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ApoE genotype-specific inhibition of apoptosis

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Abstract **Endothelial cell apoptosis can be initiated by withdrawing growth factors or serum, and is inhibited by HDL. Our results show that the total lipoprotein population from apolipoprotein E 4/4 (APOE4/4) sera is less anti-apoptotic than total lipoproteins from other APOE genotypes, as measured by caspase 3/7 activity. Moreover, APOE4/4 VLDL antagonizes the antiapoptotic activity of HDL by a mechanism requiring binding of apoE4 on VLDL particles to an LDL family receptor. This ability of APOE4/4 VLDL to inhibit the antiapoptotic effects of HDL presents a potential mechanism by which the expression of several diseases, including atherosclerosis, is enhanced by the APOE4 genotype.**—DeKroon, R. M., M. Mihovilovic, Z. V. Goodger, J. B. Robinette, P. M. Sullivan, A. M. Saunders, and W. J. Strittmatter. **ApoE genotype-specific inhibition of apoptosis.** ***J. Lipid Res.*** **2003.** 44: **1566–1573.**

Supplementary key words apolipoprotein E • high density lipoprotein • very low density lipoprotein • low density lipoprotein • atherosclerosis • endothelial cell

Endothelial cells undergo apoptotic cell death in response to varied stressors, including growth factor deprivation, oxidized- and minimally-oxidized low density lipoproteins, homocysteine, hyperglycemia, and reactive oxygen species, all of which are involved in cellular mechanisms producing atherosclerosis (1–3). In contrast, other factors, such as nitric oxide, estrogen, and insulin, inhibit endothelial cell apoptosis and are antiatherosclerotic (4–6). HDLs are also antiatherosclerotic, and have recently been demonstrated to inhibit endothelial cell apoptosis. Nofer et al. (7) showed that HDL potently inhibits apoptosis in endothelial cells by activating protein kinase Akt, and further demonstrated that two sphingolipids in HDL, sphingosylphosporylcholine and lysosulfatide, mimicked this antiapoptotic activity of HDL. These sphingolipids are agonists of the endothelial differentiation gene receptor family that act through the PI3 kinase-Akt pathway, which in turn inactivates caspases (7, 8).

The apolipoprotein E (APOE, gene; apoE, protein) genotype profoundly affects lipoprotein particle class distribution and metabolism, and is also associated with varying risk of atherosclerosis (9, 10). Compared with individuals with the APOE3 allele, individuals possessing the APOE4 allele have increased risk of atherosclerosis. The apoE4 and apoE3 isoforms bind the LDL family of receptors with similar avidity (11), but are differentially associated with the various populations of lipoprotein particles, and influence the serum concentrations of these particles (12, 13). In humans, apoE4 preferentially associates with VLDL, while apoE3 preferentially associates with HDL. In transgenic (TG) mice, however, both apoE3 and apoE4 are largely associated with VLDL and IDL. The molecular mechanism of apoE isoform-specific interactions with lipoprotein particle classes has been extensively studied (14, 15). Approximately 15% of APOE2 homozygote individuals accumulate β-VLDL, have increased plasma cholesterol and triglycerides, and also manifest premature atherosclerosis, while the remaining 85% are slightly hypercholesterolemic compared with APOE3 individuals (16). ApoE2 binds the LDL family of receptors with an affinity of ~10-fold less than apoE3 (11). Because HDL inhibits endothelial cell apoptosis, and the various alleles of APOE partially determine the distribution of lipoprotein classes, we examined whether lipoproteins from sera of different APOE genotypes differentially inhibit apoptosis.

MATERIALS AND METHODS

Cell culture and induction of apoptosis

Human umbilical vein endothelial cells (HUVECs) were obtained from American Type Culture Collection (ATCC; Manassas, VA) and used between passages 21 and 29. Cells were grown in 96 well plates (Phoenix Research Products, Hayword, CA) in EBM-2 Clonetics media (BioWhittaker Inc., Walkersville, MD)

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supplemented with 2% fetal calf serum (FCS) at 37°C in an atmosphere of 5% CO_2. Each well received 0.1 ml of cell suspension containing 10^6 cells/ml, and cells were grown at 37°C for 48 h.

To initiate apoptosis, cells grown to confluence were washed twice with RPMI 1640 (Invitrogen Life Technologies, Carlsbad, CA) and received 0.070 ml of either *a*) sera-free media (SFM) consisting of RPMI 1640 supplemented with 1.0 μg/ml heparin, 0.2 μg/ml hydrocortisone, and 1.0 μg/ml ascorbic acid (Sigma, St. Louis, MO); *b*) SFM supplemented with 20% characterized FCS (HyClone, Logan, UT); or *c*) SFM supplemented with the indicated concentrations of lipoproteins in saline (NaCl 0.9%, pH 7.5). The volume of lipoprotein solution added never exceeded 20% of the incubation volume; SFM blanks and sera-containing media received the appropriate amount of saline. Experimental end points were obtained after 9 h of sera deprivation.

Statistical analysis

Results are presented as means ± SE. Unpaired two-tailed Student's *t*-test was used to compare data groups.

Caspase 3/7 activity assay

Caspase 3/7 activity was measured using the Apo-ONE assay developed by Promega (Madison, WI) using a 1:200 dilution of substrate. The Apo-ONE substrate is a profluorescent peptide linked to a rhodamine 110, leaving a group that, when cleaved by activated caspases-3 and -7, becomes fluorescent (Ex 485 nm, Em 520 nm). Caspase activity (%) was determined by subtracting the relative fluorescence units obtained in the presence of 20% FCS from that obtained in serum-free medium, and assigning 100% caspase activation to this difference. Caspase activity (%) obtained in the presence of lipoproteins was expressed relative to this difference.

Preparation of lipoprotein fractions

C57BL mice expressing wild-type apoE mice (APOE-WT), mice null for APOE (APOE-KO), APOE-KO mice made TG to express human apoE2, apoE3, or apoE4 (17), and targeted replacement (TR) mice homozygous for APOE2, APOE3, or APOE4 (20, 21) were kept on a normal chow diet. Lipoproteins were purified from pooled sera of fasted adult mice as previously described (18, 19). Sera was obtained within 2 h of bleeding and adjusted to 5 mM EDTA by the addition of 0.5 M EDTA (pH 7.5). Total lipoproteins and lipoprotein fractions were purified through isopycnic flotation of pooled sera. Densities were adjusted through the addition of dehydrated KBr. VLDL fractions were floated at a density of 1.006 g/ml; IDL (corresponding to human IDL-LDL) fractions at a density >1.006 g/ml and <1.063 g/ml; HDL fractions at a density >1.1365 g/ml and <1.25 g/ml; and total lipoproteins at a density of 1.25 g/ml. KBr densities for HDL were selected to avoid contamination of the HDL particles with IDL particles; the latter are particularly abundant in genotypes rich in VLDL and IDL species. Lipoproteins were dialyzed against NaCl 0.9%, 5 mM EDTA (pH 7.5), to eliminate KBr, and further dialyzed for a maximum of 24 h against NaCl 0.9% (pH 7.5). Recovered lipoproteins were filtered through Millex-GV 0.22 μm filters (Millipore, Bedford, MA), snap frozen in liquid nitrogen, and kept at −80°C. The antiapoptotic activity of lipoproteins was not altered by storage at −80°C. In addition, there was no significant difference in antiapoptotic activity between WT lipoproteins stored at 4°C or −80°C. Based on cholesterol concentration, the average yield for total lipoproteins was 76.60 ± 8.84%, and for lipoprotein fractions (HDL, IDL, and VLDL combined) was 72.83 ± 10.55%.

Lipoprotein concentrations are expressed as the percent of the concentration (by volume) of lipoproteins found in sera of a particular APOE genotype. The ratio of the volume of sera to the volume of the KBr-floated lipoproteins from that sera represents the fold increase in lipoprotein concentration, and was corrected for losses during purification by determining the percent recovery of cholesterol from the original serum sample.

Lipoprotein cholesterol determinations

Cholesterol concentration was determined using a cholesterol oxidase-based methodology (Sigma). Samples of the purified lipoprotein fractions were added to 0.5 ml of cholesterol reagent to give a final volume of 0.505 ml in each assay (volumes were equalized by the addition of 0.9% NaCl, pH 7.5). The absorbance of each sample was measured at 500 nm, following incubation at 37°C for 5 min, and compared with cholesterol standards in the range from 0 to 10 μg cholesterol.

HPLC analysis of lipoprotein preparations

Phospholipids were extracted from total lipoprotein preparations (resuspended in PBS to a concentration equivalent to 16% of that found in serum) from APOE-WT, APOE-KO, and apolipoprotein E 4/4 (APOE4/4) mice using acetonitrile extraction, with a volume ratio of lipoprotein to acetonitrile of 1:1, according to the methods described by Nofer et al. (7). Lipid fractions were resolved by HPLC on a 5 μm Nucleosil 100 silicon column (MetaChem Technologies, Lake Forest, CA) using an acetonitrile gradient extending from 100% acetonitrile to 80% acetonitrile/20% water with a running time of 63 min. Phospholipids were detected by absorbance at 193 nm and elution profiles were compared with analytical-grade standards for sphingosylphosphorylcholine (SPC), sphingosine-1 phosphate, lysophosphatidic acid (Avanti Polar Lipids, Alabaster, AL), and lysosulfatides (LSFs) (Sigma).

HPLC-resolved fractions were dried under nitrogen, resuspended in 20 μl of ethanol, and diluted to 1 ml in RPMI (serum-free medium). SPC concentrations were determined through integration of the area under the SPC absorbance peaks using the

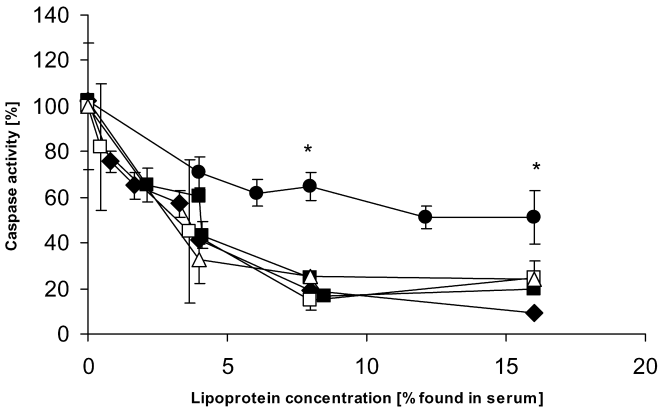

Fig. 1. The effects of serum lipoproteins on caspase activity in Human umbilical vein endothelial cells (HUVECs). Effects of total serum lipoproteins from wild-type apoE (APOE-WT) (triangles), mice null for APOE (APOE-KO) (open squares), APOE 2/2 targeted replacement (TR) (triangles), APOE 3/3 (TR) (open squares), and APOE 4/4 (TR) (circles) mouse sera on HUVEC caspase-3 and -7 activity of serum-depleted HUVECs. Lipoprotein concentrations are expressed as the percentage of lipoproteins found in sera, and caspase activity is expressed as a percentage of the activity obtained when no lipoprotein was added. APOE4/4 lipoproteins are clearly less protective than the other apoE genotypes ($P < 0.05$), while APOE-WT, APOE-KO, APOE 3/3, and APOE 2/2 show no significant difference from each other. Results are presented as means ± SE. * $P < 0.05$.

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Star Chromatography Workstation, version 5.51. The biological activity of these fractions was then assayed for their ability to protect cells from apoptosis using the caspase 3/7 activity assay.

Electrophoresis and Western blots of lipoprotein

Two microlitres of sera from each genotype were separated on the Titan lipoprotein gel system (Helena Laboratories, Allen Park, MI) according to the manufacturer's instructions. Lipids were visualized with 0.1% Fat Red stain. Western transfer was performed by capillary transfer onto polyvinylidene difluoride membrane and was followed by overnight blocking at 4°C with 5% skim milk in TBS (10 mM Tris, 150 mM NaCl, pH 7.5). ApoE was detected by indirect immunochemistry using goat anti-human apoE (1:2,000) (Calbiochem) followed by HRP-conjugated donkey anti-goat IgG (1:2,500) (Santa Cruz Biotechnologies, Santa Cruz, CA). Washes of 5 min each in TBS, followed by TBS-0.1% Nonidet P40, and then TBS followed each incubation in antisera. Blots were developed using enhanced chemiluminescence detection (Amersham, Piscataway, NJ) according to the manufacturer's instructions.

RESULTS

Inhibition of apoptosis by total lipoproteins is APOE genotype specific

Mice TG for the three human APOE alleles, APOE2, APOE3, and APOE4, have been produced in APOE-KO mice by both pronuclear injection of DNA and by targeted gene replacement. These lines have been extensively characterized for genotype-specific lipoprotein particle class distribution (12, 20, 21, and supplemental data). We isolated serum lipoproteins from mice expressing APOE-WT, from APOE-KO mice, and from mice (both TG and TR) homozygous for APOE2, APOE3, or APOE4. Since apoE protein levels, lipid levels (cholesterol, triglycerides), and the distribution of lipoprotein classes (VLDL, IDL/LDL, HDL) vary with APOE genotype (12, 13, 20, 21), lipoprotein concentrations for each APOE genotype

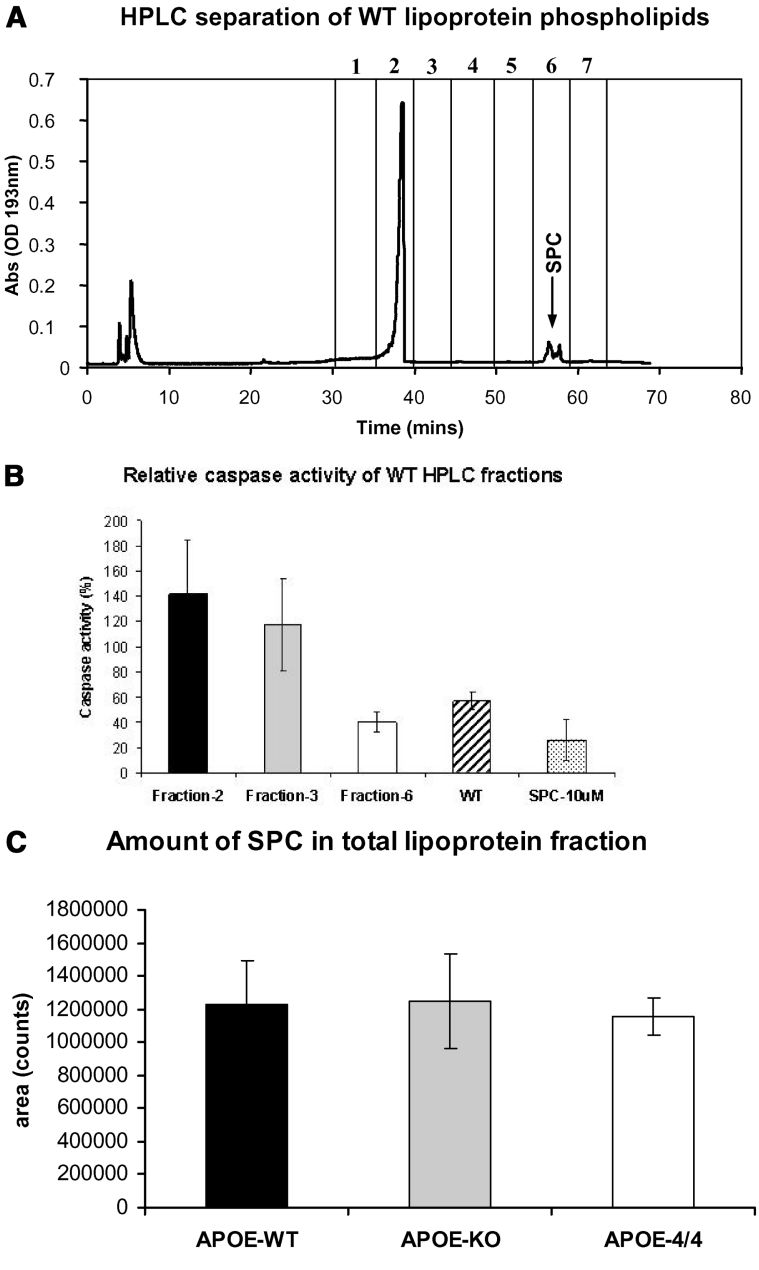

Fig. 2. Lysophospholipid concentration and antiapoptotic activity. A: HPLC separation of WT total lipoprotein phospholipids. Sphingosylphosphorylcholine (SPC) was identified in fraction 6, while lysosulfatide (LSF) was not detected. B: Fractions 1–7 were assayed for their ability to protect HUVECs from apoptosis. Only fraction 6 provided antiapoptotic activity. Ten micromoles of SPC standard and 16% APOE-WT total lipoproteins were included as positive controls for antiapoptotic activity. C: The amount of SPC in total lipoprotein from APOE-WT, APOE-KO, and APOE4/4 sera, obtained through integration of the area under the peak, showed no significant difference between genotypes.

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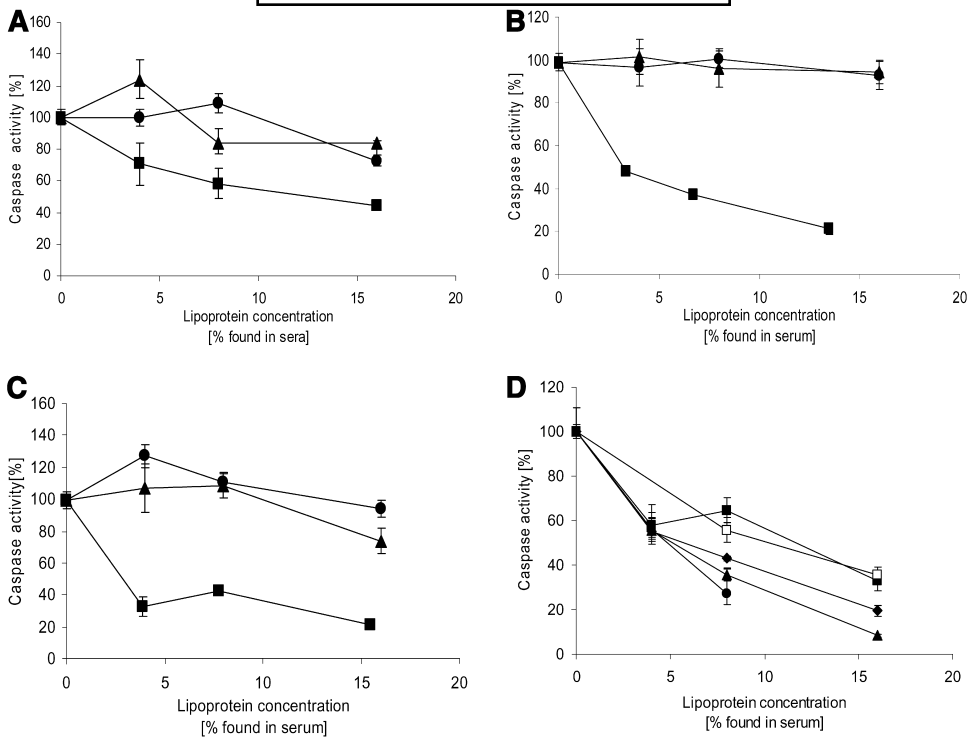

Fig. 3. Effects of different lipoprotein fractions on caspase activity in sera-depleted HUVECs. The suppression of caspase activity by HDLs (closed squares) from (A) APOE2/2 (TR), (B) APOE3/3 (TR), and (C) APOE4/4 (TR) mice was always much greater than that obtained with IDL/LDLs (triangles) or VLDLs (circles). In addition, the HDL lipoproteins (D) from APOE-WT (triangle), APOE-KO (open squares), APOE2/2 (TR) (closed squares), APOE 3/3 (TR) (diamonds), and APOE 4/4 (TR) (circle) mouse sera inhibited caspase activation to a similar extent. Lipoprotein fractions from transgenic (TG) mice showed the same trend (HDL > IDL/LDL = VLDL) and are presented in supplemental data. Results are presented as mean ± SE.

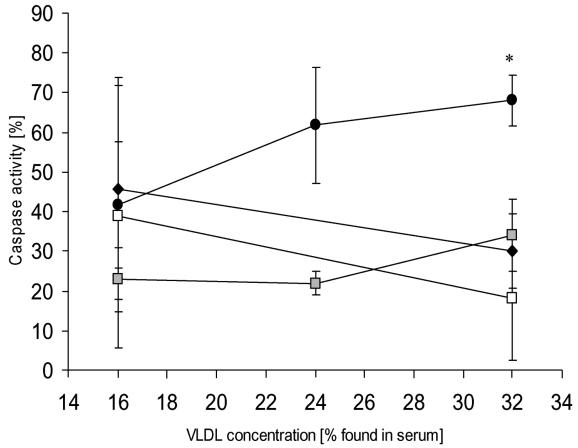

Fig. 4. VLDL inhibition of HDL activity. Addition of increasing amounts of VLDL to a fixed amount of HDL was tested for its effect on HDL suppression of caspase activity. VLDL from APOE4/4 (TG) (circles) mouse sera, but not from APOE2/2 (TR) (diamonds), APOE3/3 (TG) (closed squares), or APOE-KO (open squares) sera, inhibit the suppression of caspase activity by HDL. Results are presented as mean ± SE. * Indicates a significant difference ($P < 0.05$) between 8% APOE4/4 HDL alone and 8% APOE4/4 HDL + 32% APOE4/4 VLDL. No other significant differences were observed between HDL with or without VLDL of other APOE genotypes.

are expressed as the percentage of lipoproteins found in the sera. Total lipoproteins added to the media of serum-deprived HUVECs prevented effector caspase activation and subsequent apoptosis. Total lipoproteins from APOE-WT, APOE-KO, APOE3/3, and APOE2/2 sera were approximately equipotent in suppressing apoptosis (**Fig. 1**). Total lipoproteins from APOE4/4 sera, however, were markedly less potent in inhibiting apoptosis ($P < 0.05$, comparing caspase activity of APOE4/4 at 16% and 8% of original sera to that of other apoE genotypes).

Lysophospholipid concentration and antiapoptotic activity are not APOE genotype dependent

Nofer et al. (7) demonstrated that the ability of human lipoproteins to protect HUVECs from apoptosis is mediated in part by lysophospholipids in lipoproteins, including SPC and LSF. We therefore resolved lysophospholipids in mouse lipoproteins through HPLC to determine if reduced amounts of these lipids in APOE4/4 lipoproteins explained the reduced ability of these lipoproteins to suppress apoptosis. **Figure 2A** shows the HPLC elution profiles of phospholipids from APOE-WT lipoproteins. The SPC standard had the same retention time as the peaks in fraction 6; however, none of the other lysophospholipid standards corresponded to peaks in mouse lipoprotein elution profiles.

HPLC fractions were subsequently assayed for their ability to protect HUVECs from apoptosis. Only fraction 6

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(corresponding to the SPC standard) contained significant antiapoptotic activity (Fig. 2B). SPC content of total lipoproteins (of equivalent percent found in serum) from APOE-WT, APOE-KO, and APOE4/4 mice showed no significant differences (Fig. 2C).

The majority of antiapoptotic activity of total lipoproteins resides in HDL; HDL antiapoptotic activity is not dependent on APOE genotype

Since the levels of SPC present in total lipoproteins did not show a significant difference amongst the APOE genotypes, we next tested the various fractions of lipoproteins (VLDL, IDL/LDL, and HDL) isolated from APOE-WT and APOE-KO, and APOE2/2, APOE3/3, and APOE4/4 mouse sera for their ability to suppress apoptosis. Most of the antiapoptotic activity associated with the HDL fraction for each APOE genotype (**Fig. 3**). The ability of HDL from APOE-KO mice to suppress apoptosis demonstrates that inhibition of apoptosis by HDL does not require apoE itself (Fig. 3D). VLDL and IDL/LDL lipoproteins from all APOE genotypes showed only a minor inhibition of apoptosis. Importantly, the ability of HDL from APOE4/4 mice to inhibit apoptosis was similar to that of HDL from other APOE genotypes (Fig. 3D). In addition, the relationship between the activities of the lipoprotein fractions (HDL $>$ IDL/LDL $\geqslant$ VLDL) was preserved for all genotypes, whether from TR or TG mice (Fig. 3, supplemental data).

APOE4/4 VLDL inhibits the antiapoptotic activity of HDL

We next determined whether VLDL from different APOE genotypes suppressed the antiapoptotic activity of HDL. Addition of increasing amounts of APOE4/4 VLDL to a fixed amount of APOE4/4 HDL increasingly suppressed this activity of HDL ($P < 0.05$ at 32% APOE4/4 VLDL). In contrast, APOE2/2, APOE3/3, or APOE-KO VLDL had no effect on syngenic HDL activity (**Fig. 4**). These data suggest that the reduced inhibition of apoptosis by the total lipoproteins in APOE4/4 sera is due to the ability of APOE4/4 VLDL to suppress the antiapoptotic activity of HDL. As shown in Fig. 3, the antiapoptotic activity of HDL alone is not significantly different between APOE genotypes.

Receptor-associated protein prevents APOE4/from inhibiting HDL antiapoptotic activity

APOE4/4 VLDL might suppress HDL activity by a mechanism requiring the presence of the apoE4 protein isoform on VLDL. To test this possibility, we examined whether the receptor-associated protein (RAP), which inhibits binding of apoE to the LDL family of cell surface receptors (22), had any effect on the ability of APOE4/4 VLDL to inhibit HDL. RAP (10 μg/ml) prevented APOE4/4 VLDL from inhibiting HDL antiapoptotic activity, while it had no direct effect on the antiapoptotic activity of HDL itself (**Fig. 5**), suggesting that the effects of APOE4/4 VLDL require binding of apoE4 to LDL receptor family member(s).

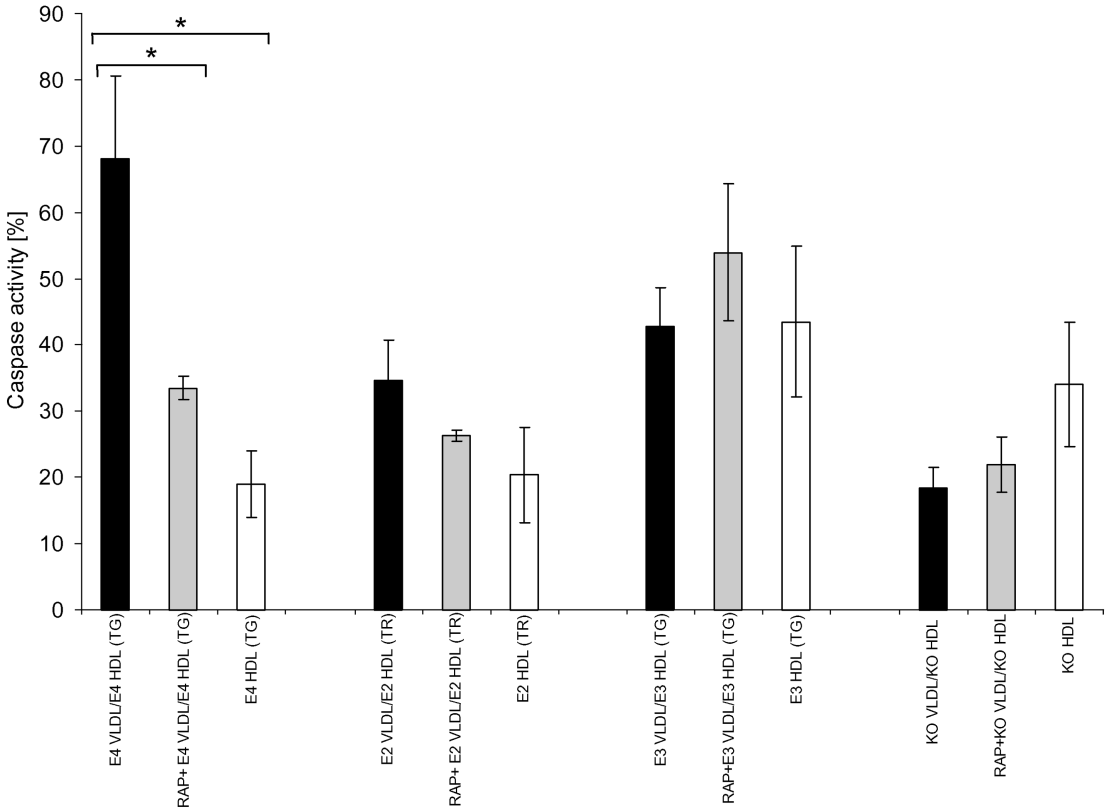

Fig. 5. The effects of receptor-associated protein (RAP) on APOE4/4 VLDL inhibition of HDL activity. RAP prevents inhibition of HDL activity by APOE4/4 VLDL (TG) but has no effect on VLDL from APOE3/3 (TG), APOE2/2 (TR), or APOE-KO. In addition, RAP does not appear to inhibit HDL activity from any of the APOE genotypes. Results are presented as mean ± SE. * $P < 0.05$.

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The inability of APOE3/3 VLDL to suppress HDL activity may be due to the concentration of apoE in VLDL

The absence of apoE in APOE-KO VLDL and the reduced ability of apoE2 to bind to the LDL-receptor family can account for their inability to suppress HDL activity. However, APOE3/3 VLDL also failed to significantly inhibit HDL antiapoptotic activity, although apoE3 binds the LDL receptor family with high affinity. Therefore, APOE4/4-specific VLDL inhibition of HDL activity may result from either a higher concentration of VLDL particles or a higher apoE4 concentration in the VLDL compared with APOE3/3 VLDL.

To test the first possibility, we expressed the data for APOE3/3 and APOE4/4 VLDL (Fig. 4) based on equal cholesterol concentrations (**Fig. 6A**) because cholesterol concentration correlates with the number of VLDL particles. Only APOE4/4 VLDL inhibited HDL activity, compared with the equivalent number of APOE3/3 VLDL particles, which did not inhibit HDL.

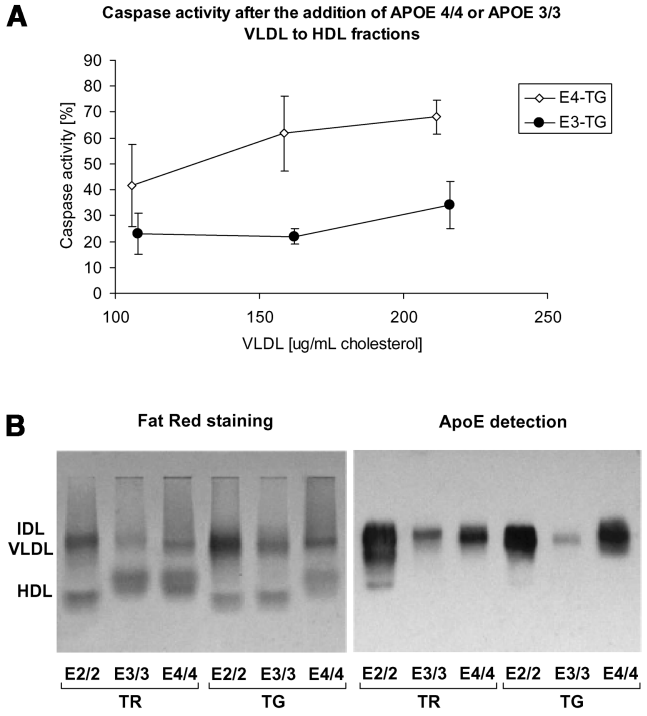

Fig. 6. Comparison of APOE4/4 and APOE3/3 lipoproteins. A: Comparison of APOE4/4 and APOE3/3 VLDL inhibition of HDL antiapoptotic activity based on cholesterol concentration of VLDL preparations. Only the APOE4/4 VLDL lipoproteins show inhibition of HDL activity, although both APOE4/4 and APOE3/3 VLDL preparations have similar cholesterol content, and thus a similar number of particles. The APOE3/3 and APOE4/4 curves are comparable to those seen when VLDL is expressed as percentage found in serum (Fig. 4). APOE2/2 and APOE-KO VLDL added at higher cholesterol concentrations, from 300 μg/ml to 1,600 μg/ml, had no effect on HDL activity (data not shown). B, Left: Fat Red staining of serum lipoproteins from TR and TG mice demonstrate a high content of VLDL in APOE2/2 mice, and similar content in APOE3/3 and APOE4/4 mice. B, Right: Western blot of an identical gel showed that APOE2/2 and APOE4/4 VLDLs contain substantially more apoE than APOE3/3 VLDL (in both TR and TG mice).

To determine whether differences in the amount of apoE associated with VLDL particles could explain our results, we estimated apoE content through Western analysis of lipoproteins from equivalent amounts of serum. Both APOE2/2 and APOE4/4 VLDLs from TR and TG mice contained significant amounts of apoE (Fig. 6B). In contrast, APOE3/3 VLDL from TR and TG mice contained reduced amounts of apoE, with a pronounced reduction in the TG APOE3/3 mice. Therefore, the inability of APOE3/3 VLDL to inhibit HDL activity may be due to its lower concentration of apoE3.

DISCUSSION

In agreement with previous work by Nofer et al. (7), our observations indicate that HDL suppresses endothelial cell apoptosis. HDL fractions are the most potent lipoprotein fractions suppressing apoptosis in all APOE genotypes tested. Suppression of apoptosis by HDL from APOE-KO mice demonstrates that the antiapoptotic activity of HDL does not require apoE. In addition, our demonstration that SPC in mouse lipoproteins inhibits apoptosis supports the notion of Nofer et al. (7) that specific lysophospholipids in human lipoproteins mediate HDL antiapoptotic activity. We found that suppression of apoptosis by total lipoproteins depends on APOE genotype, with APOE4/4 lipoproteins inhibiting apoptosis significantly less than other APOE genotypes.

The reduced potency of APOE4/4 lipoproteins is attributed to the ability of APOE4/4 VLDL to suppress HDL activity by a mechanism requiring interaction of apoE4 itself with a RAP-inhibitable LDL family receptor. The inability of APOE2/2 VLDL to inhibit HDL also supports the conclusion that an apoE-LDL receptor family interaction is required, since apoE2 does not effectively bind this receptor family. ApoE3 binds the LDL-receptor family with high affinity, but APOE3/3 VLDL failed to significantly inhibit HDL antiapoptotic activity. The inability of APOE3/3 VLDL to inhibit HDL could not be explained simply by fewer APOE3/3 VLDL particles, since when APOE3/3 and APOE4/4 VLDL activities were compared by equivalent cholesterol concentrations (reflecting an equivalent number of VLDL particles), only APOE4/4 VLDL inhibited HDL activity. The concentration of apoE protein in APOE3/3 VLDL, however, is lower than that in APOE4/4 VLDL (in both the TR and TG mice) (13) (Fig. 6B). Therefore, the lower concentration of apoE3 in VLDL may account for the inability of APOE3/3 VLDL to inhibit HDL activity.

The HDL fraction itself is the most potent lipoprotein fraction suppressing apoptosis in all APOE genotypes, including that from APOE-KO sera. In addition, the inability of RAP to alter the antiapoptotic effects of HDL further supports the conclusion that HDL activity does not require apoE or HDL interaction with an LDL family receptor.

Together, these data suggest that binding of apoE4-bearing VLDL to an LDL family receptor may sterically in-

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hibit the ability of HDL to bind its cell surface receptors. Alternatively, binding of apoE4-VLDL to an LDL family receptor may suppress intracellular pathways activated by HDL. Elucidating the molecular mechanism by which APOE4/4 VLDL inhibits HDL antiapoptotic activity is the focus of current experiments.

Decreased antiapoptotic activity of APOE4/4 lipoproteins could be clinically important, because the APOE4 allele is associated with a number of disorders, including diseases of the vasculature and central nervous system. APOE4-bearing individuals are at increased risk of atherosclerosis compared with APOE3-bearing individuals (9, 10), and possession of the APOE4 allele is also associated with higher risk and earlier development of sporadic and late-onset familial Alzheimer's disease (AD) (23–25). The association of the APOE4 allele with these disorders may be related to the decreased ability of APOE4 lipoproteins to inhibit apoptotic cell death, since apoptosis occurs in both atherosclerosis and AD (3, 26). The distribution of lipoprotein classes and their composition differ markedly between sera and cerebrospinal fluid (CSF). Moreover, the effects of the APOE genotype on the distribution and metabolism of CSF lipoprotein classes are not completely characterized (27–30). Therefore, to extend our findings to apoptosis in neurons, lipoproteins from the CSF need to be examined.

In conclusion, this work demonstrates that inhibition of apoptosis by lipoproteins depends, in part, on APOE genotype, with lipoproteins from the APOE4/4 genotype inhibiting apoptosis less than those from other genotypes. In addition, the antiapoptotic activity of HDL is suppressed by VLDL bearing the apoE4 isoform. These observations may help explain how the APOE genotype modifies disease expression, and may be the basis for new therapeutic strategies.

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