ApoC-III content of apoB-containing lipoproteins is associated with binding to the vascular proteoglycan biglycan

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Abstract Retention of apolipoprotein (apo)B and apoE-containing lipoproteins by extracellular vascular proteoglycans is critical in atherogenesis. Moreover, high circulating apoC-III levels are associated with increased atherosclerosis risk. To test whether apoC-III content of apoB-containing lipoproteins affects their ability to bind to the vascular proteoglycan biglycan, we evaluated the impact of apoC-III on the interaction of [^{35}S]SO_4-biglycan derived from cultured arterial smooth muscle cells with lipoproteins obtained from individuals across a spectrum of lipid concentrations. The extent of biglycan binding correlated positively with apoC-III levels within VLDL ($r = 0.78$, $P < 0.01$), IDL ($r = 0.67$, $P < 0.01$), and LDL ($r = 0.52$, $P < 0.05$). Moreover, the biglycan binding of VLDL, IDL, and LDL was reduced after depletion of apoC-III-containing lipoprotein particles in plasma by anti-apoC-III immunoaffinity chromatography. Since apoC-III does not bind biglycan directly, enhanced biglycan binding may result from a conformational change associated with increased apo C-III content by which apoB and/or apoE become more accessible to proteoglycans. This may be an intrinsic property of lipoproteins, since exogenous apoC-III enrichment of LDL and VLDL did not increase binding. ApoC-III content may thus be a marker for lipoproteins characterized as having an increased ability to bind proteoglycans.—Olin-Lewis, K., R. M. Krauss, M. La Belle, P. J. Blanche, P. H. R. Barrett, T. N. Wight, and A. Chait. **ApoC-III content of ApoB-containing lipoproteins is associated with binding to the vascular proteoglycan biglycan.** ***J. Lipid Res.*** **2002.** 43: **1969–1977.**

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A critical step in the pathogenesis of atherosclerosis is the deposition and retention of lipoproteins by vascular extracellular matrix molecules, particularly proteoglycans (1–4). Arterial wall proteoglycans play a key role in the development of atherosclerosis by interaction of negatively charged groups on their glycosaminoglycan side chains with clusters of positively charged residues on apolipoproteins (apo)B and apoE (5–7). Kinetic analyses suggest that the trapping of lipoproteins by arterial wall proteoglycans is due to selective retention rather than increased delivery of the lipoproteins (4, 8). Thus, it is important to understand the factors that mediate the binding of lipoproteins to proteoglycans.

High concentrations of apoC-III in plasma and on apoB-containing lipoproteins are associated with increased cardiovascular disease risk (9–11). However, unlike apoB and apoE, apoC-III lacks clusters of positively charged amino acid residues (12) and thus does not contain any putative heparin or proteoglycan binding regions. Consistent with this we have shown that apoE-free HDL, which contain abundant apoC-III, do not bind to proteoglycans(13, 14), thus further indicating that apoC-III does not bind directly to proteoglycans. The cardiovascular risk associated with high levels of apoC-III is thought to relate, in part, to its association with elevated triglyceride concentrations that are due to apoC-III-mediated impairment of lipolysis (15, 16). However, several investigators have reported that the presence of apoC-III on lipoproteins may affect the accessibility of apoB and/or apoE for the low density lipoprotein receptor (LDLR) (16–19). In addition, VLDL from apo-CIII transgenic mice was found to bind less well to heparin Sepharose compared to VLDL from non-transgenic animals, which could result in reduced exposure of apoC-III-rich VLDL to lipoprotein lipase, and in turn impaired lipolysis (16,

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20). Thus, there may be other ways in which apoC-III affects lipoprotein metabolism and atherogenesis. Since lipoproteins interact with the LDLR, glycosaminoglycans, and proteoglycans via similar (i.e. ionic) mechanisms, we speculated that the presence of apoC-III on lipoproteins also might affect how lipoproteins interact with proteoglycans.

Therefore, we investigated whether the content of apoC-III on apoB-containing lipoproteins (VLDL, IDL, and LDL) affected their ability to bind to the extracellular vascular proteoglycan biglycan. We evaluated lipoprotein binding to biglycan because biglycan appears to play an especially significant role in the trapping and retention of lipoproteins, as it was found to co-localize with apoB and apoE in human atherosclerotic lesions (13).

MATERIALS AND METHODS

Lipoprotein isolation

Fasting plasma samples were obtained after an overnight fast from clinically healthy subjects (n = 28) with a wide range of lipid values (**Table 1**). Subjects were not on any medication known to alter lipid metabolism and none of the subjects were smokers. All subjects gave their informed consent to participate in this study, which was approved by the Committee for Protection of Human Subjects at the University of California, Berkeley. Blood was collected by venipuncture into vacutainers containing 1 mg of EDTA per ml and 10 μM Trolox, a water-soluble vitamin E analog. LDL (d = 1.019–1.063 g/ml) was isolated by sequential ultracentrifugation from all subjects. VLDL (d <1.006 g/ml) and IDL (d = 1.006–1.019 g/ml) were isolated from 24 of the subjects. Subfractions of LDL were prepared by discontinuous density gradient ultracentrifugation as described previously in four of the subjects (21, 22).

Lipoprotein particle size measurements

Particle size measurement of apoB-containing lipoproteins was performed on plasma and isolated lipoprotein fractions using non-denaturing 2–14% polyacrylamide gradient gel electrophoresis (GGE) and standardized conditions (23). Following electrophoresis, lipoproteins were lipid-stained with Oil Red-O and the protein calibration standards were stained with Coomassie R-250. Gels were analyzed using computer-automated densitometry, and calculations of peak particle sizes were based on the migration of reference standards of known particle size. Based on performance of control lipoprotein samples, coefficient of variation for measurements made in the IDL and LDL size range was within 2.5 Å. Lipid-stained area of seven LDL subclasses was measured within particle-size boundaries and calculated as percent area/subclass.

TABLE 1. Plasma triglyceride and cholesterol concentrations, and LDL particle sizes

		Cholesterol			Peak LDL Particle Diameter
	Triglycerides	Total	HDL	LDL	
	mg/dl				*Å*
Mean ± SEM	132 ± 12.9	209 ± 6.8	47 ± 2.8	135 ± 6.1	260 ± 1.9
(Range)	(48–267)	(144–283)	(31–92)	(86–210)	(244.4–276.8)

n = 28.

Compositional measurements

Total and unesterified cholesterol and triglyceride were analyzed using enzymatic-endpoint reagent kits (Ciba-Corning Diagnostics Corp., Oberlin, OH), according to the manufacturer's instructions. Phospholipids were measured using a standardized colorimetric method (24) and a phosphorous calibrator (Sigma Chem. Co., St. Louis, MO). LDL protein concentrations were determined by a modification of the method of Lowry, et al. (25) using BSA as the standard. ApoB, apoE, and apoC-III were measured using standardized sandwich-style ELISA with detection using biotinylated antibodies prepared from purified apolipoprotein-specific antisera (ICN Biomedicals, Costa Mesa, CA; Genzyme Diagnostics, Cambridge, MA), streptavidin-conjugated horseradish peroxidase, and o-phenylenediamine (Sigma Chem. Co. St. Louis, MO). Apolipoprotein calibrators were standardized using CDC #1883 serum reference material (Center for Disease Control, Atlanta, GA) and pooled reference sera (Northwest Lipid Research Clinic, Seattle, WA). Relative electrophoretic mobility of lipoproteins was measured in agarose gels as described previously (26). Charge characteristics of the lipoproteins, such as valence, surface potential, and surface charge density were calculated using electrophoretic migration and particle size values, as described previously (27, 28).

ApoC-III-immunoaffinity chromatography

Plasma, collected in tubes containing a preservative cocktail of protease and bacterial inhibitors [final concentrations: 0.15% (w/v) EDTA; 1.5 μM PPACK; 75 μg/l gentamycin sulfate; 75 μg/ml chloramphenicol; 0.15% (w/v) sodium azide; 25 KU/ml aprotinin], was separated from red cells, adjusted to 10 μM Trolox, and passed through a 0.45 μM filter. Lipoproteins without apoC-III were prepared by incubating 2 ml plasma overnight at 4°C, rocking, with affinity-purified apoC-III antibody (International Immunology Corp., Murrieta, CA) conjugated to Affigel-10 (BioRad Laboratories, Hercules, CA) in a sealed column. Plasma, as an antibody-unbound fraction and depleted of apoC-III, was removed from the column by washing the gel matrix with 5 column volumes of 20 mM Tris-saline, pH 8. The unbound fractions then were concentrated to the initial 2 ml plasma volume using Centriprep-30 concentrators, molecular weight cut-off 30 kDa (Centriprep-30, Amicon, Beverly, MA). Lipoprotein fractions (VLDL, IDL, and LDL) then were prepared from total and apoC-III-depleted plasma by sequential ultracentrifugation, as described previously (29). It is important to note that this procedure removed apoC-III-containing *particles*, and not just free apoC-III.

Exogenous apoC-III enrichment

To test if adding exogenous apoC-III to lipoproteins might result in increased biglycan binding, equal protein levels of apoC-III (Academy Bio-Medical, Houston, TX) were added to VLDL or LDL as described previously (30).

Proteoglycan isolation

[^{35}S]SO_4-biglycan was derived from cultured human arterial smooth muscle cells, as described previously (31). Briefly, the cell medium was concentrated on DEAE-Sephacel mini-columns equilibrated in 8 M urea, 0.25 M NaCl, and 0.5% (w/v) CHAPS. [^{35}S]SO_4-proteoglycans were eluted with 8 M urea, 3 M NaCl, and 0.5% (w/v/) CHAPS and applied to a Sepharose CL-2B column equilibrated in 8 M urea and 0.5% (w/v) CHAPS (32). Radiolabeled material eluting at K_{av} 0.44–0.60 was pooled for biglycan, and dialysed into the buffer used for the gel mobility-shift assay. Purity was confirmed by Western blot analysis using a monospecific antibody against the core protein of biglycan (LF-51) (33) (a gift from Larry Fisher, Bone Research

TABLE 2. Constituents of lipoproteins (expressed per mole apoB)

	Mean ± SEM	Range
VLDL (n = 24)		
ApoC-III (moles)	39.6 ± 2.8	(15.5–73.4)
ApoE (moles)	0.99 ± 0.25	(0.03–5.31)
Triglyceride (mmoles)	5.61 ± 0.29	(2.59–8.03)
Free cholesterol (mmoles)	1.71 ± 0.13	(0.69–3.60)
Cholesteryl ester (mmoles)	0.89 ± 0.07	(0.41–1.82)
Phospholipid (mmoles)	2.36 ± 0.03	(1.60–5.44)
IDL (n = 24)		
ApoC-III (moles)	6.54 ± 0.80	(1.23–17.12)
ApoE (moles)	0.48 ± 0.09	(0.05–1.64)
Triglyceride (mmoles)	1.18 ± 0.11	(0.56–3.21)
Free cholesterol (mmoles)	1.22 ± 0.06	(0.53–1.84)
Cholesteryl ester (mmoles)	1.07 ± 0.08	(0.51–1.57)
Phospholipid (mmoles)	1.23 ± 0.06	(0.75–1.87)
LDL (n = 28)		
ApoC-III (moles)	0.80 ± 0.08	(0.33–1.91)
ApoE (moles)	0.07 ± 0.004	(0.007–0.108)
Triglyceride (mmoles)	0.14 ± 0.002	(0.073–0.287)
Free cholesterol (mmoles)	0.71 ± 0.04	(0.47–1.21)
Cholesteryl ester (mmoles)	1.02 ± 0.04	(0.62–1.37)
Phospholipid (mmoles)	0.77 ± 0.05	(0.47–2.07)

Branch, NIDR, National Institutes of Health, Bethesda, MD) and enhanced chemiluminescence (Western-Light Chemiluminescent Detection System with CSPD substrate; Tropix, Bedford, MA).

Gel mobility-shift assay

The ability of lipoprotein preparations (whole lipoproteins or LDL subfractions) to bind purified biglycan was assessed using a modification of the gel mobility-shift assay described by Camejo et al (31, 34, 35). Advantages of this assay are that the interaction step is performed under physiologic conditions (e.g., pH, salt and ion concentrations, and temperature) and that affinity constants (K_a) for the binding can be determined. Prior to the assay, the [^{35}S]SO_4-biglycan and lipoprotein preparations were dialyzed extensively at 4°C against HEPES sample buffer (10 mM HEPES, 150 mM NaCl, 5 mM $CaCl_2$, 2 mM $MgCl_2$, pH 7.4) with 25 μM BHT, and stored at 4°C. Increasing concentrations of the lipoproteins were mixed with a fixed amount of [^{35}S]SO_4-labeled biglycan in a total volume of 20 μl of HEPES buffer A for 1 h at 37°C. Three microliters of 0.03% (w/v) xylene cyanol dye-glycerol (1:1, v/v) were added to the samples and 20 μl were applied to wells in 0.7% NuSieve (FMC BioProducts, Rockland, ME) agarose gels prepared on Gel-Bond film (FMC Bioproducts), and electrophoresed in HEPES running buffer (10 mM HEPES, 3 mM $CaCl_2$, 5 mM $MgCl_2$, pH 7.2) at 60 V for 3 h at 4°C. The gels were fixed in cetyl pyridium chloride [0.1% (w/v) in 70% ethanol] for 1 h, air-dried, and exposed to either Hyper-Film MP (Amersham) for autoradiography or a phosphor screen for phosphorimage analysis (Packard, Meriden, CT). [^{35}S]SO_4-biglycan bound to lipoprotein migrated partially into the gel and/or remained at the origin, whereas free biglycan migrated to the gel's front. Lipoprotein binding to [^{35}S]SO_4-biglycan was confirmed

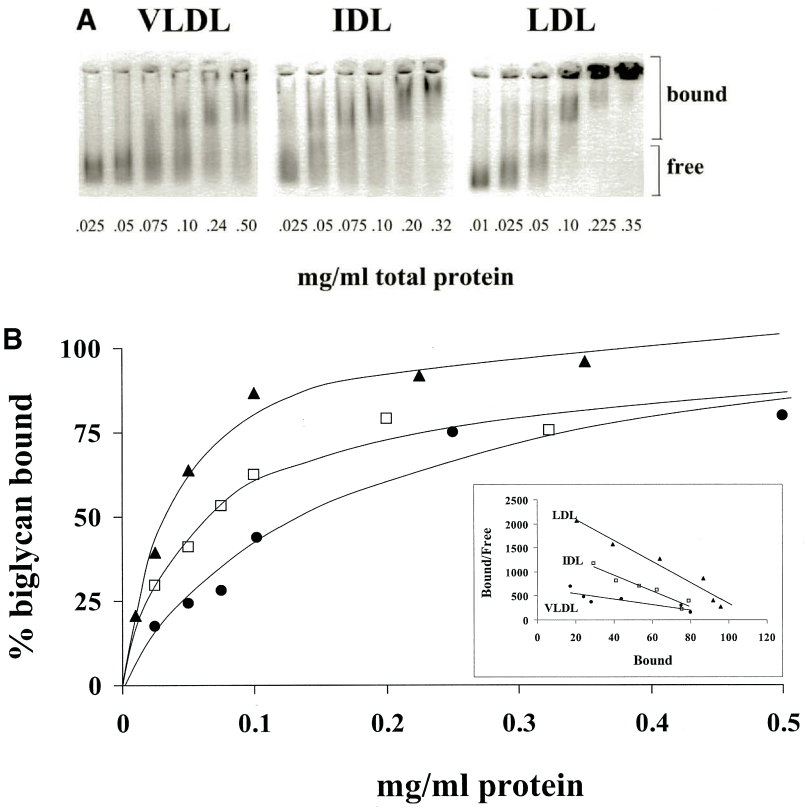

Fig. 1. LDL binds to biglycan with high affinity. A: Autoradiograph of a gel from the electrophoretic gel mobility shift assay is shown. Increasing concentrations of VLDL, IDL, and LDL from a subject were incubated with a fixed amount (0.19 μM) of [^{35}S]SO_4-labeled biglycan for 1 h at 37°C prior to electrophoresis in agarose, as described in Materials and Methods. Biglycan that binds lipoproteins remains at the origin, or migrates a short distance into the gel. Free, unbound [^{35}S]SO_4-biglycan migrates to the front of the gel. B: The binding curves for the interaction of VLDL (circle), IDL (square), and LDL (triangle) and [^{35}S]SO_4-labeled biglycan that were shown in A. The amount of bound versus free [^{35}S]SO_4-biglycan for each lane was calculated, and plotted against the concentration of total protein for that lipoprotein sample. The inset shows the same data as a Scatchard plot. Data are from a single experiment that is representative of lipoprotein samples from 24 subjects.

by staining the gel with Oil Red-O (final concentration 0.3%, w/v). The amount of complexed versus free biglycan in each lane was quantitated using a scanner (Hewlett-Packard ScanJet II cx) and the computer program ImageQuant (Molecular Dynamics) for autoradiograms; phosphorimages were quantitated using OptiQuant (Packard). K_a (amount of lipoprotein required to achieve 50% maximum binding) was calculated for each lipoprotein-proteoglycan interaction using SAAM II computer software (SAAM Institute; Seattle, WA). For some experiments, binding also was evaluated by determining the amount of biglycan that binds to a single concentration of lipoprotein.

Statistical analyses

Significant relationships for binding affinities and compositional parameters were analyzed by multiple linear and hyperbolic regression analyses. Significance of differences was assessed by Student's *t*-test and ANOVA. $P < 0.05$ were considered statistically significant.

TABLE 3. Affinity constants (K_a) and percent binding for the interaction of lipoproteins with $[^{35}S]SO_4$-biglycan

	Mean ± SEM
K_a ($M \times 10^{-7}$)[a]	
VLDL (n = 21)	2.08 ± 0.53
IDL (n = 22)	1.35 ± 0.45
LDL (n = 28)	1.03 ± 0.10
Percent $[^{35}S]SO_4$-biglycan bound to 0.05 mg/ml protein[a]	
VLDL (n = 21)	42.0 ± 5.5
IDL (n = 22)	50.3 ± 5.7
LDL (n = 28)	58.9 ± 3.6
LDL fractions (n = 4 for each LDL subfraction)[b]	
LDL 1 (1.024 g/ml)	49.5 ± 5.5
LDL 2 (1.031 g/ml)	53.0 ± 1.0
LDL 3 (1.036 g/ml)	57.5 ± 3.5
LDL 4 (1.041 g/ml)	63.5 ± 1.5
LDL 5 (1.049 g/ml)	67.0 ± 2.0
LDL 6 (1.060 g/ml)	72.5 ± 2.5
LDL 7 (1.066 g/ml)	71.0 ± 1.0

[a] Not significantly different.
[b] $P < 0.05$ for trend.

RESULTS

LDL was isolated from 28 individuals; VLDL and IDL were isolated from 24 of these individuals (**Table 2**). Three VLDL samples and two IDL samples did not contain a sufficient amount of protein to use in the gel mobility shift assay. From representative examples of gels using this assay, it can be seen that with increasing concentrations of lipoprotein and a constant amount of $[^{35}S]SO_4$-biglycan in each lane, there is an increasing amount of $[^{35}S]SO_4$-biglycan retained at the origin (representing "bound" biglycan) and a decreasing amount of $[^{35}S]SO_4$-biglycan at the gel's front, which is "free" biglycan (**Fig. 1A**). Binding curves generated for the interactions demonstrated that VLDL, IDL, and LDL all bound biglycan with various degrees of affinity (Fig. 1B). Linear Scatchard plots (Fig. 1B, inset) are consistent with the idea that there is one class of binding sites for the interaction of apolipoproteins and proteoglycans (5). The extent of biglycan binding was evaluated in two additional ways: first, the mean percent biglycan that bound to 0.05 and 0.1 mg total protein/ml lipoprotein was determined. Second, affinity constants (K_a) for the binding interaction were calculated. Using both methods of analysis, LDL demonstrated the greatest ability to bind biglycan, followed by IDL and VLDL (**Table 3**). In addition, we observed that within an individual subject, biglycan binding to the smaller, denser LDL subfractions was greater than to the large, buoyant subfractions (Table 3). Taken together, these data suggest that the density of lipoproteins was related to their affinity for biglycan. In addition, the density of the lipoproteins and LDL subfractions were inversely correlated with their valence, surface potential, and charge density ($r = -0.71$, $P < 0.001$ for each). It should be noted that the lower the charge value, the more positively charged the particle is. Thus, consistent with previous reports (28), a lipoprotein's density is related to its overall charge, such that VLDL was the least positively charged lipoprotein, followed by IDL and then LDL. Furthermore, the amount of biglycan that bound to the lipoproteins was inversely correlated with valence, surface potential, and charge density ($r = -0.69$, $P < 0.001$ for each). These data are consistent with the idea that the interaction between lipoproteins and proteoglycans is ionic in nature, such that lipoproteins with greater positive charge bind better to negatively charged glycosaminoglycan side chains on proteoglycans (6, 7, 36).

For each lipoprotein class, the amount of biglycan bound was found to be significantly related to apoC-III content (**Fig. 2**). A significant inverse relationship also was found between K_a and the amount of apoC-III per lipoprotein particle (**Fig. 3**). Thus, within each of the lipoprotein classes (VLDL, IDL, and LDL), we observed that the higher the apoC-III content, the greater the ability of the lipoprotein to bind the extracellular arterial proteoglycan biglycan in vitro, suggesting that apoC-III content played a

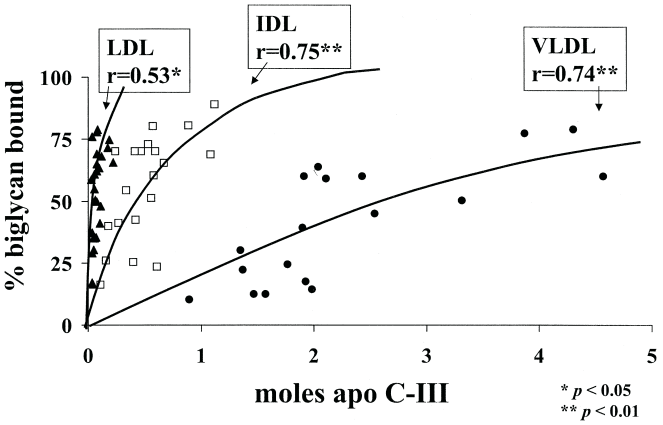

Fig. 2. Within each lipoprotein class, the amount of apoC-III correlates positively with the ability of the lipoprotein to bind biglycan. Hyperbolic regression curves are shown to demonstrate the correlation of a lipoprotein's apoC-III content with the amount of biglycan bound to samples of lipoprotein (VLDL, circle; IDL, square; LDL, triangle) that contained 0.05 mg/ml total protein. For the interaction, samples were incubated with a fixed amount (0.19 μM) of $[^{35}S]SO_4$-labeled biglycan for 1 h at 37°C prior to electrophoresis in agarose, as described in Materials and Methods.

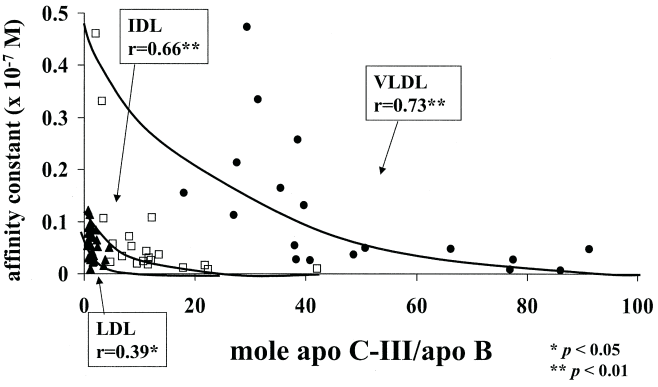

Fig. 3. Within each lipoprotein class, the amount of apoC-III correlates with the affinity constant (K_a) for the interaction of the lipoprotein with biglycan. Hyperbolic regression curves are shown to demonstrate the correlation of a lipoprotein's apoC-III content with the affinity constant (K_a) calculated from the interaction with biglycan (VLDL, circle; IDL, square; LDL, triangle). For the interaction, samples were incubated with a fixed amount (0.19 μM) of [^{35}S]SO_4-labeled biglycan for 1 h at 37°C prior to electrophoresis in agarose, as described in Materials and Methods.

role in lipoprotein binding. Furthermore, LDL particle size was inversely correlated with the apoC-III content ($r = -0.57$; $P = 0.002$). In other words, the smaller, denser LDL particles had a greater amount of apoC-III per particle.

The role of apoC-III in mediating the interaction of lipoproteins with biglycan was further supported in studies using VLDL, IDL, and LDL isolated from plasma that was substantially depleted (>99%) of apoC-III-containing particles by passage through an anti-human apoC-III column. For all lipoprotein fractions, biglycan binding was reduced significantly after the plasma from which the lipoprotein fractions were isolated was depleted of apoC-III-containing particles (**Fig. 4A–C**). When comparing the biglycan binding curves for lipoproteins before and after apoC-III depletion, VLDL showed the greatest difference (~20%) (Fig. 4A), followed by IDL (~15%) (Fig. 4B) and LDL (~10%) (Fig. 4C). This follows the trend for the amount of apoC-III in the lipoproteins, with VLDL containing the greatest content of apoC-III, followed by IDL and LDL (Table 2). As assessed by gradient gel electrophoresis, there were no significant differences in the peak particle diameters of lipoprotein fractions isolated from plasma before and after it had been passed over the anti-human apoC-III column (data not shown).

The sum of the moles of apoB and apoE in VLDL and LDL was found to correlate positively with the amount of biglycan bound to each of these lipoproteins ($r = 0.62$ and 0.54, respectively; $P < 0.05$). Thus, perhaps not surprisingly, the amount of apolipoproteins previously identified to contain proteoglycan binding regions (e.g., apoB and apoE) (36) is associated with an increased ability of a lipoprotein to bind biglycan. However, there were no correlations between the extent of lipoprotein binding to biglycan and lipoprotein content of apoE, apoC-III:E, or apoE:B molar ratios (data not shown).

To investigate if apoC-III-enhanced binding of lipoproteins to biglycan could be explained by the charge on lipoproteins, we evaluated the relationship between lipoprotein charge, lipoprotein binding, and apoC-III content. When all of the lipoprotein classes were evaluated together, apoC-III content was positively associated with lipoprotein charge density, surface potential and valence ($r = 0.84$, $P < 0.001$). This would be expected, since VLDL contains the greatest amount of apoC-III and is the most negatively charged of these lipoproteins. Within LDL there was a weaker, but also positive correlation between charge density and apoC-III content ($r = 0.54$, $P = 0.03$). There was no significant association between apoC-III content and charge density for VLDL and IDL (data not shown). In addition, lipoprotein fractions isolated from plasma that had been depleted of apoC-III-containing particles by immunoaffinity chromatography had similar charge characteristics compared to lipoprotein fraction isolated from apoC-III-containing plasma (data not shown). Taken together, these data further support our hypothesis that apoC-III is not acting directly to mediate binding, since those lipoproteins with the highest apoC-III content (i.e., VLDL) have the highest charge density and thus, the greatest the negative charge and least amount of biglycan binding. Therefore, the apoC-III-mediated enhanced binding of lipoproteins to biglycan was not related to charge, as assessed by surface potential, valence, and surface charge density.

DISCUSSION

ApoC-III is present on all lipoprotein classes, and plays a role in normal lipid metabolism (15, 37). However, high levels of apoC-III on apoB-containing lipoproteins and in plasma are independent risk factors for cardiovascular disease in individuals (9–11). In normotriglyceridemic subjects, apoC-III is associated primarily with HDL; however, there is redistribution of apoC-III to VLDL and a concomitant increase in total plasma levels of apoC-III in hypertriglyceridemia (15, 38). Since high apoC-III levels are associated with alterations in lipoprotein composition and the conformation of apoB and E on lipoproteins (39), we determined whether the content of apoC-III was important in mediating the interaction of VLDL, IDL, and LDL with extracellular arterial proteoglycans. We chose to use biglycan because it appears to play an especially significant role in the trapping and retention of lipoproteins (13). Indeed, intrinsic apoC-III content was found to be associated with biglycan binding for each of the lipoprotein classes studied, despite the fact that apoC-III does not bind to biglycan itself. Differing contents of apoC-III on the lipoprotein particles may account for the observation that the correlations of biglycan binding for VLDL and IDL with apoC-III content were strongest, followed by LDL, which contains relatively little apoC-III.

A role for apoC-III in enhancing the binding of lipoproteins to biglycan was further evaluated in experiments in which we measured biglycan binding to lipoproteins iso-

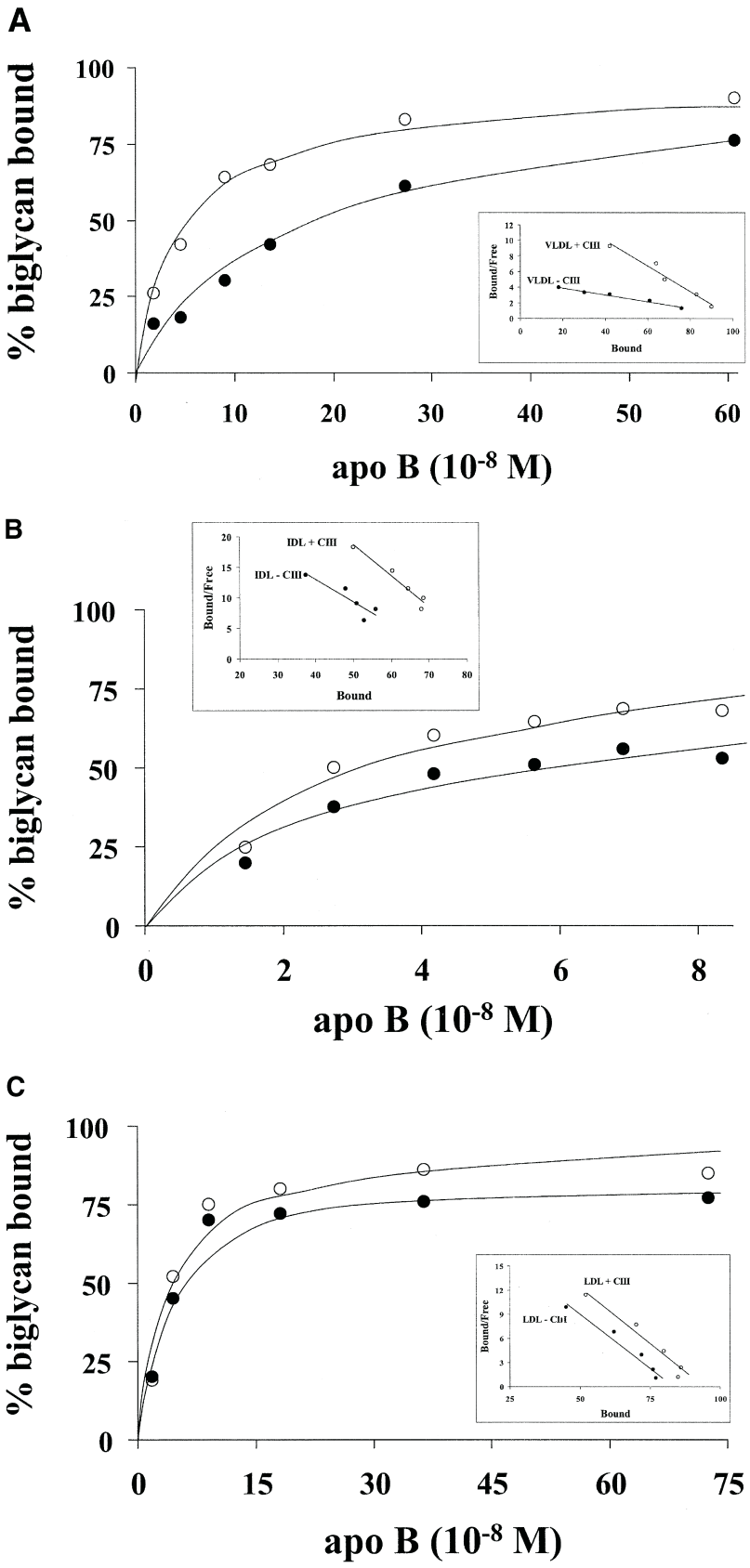

Fig. 4. Increased binding of biglycan to lipoproteins that contain apoC-III compared to apoC-III-depleted lipoproteins. Binding curves are shown for the interaction of biglycan and apoC-III-containing (A) VLDL, (B) IDL, and (C) LDL (open circle), or VLDL, IDL, and LDL isolated from the same plasma sample that had been passed over an anti-human apoC-III column (closed circle). Equal numbers of lipoprotein particles were incubated with a fixed amount (0.19 μM) of [^{35}S]SO_4-labeled biglycan for 1 h at 37°C prior to electrophoresis in agarose, as described in Materials and Methods. The insets show the same data as Scatchard plots. The data are from a single experiment that is representative of two independent experiments.

lated before and after depleting apoC-III-containing particles from plasma by immunoaffinity chromatography. ApoC-III-depleted VLDL bound significantly less (~20%) to biglycan than apoC-III-containing VLDL. ApoC-III-depleted IDL and LDL also showed an impaired ability to bind biglycan (~15% and 10% less, respectively), although the effect of removing apoC-III-containing particles from these lipoproteins was not as strong as found for VLDL. In contrast, when purified apoC-III was added to lipoproteins in vitro, this exogenous apoC-III-enrichment did not result in increased biglycan binding, but rather mildly inhibited it by ~5–15% (data not shown). Thus, the results from these experiments further support the idea that it is the *intrinsic* apoC-III content that is associated with the ability of lipoproteins to bind biglycan.

We also show that the total content of apoB plus apoE on VLDL and LDL affect the degree to which these lipoproteins bind biglycan. This is consistent with the presence of heparin/proteoglycan binding regions on both apoB and apoE (5, 36). However, we observed that the amount of apoC-III on a lipoprotein was an even greater predictor of its ability to bind biglycan. This is surprising, since apoC-III does not contain any known heparin/proteoglycan binding regions, nor does it have clusters of positively charged amino acid residues (e.g., lysine and arginine) that could perhaps act as binding regions for the highly negatively charged glycosaminoglycan side chains on proteoglycans (12).

Several lines of evidence suggest that apoC-III is not acting *directly* in enhancing the binding of lipoproteins to biglycan. VLDL contains 40–60 times as much apoC-III per particle as LDL, yet for a given amount of protein, biglycan binding is similar for VLDL and LDL. Further, we have demonstrated previously that apoE-free HDL does not bind to biglycan at all (13), despite high apoC-III content. It is possible that apoC-III, when present in its physiological state within lipoproteins, is acting indirectly by inducing a conformational change in lipoproteins, increasing the accessibility of apoB and/or E to proteoglycan binding. Alternatively, such a conformational change may not be caused by apoC-III, but may reflect an intrinsic property of the particle that affects both proteoglycan binding and apoC-III content. In contrast to our results for proteoglycan binding, apoC-III-enriched lipoproteins previously have been reported to have a decreased affinity for the LDLR (16–18). The nature of the interaction of apolipoproteins with the LDLR is similar to apolipoprotein-proteoglycan interactions, such that they are both primarily ionic. However, in studies using mice expressing wild-type or mutant human apoB, Borén et al. demonstrated that specific amino acid substitutions in the putative LDLR binding region on apoB resulted in reduced binding of LDL to vascular proteoglycans, but not to the LDLR (40), suggesting that there are differences in the binding of apolipoproteins to proteoglycans and the LDLR. The differences in the association of apoC-III content with LDLR and proteoglycan binding may be another example of how lipoprotein interactions with the LDLR and proteoglycans are similar, but not identical. These data support the notion that apoC-III affects the ability of lipoproteins to interact with the LDLR and extracellular proteoglycans, both of which participate in the metabolism of apoB-containing lipoproteins (41, 42).

Previous work has suggested that in human LDL, compositional parameters such as an elevated cholesterol-protein ratio, a low triglyceride-protein ratio, and a high isoelectric point increase its affinity for chondroitin sulfate proteoglycans (43, 44). In the present investigation, while LDL charge was a major determinant of biglycan binding, we found no relationships with LDL lipid content (data not shown). Although overall lipoprotein charge was related to biglycan binding in our study, we found that the apoC-III-mediated enhanced binding of lipoproteins to biglycan was not related to charge. It may be that the content of apoC-III on lipoproteins may affect the accessibility of *specific* lysine and arginine residues on apoB and apoE, as there have been numerous reports that the size, density, and composition of lipoproteins do in fact affect the microenvironments surrounding specific lysine residues, which affect apolipoprotein accessibility and LDLR binding (45, 46). It appears, however, from these data that at least *overall* charge is not making a major contribution to the enhanced ability of apoC-III-containing lipoproteins to bind biglycan.

Size and density of lipoproteins also can affect the accessibility and conformation of apoB and apoE on lipoproteins (7, 46, 47). In humans, LDL displays a large degree of heterogeneity with regard to size, density, and composition. Individuals with a predominance of small, dense LDL have a 3–7-fold increase in myocardial infarction risk (48, 49). Along with being more susceptible to oxidative modification than large LDL (50, 51), small, dense LDL particles have been hypothesized to have greater permeability and have been suggested to have enhanced affinity for arterial wall proteoglycans (7, 52). When we fractionated LDL, we also observed an effect of particle size on biglycan binding, such that the smaller, denser subfractions bound biglycan better than large, buoyant subfractions. In addition, these smaller, denser LDL subfractions have a higher content of apoC-III and a greater positive charge as assessed by lower valence, surface potential, and charge density values compared to large, buoyant subfractions.

Individuals with high apoC-III levels have a combination of high circulating triglycerides as well as having a greater number of small, dense LDL and apoC-III-rich lipoproteins, both of which we show here are associated with an enhanced affinity for arterial proteoglycans. Therefore, this may put these individuals at a great risk of developing atherosclerosis. The combination of decreased clearance from the circulation via the LDLR along with increased binding to extracellular vascular proteoglycans, such as biglycan, suggests that these lipoproteins would be particularly atherogenic. This may in part explain the increased risk of cardiovascular disease that is observed in subjects with high plasma levels of apoC-III.

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