples (2 μl/lane) were applied onto Titan agarose gels and electrophoresed at 80 V for 45 min. After electrophoresis, lipoproteins were visualized by staining with Fat Red 7B (fat stain). Lane 1, apoE4 (264C) treated with preprandial plasma (P0); lane 2, apoE4 treated with postprandial 3.5 h plasma (P3.5); lane 3, apoE4 treated with postprandial 6 h plasma (P6); lane 4, apoE4 treated with Tris saline buffer. B: EPR measurements of spin-labeled apoE4 associated with preprandial and postprandial plasma. From the gel experiments in A, bands of chylomicrons (CM), LDL, VLDL, and HDL were excised and solubilized into 4.5 M guanidine isocyanate by incubating at 65°C for 3 min, and gel extracts were subjected to EPR spectroscopy. The amount of free protein left after plasma treatment was calculated. The position of the free protein in the plasma-treated samples was determined by running the protein alone. Error bars represent SD.

apoE4 has a similar pattern of distribution in the preprandial and postprandial states with respect to lipoprotein affinity (VLDL > LDL).

We also tested for relative amounts of excess free protein after electrophoresis of the plasma samples to which apoE4 (264C) had been added. The position of the free protein on the gel was determined by running the protein alone. Before loading the samples onto the gel, the apoE4 (264C) was mixed with plasma lipids in the same ratios as for the EPR experiments and incubated at 37°C for 1 h. In both preprandial and postprandial samples, ~40% of the added apoE4 migrated to the position corresponding to free apoE4 (264C) (Fig. 3B).

ApoE4 (264C) spectral changes are associated with postprandial triglycerides

As described above, spin labels located at position 264 report a decrease in broadening of the transition of apoE4 from the lipid-free state to the lipid emulsion-bound state (21). This is reflected in the narrower EPR line shapes of spin-labeled W264C protein. Because of the low spin levels (~14 μM) in the mixtures of plasma and spin-labeled apoE4, the most reliable line shape parameter indicative of the structural change is the signal intensity of the central ($m_I = 0$) EPR line (Fig. 2). Repeated scans of samples analyzed over a period of time demonstrated that this parameter provides highly reproducible values, with a total variance range of <2% for samples scanned, removed from the instrument, and rescanned more than five times. However, spectral fitting and deconvolution, processes that can provide quantitative assessment of changes in spin label correlation times or interspin distance, are not possible at the low signal-to-noise levels of these samples. Hence, these measurements provide a qualitative marker of the amount of apoE4 that has remodeled at its C terminus in the presence of a specified plasma sample.

Blood triglycerides have been shown to be increased to maximum levels at 3.5 h after ingesting a moderate-fat meal in normal humans and decline to reach steady-state (fasting) triglyceride levels within 6–8 h (23). These triglycerides are incorporated primarily into chylomicrons, chylomicron remnants, VLDL, and VLDL remnants. To determine the dependence of C-terminal reordering in apoE4 on plasma lipid components, we combined spin-labeled apoE4 (264C) with preprandial and postprandial samples of six healthy volunteers. **Figure 4A** shows the loss in spectral broadening upon incubation of spin-labeled apoE4 (264C) with preprandial and postprandial plasma samples from individual volunteers compared with protein maintained in buffer alone. All samples contained identical amounts of spin-labeled apoE4 (264C) (0.5 mg/ml). The level of loss in spectral broadening is simply given as the percentage increase in the EPR signal intensity from the central ($m_I = 0$) line relative to the lipid-free protein. The serum triglyceride levels for each volunteer are given in **Table 1**. Within the group of six volunteers, the preprandial plasma sample of volunteer 5289, with the lowest fasting triglycerides (35 mg/dl), gave an EPR signal of 142% over the buffer control, whereas volunteer 7125, with the highest serum triglycerides (173), had an EPR signal of 174% above control (Fig. 4A). In five of the six volunteers, a higher EPR signal was obtained for apoE4 (264C) treated with postprandial plasma at 3.5 h relative to the fasting plasma controls. However, in each volunteer, the 6 h postprandial sample gave a lower EPR signal than the corresponding 3.5 h sample.

As shown in Fig. 4B, the apoE4 (264C) EPR signal amplitude correlates with the increase in postprandial triglycerides. Our results (Fig. 4B) show a linear relationship between the percentage increase in postprandial (3.5 h) triglycerides and the increased EPR signal amplitude of apoE4 (264C). Six hours after the meal, triglycerides returned almost to control levels, which correlate well with the apoE4 EPR signal amplitude (Fig. 4B). Thus, spin-labeled apoE4 at position 264 reports a loss in C-terminal interaction associated with increased plasma triglyceride content.

To test whether the loss in spectral broadening of apoE4 (264C) in plasma is sensitive to lipid removal, the spectral

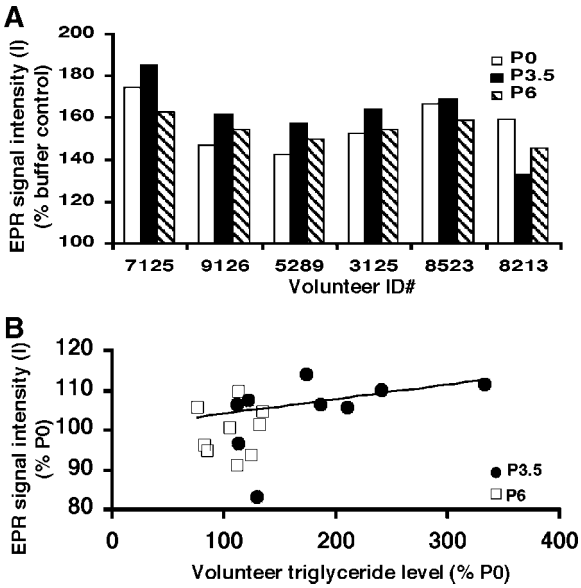

Fig. 4. Postprandial lipids induce the greatest spectral change in spin-labeled apoE4 (264C). A: The C-terminal interaction of apoE4 in plasma was measured as an increase in the spectral intensity (decrease in broadening) of the spin-labeled apoE4 EPR signal as a percentage of the buffer control. The intensities were determined from the height of the $m_I = 0$ line (see Figure 2). Data from volunteers 8523, 9126, and 5289 are averages of two independent measurements, whereas data from volunteers 8213, 3125, and 7125 are from a single experiment. B: Correlation between change in postprandial triglycerides and change in EPR signal amplitude. Preprandial and postprandial serum triglycerides of six healthy volunteers were analyzed. Percentage changes in postprandial (3.5 and 6 h) plasma triglycerides for each volunteer relative to their fasting triglycerides were calculated. The EPR signal amplitude of apoE4 (264C) upon treatment with preprandial plasma was considered as 100%. The percentage change in EPR signal after treatment with postprandial plasma samples was plotted against the percentage change in postprandial plasma triglycerides. Three of the six volunteers were invited twice for the study, and their average values are shown.

broadening of spin-labeled apoE4 in 3.5 h postprandial plasma was compared with and without delipidation. **Figure 5** shows the average spectra obtained from apoE4 combined with buffer, the 3.5 h postprandial plasma from four volunteers, or the same plasma samples treated by delipidation, which removes phospholipids, triglycerides, and cholesterol. Nine percent of nonesterified fatty acids were left after delipidation (data not shown). Compared with 3.5 h postprandial plasma, the spectral amplitude resulting from delipidated plasma is substantially less and there is increased spectral broadening, indicating that the loss in C-terminal interaction of apoE4 (264C) is dependent on the lipid component of plasma. The intermediate position of apoE4 (264C) in delipidated plasma may result from remnant solvent in solution, triggering a change in protein conformation and/or oligomeric state. It should also be noted that the presence of residual nonesterified fatty acids may contribute to the loss in spin-coupling in the delipidated sample.

TABLE 1. Serum triglycerides of the healthy human volunteers

Volunteer No.	Serum Triglycerides		
	Preprandial (0 h)	Postprandial (3.5 h)	Postprandial (6 h)
	mg/dl		
7125	173	324	217
9126	122 ± 16	233 ± 9	135 ± 22
5289	48 ± 13	145 ± 60	48 ± 1
3125	98	120	130
8523	146 ± 5	166 ± 5	125 ± 2
8213	46	60	52

Volunteers 9126, 5289, and 8523 were reinvited for the study; therefore, the data from those volunteers are averages of two independent measurements. The data from volunteers 7125, 3125, and 8213 are from a single experiment.

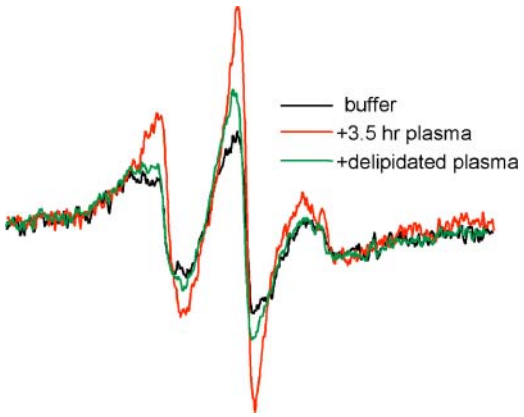

Fig. 5. Postprandial plasma delipidation diminishes C-terminal interaction in apoE4 (264C). Postprandial (3.5 h) plasma samples were subjected to delipidation by treating plasma with 2 volumes of a mixture of butanol-di-isopropyl ether (40:60, v/v). The apoE4 (264C) protein was treated with Tris saline buffer, postprandial (3.5 h) plasma, or delipidated postprandial (3.5 h) plasma. The samples then were incubated at 37°C for 1 h and subjected to EPR spectroscopy.

Lipolysis products of TGRLs mediate the monomerization of apoE4

Because increased lipolytic activity also is associated with the postprandial state, we examined the effect of isolated TGRL on the apoE4 (264C) oligomeric state and compared those changes with samples containing TGRL that were pretreated with LPL. Equal amounts of spin-labeled apoE4 were subjected to EPR spectroscopy after incubation with TGRL (triglyceride = 450 mg/dl) or the same TGRL pretreated with LPL (3 U/ml). The apoE4 added to TGRL pretreated with LPL generated a signal intensity 30% greater than that generated by adding TGRL alone (**Fig. 6**). No direct effect of LPL on the spectrum was observed when the LPL was incubated with apoE4 (W264C) in buffer. To test whether or not lipolysis products are responsible for the increase in signal intensity, we added 0.5 mM palmitate to isolated VLDL (triglyceride = 415 mg/dl). The addition of palmitate-BSA to VLDL generated 20% greater signal intensity than in the presence of VLDL alone with an equivalent amount of BSA

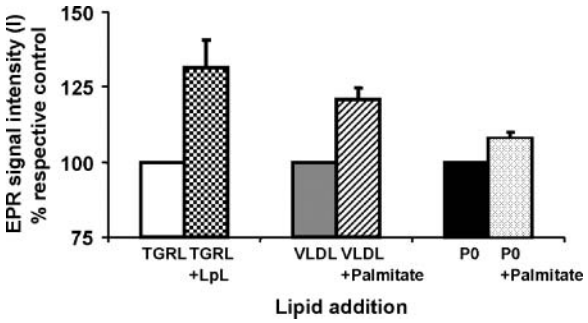

Fig. 6. Effect of triglyceride-rich lipoproteins (TGRLs) and TGRL lipolysis products on apoE4 (264C). TGRL and VLDL were isolated from postprandial (3.5 h) plasma of three healthy volunteers by ultracentrifugation. ApoE4 (264C) at a final concentration of 0.5 mg/ml was incubated with TGRL with and without LPL or with VLDL or preprandial plasma (P0) with and without 0.5 mM palmitate. EPR signal amplitudes of their respective controls were used as the 100% controls. Error bars represent SD.

added. We also added palmitate to preprandial plasma, which resulted in an increase of 7% over the signal obtained from apoE4 (264C) treated with preprandial plasma alone (Fig. 6). However, adding palmitate alone to the protein without adding any of the above lipids did not change the signal intensity (data not shown). These data suggest that lipolysis products generated during the postprandial state influence the lipoprotein preference of apoE4.

DISCUSSION

Lipid binding is known to induce the structural rearrangement of apolipoproteins (19, 20). Previous studies have examined the conformational changes of apoE upon lipid binding, which is required for receptor-mediated lipoprotein metabolism (33, 34). Our interest in this study was to evaluate whether elements of apoE4 structure are sensitive to postprandial lipids. No prior study has reported on the structural properties of apolipoproteins during the postprandial state and their preferential association with lipoprotein fractions.

Epidemiologically, apoE4 is associated with increased cardiovascular and Alzheimer's diseases, and the basis for this difference is an area of intense study (12–14, 35). Because of the preferential association of apoE4 with VLDL (10, 13), a pathophysiological lipoprotein profile is observed in apoE4 homozygotes. In our study, we found much more apoE4 association with VLDL and LDL fractions than with the HDL fraction. Furthermore, apoE4's preference for VLDL was much higher during the 3.5 h postprandial state (Fig. 3B). Here, we describe a simple approach for observing apoE4 lipoprotein preferences in plasma in real time.

In some individuals and in some patient populations (e.g., diabetics), postprandial hyperlipemia occurs several times daily. Postprandial lipoproteins could exert effects on the arterial endothelium and can cause arterial inflammation (3, 4). ApoE is involved in important interactions between the lipoproteins and the endothelium. Because postprandial hyperlipemia has been implicated in the development of atherosclerosis via repetitive injury to the arterial endothelium (36), understanding the role of the apoE isoform and conformation in modulating these proinflammatory processes is a pressing objective.

In this study, the apoE4 (264C) EPR signal amplitude correlated with the increase in postprandial triglycerides. Six hours after the meal, triglycerides returned almost to control levels, as did the apoE4 EPR signal amplitude. Our findings suggest that postprandial increases in triglycerides can affect the oligomeric state of apoE4. Moreover, our experiments with delipidated plasma suggest that the lipid component of the plasma is largely responsible for the change in the apoE4 oligomeric state seen with the 3.5 h postprandial treatment.

The lipid-free state of spin-labeled W264C used in these experiments was determined to be >80% tetrameric (21). Previous studies have shown that lipid-free apoE associates primarily through the C terminus (15, 37, 38). To obtain a monomeric C-terminal domain, Fan et al. (28) identified five bulky hydrophobic residues, including W264, within the C terminus where substitution as a group leads to a stable monomeric protein. Although it is likely that W264 directly participates in the interaction of C termini, a single substitution of W264R did not affect functional properties, including the C-terminal domain-dependent lipid association of apoE. Our findings show that changes in the self-association of the apoE4 C terminus are useful markers for discriminating between specific apolipoprotein particle binding. By exploiting the loss in interaction between these spin labels in the apoE4 C terminus, the approach described here can discriminate between apoE4 binding to lipid particles of differing composition. In this specific case, the loss in spectral broadening of apoE4 treated with postprandial plasma can be linked to a protein shift from LDL to VLDL. The EPR approach of measuring apoE self-association may be especially useful for examining the mechanisms of apoE-associated pathologies, because the higher self-aggregation propensity of apoE4 is a fundamental difference between apoE isoforms (38).

Despite having similar amounts of apoE4 bound to plasma lipids from fasting and postprandial samples, we saw significant loss in dipolar interaction only when the fraction of bound apoE4 was shifted much more to the VLDL species (i.e., the 3.5 h postprandial state). This suggests that the C-terminal interaction is maintained when associated with the LDL and diminished when bound to the VLDL. Studies of artificial lipid particles may provide insights on the VLDL requirement for diminishing interaction between spin labels located within the apoE C-terminal domain. The spin interaction between labels located at position 264 is maintained in DMPC, indicating that this position remains proximal among neighboring proteins on discoidal DMPC particles. A recent Fourier transform infrared and cross-linking study of the C-terminal domain of DMPC particles is consistent with a parallel-running dimer in the belt orientation (22).

The same group has postulated different arrangements of the E3 and E4 proteins on discoidal DMPC particles, where E3 may prefer an extended linear belt (39) and E4 a hairpin belt configuration (40). Because spin interaction is lost when the protein is combined with lipid emulsions, apoE may assume a monomeric arrangement on these particles, as has been reported for the protein bound on the VLDL particle (41, 42). Given that LPL treatment of TGRL causes spin interaction via the C terminus to be lost entirely, it is possible that the VLDL effect is dependent on its level of free fatty acids.

Postprandial lipoprotein metabolism is largely dependent upon lipolysis of TGRL by lipoprotein lipase. In healthy human subjects, the specific activity of LPL has been shown to be significantly increased postprandially when both heparinized (43) and nonheparinized (44) plasma samples are analyzed. We tested whether lipolysis products are responsible for the change in oligomeric state of apoE4 during the postprandial state. Treatment with lipolyzed TGRL resulted in a complete loss of dipolar broadening among spin labels at the C terminus of apoE4, yielding an EPR signal amplitude 30% higher than that from apoE4 combined with untreated TGRL. TGRL treated with LPL had 5- to 10-fold higher levels of nonesterified fatty acid content compared with untreated TGRL (data not shown). We incubated the protein with VLDL plus palmitate, a free fatty acid, to determine whether the added free fatty acid can simulate the effect of lipolysis products. Palmitate in the presence of VLDL did amplify the signal intensity of the protein 264C. These findings suggest that lipolysis products enhance the binding of apoE4 to VLDL or promote a more monomeric arrangement of the protein on VLDL particles.

Conclusions

Our study shows a dramatic shift of apoE4 preference in lipoprotein binding during the preprandial and postprandial periods. Furthermore, lipolysis products of TGRL diminish the C-terminal interaction of apoE4 during the postprandial state. Future studies will explore whether the system described here can detect isoform-dependent differences in the association of spin-labeled apoE with plasma apolipoproteins and vascular cells, such as endothelium. Such studies help us to understand the relevance of postprandial-mediated structural changes to disease cause and progression.

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