SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Chemical analysis

Blood and bile samples

All blood samples were collected in fasting state. C-reactive protein (CRP), creatine kinase (CK), ApoE and safety assessment analyses (e.g. AST and ALT) in addition to biliary total bile acids were performed by certified routine assays at the Karolinska University Laboratory, Stockholm, Sweden.

For lipoprotein profiling the total and free cholesterol, and TG content in the different lipoprotein fractions corresponding to VLDL, LDL and HDL were determined after separation by size-exclusion chromatography[1]. Detailed analysis of lipoprotein subclasses as well as ApoA1 and ApoB were performed with Nuclear Magnetic Resonance spectroscopy (NMR) at Nightingale Health Ltd (formerly known as Brainshake) Laboratory, Vantaa, Finland[2].

ApoCIII was analyzed using an enzyme-linked immunosorbent assay (Abcam, Cambridge, USA) following the manufacturer's instructions. Plasma and biliary lathosterol and campesterol were determined by isotope dilution-mass spectrometry after addition of deuterium labeled internal standards[3, 4]. The campesterol/cholesterol and the lathosterol/cholesterol ratios were calculated as biomarkers for intestinal cholesterol absorption and total body cholesterol synthesis, respectively[5-8]. The intermediate in bile acid

synthesis 7α-hydroxy-4-cholesten-3-one (C4) was analyzed in plasma samples by LC-MS/MS as described[9].

Biliary lipids were extracted using the Bligh-Dyer method[10]. Cholesterol and phospholipid concentrations were determined by enzymatic methods with commercially available kits (Roche Diagnostics, Mannheim, Germany). Cholesterol saturation index (CSI) was calculated according to the critical tables described by Carey[11]. The individual bile acids were analyzed using GC-MS as previously described[12]. Briefly, bile was diluted with water, ethanol, KOH and hydrolyzed overnight after addition of deuterium labeled bile acids as internal standards. After acidification with HCl to pH 1 the deconjugated bile acids were extracted with diethyl ether and trimethylsilylated prior injection into GC-MS (Agilent Technologies).

PG binding

In this study we used a solid phase binding procedure that has been found reproducible for evaluation of affinity of isolated, or in plasma, apoB-lipoproteins for human arterial proteoglycans or purified proteoglycans preparations[13, 14]. Human aortic PG were isolated from the intima-media of human aortas essentially by the method of Hurt-Camejo *et al*[15 16]. Glycosaminoglycans were quantified by the method of Bartold and Page[17], and the amounts of PG are expressed in terms of their glycosaminoglycan content. The wells of polystyrene 96-well plates (Thermo Fisher Scientific) were coated with 100 µL of the PGs (25 µg/mL in PBS) by incubation at 4°C overnight. Wells were blocked with 1% BSA in PBS for 1 h at 37°C. Wells without PG served as controls. One microliter of plasma was added to the wells in a buffer containing 140 mM NaCl, 2 mM $MgCl_2$, 5 mM $CaCl_2$ and 10 mM MES, pH 5.5 and incubated for 1 h at 37°C. The wells were washed with 10 mM MES-50 mM NaCl, pH 5.5, and the amount of bound cholesterol was determined using the Amplex Red cholesterol kit (Molecular Probes).

RNA extraction, cDNA synthesis and real-time RT-PCR

Total RNA was extracted and transcribed into cDNA using Omniscript (Qiagen, Sollentuna, Sweden). The mRNA expression levels were then quantified and using specific primers (Table 3), arbitrary units were calculated by linearization of the CT values and normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*).

Table S1. Lipid contribution in lipoprotein fractions used in Figure 2

Data show mean ± SEM. Plasma lipoproteins were subjected to analysis by nuclear magnetic resonance.

LDL: low density lipoprotein, HDL: high density lipoprotein, Remnant: non-HDL and non-LDL, P: placebo; S: simvastatin, E: ezetimibe, S+E: simvastatin plus ezetimibe.

	Cholesterol				**Cholesteryl ester**				**Triglycerides**			
Pre-	**P**	**S**	**E**	**S+E**	**P**	**S**	**E**	**S+E**	**P**	**S**	**E**	**S+E**
Remnant (mmol/L)	1.4±0.2	1.5±0.2	1.2±0.1	1.4±0.1	0.9±0.1	1.0±0.1	0.8±0.1	0.9±0.1	0.9±0.1	1.2±0.1	0.9±0.1	0.9±0.1
LDL (mmol/L)	1.7±0.2	1.8±0.1	1.5±0.1	1.6±0.1	1.2±0.1	1.3±0.1	1.1±0.1	1.2±0.1	0.17±0	0.24±0	0.17±0	0.18±0.0
HDL (mmol/L)	1.6±0.1	1.4±0.1	1.4±0.1	1.3±0.1	1.2±0.1	1.1±0.0	1.1±0.1	1.0±0.0	0.14±0	0.16±0	0.13±0	0.14±0
Post-												
Remnant (mmol/L)	1.4±0.1	0.8±0.1	1.1±0.1	0.5±0.1	0.9±0.1	0.5±0.1	0.7±0.1	0.3±0.0	1.0±0.1	0.7±0.1	0.8±0.1	0.5±0.1
LDL (mmol/L)	1.7±0.2	0.9±0.1	1.4±0.1	0.6±0.1	1.3±0.1	0.6±0.1	1.0±0.1	0.3±0.1	0.18±0	0.14±0	0.15±0	0.10±0.0
HDL (mmol/L)	1.6±0.1	1.4±0.1	1.4±0.1	1.4±0.1	1.3±0.1	1.1±0.1	1.1±0.1	1.1±0.0	0.15±0.0	0.12±0.0	0.12±0.0	0.10±0.0

Table S2. Contribution of different subclasses for their respective fraction baseline.

	Percentage contribution of remnant subclasses (Non-LDL; Non-HDL) to overall remnant fraction						
	XXL-VLDL	**XL-VLDL**	**L-VLDL**	**M-VLDL**	**S-VLDL**	**XS-VLDL**	**IDL**
Particle number	0.1%	0.3%	2.0%	8.0%	14%	20%	55%
Triglyceride	2.0%	3.0%	14%	30%	25%	13%	14%
Cholesteryl ester	0.3%	0.6%	3.0%	9.0%	14%	18%	55%

	Percentage contribution of LDL subclasses to overall LDL fraction		
	L-LDL	**M-LDL**	**S-LDL**
Particle number	36%	29%	34%
Triglyceride	56%	27%	17%
Cholesteryl ester	52%	30%	18%

	Percentage contribution of HDL subclasses to overall HDL fraction			
	XL-HDL	**L-HDL**	**M-HDL**	**S-HDL**
Particle number	5%	13%	30%	58%
Triglyceride	10%	22%	32%	36%
Cholesteryl ester	12%	24%	32%	32%

Baseline contribution of lipoprotein subclasses for their respective fraction.

XXL-VLDL, extra extra large very low density lipoproteins; XL-VLDL, extra large-VLDL; L-VLDL, large-VLDL; M-VLDL, medium-VLDL; S-VLDL, small-VLDL; XS-VLDL, extra small-VLDL; IDL, intermediate density lipoprotein; L-LDL, large low density lipoprotein; M-LDL, medium-LDL; S-LDL, small-LDL; XL-HDL, extra-large high density lipoprotein; L-HDL, large-HDL; M-HDL, medium-HDL; S-HDL, small-HDL.

Table S3. Correlation of percentage changes of proteoglycan (PG) binding and lipoprotein particle number and composition.

	Percentage change correlation of PG binding with remnant (Non-LDL; Non-HDL) lipoprotein subclasses						
	XXL-VLDL	**XL-VLDL**	**L-VLDL**	**M-VLDL**	**S-VLDL**	**XS-VLDL**	**IDL**
Particle number (r^2; *p* value)	0.52; <0.001	0.58; <0.001	0.42; <0.001	0.45; <0.001	0.51; <0.001	0.55; <0.001	0.60; <0.001
Triglyceride (r^2; *p* value)	0.51; <0.001	0.55; <0.001	0.38; <0.001	0.39; <0.001	0.42; <0.001	0.43; <0.001	0.34; <0.001
Cholesteryl ester (r^2; *p* value)	0.62; <0.001	0.61; <0.001	0.23; <0.01	0.59; <0.001	0.65; <0.001	0.59; <0.001	0.54; <0.001

	Percentage change correlation of PG binding with LDL lipoprotein subclasses		
	L-LDL	**M-LDL**	**S-LDL**
Particle number (r^2; *p* value)	0.49; <0.001	0.49; <0.001	0.50; <0.001
Triglyceride (r^2; *p* value)	0.32; <0.001	0.35; <0.001	0.48; <0.001
Cholesteryl ester (r^2; *p* value)	0.52; <0.001	0.49; <0.001	0.46; <0.001

	Percentage change correlation of PG binding with HDL lipoprotein subclasses			
	XL-HDL	**L-HDL**	**M-HDL**	**S-HDL**
Particle number (r^2; *p* value)	0.06; NS	0.06; NS[#]	0.01; NS	0.17; <0.05[#]
Triglyceride (r^2; *p* value)	0.33; <0.001	0.13; <0.05	0.40; <0.05	0.15; <0.05
Cholesteryl ester (r^2; *p* value)	0.06; NS	0.11; <0.05[#]	0.04; NS[#]	0.16; <0.05

Pearson correlation between percentage changes of PG binding and lipoprotein subclasses particle number and composition. [#] Inverse correlation

XXL-VLDL, extra extra large very low density lipoproteins; XL-VLDL, extra large-VLDL; L-VLDL, large-VLDL; M-VLDL, medium-VLDL; S-VLDL, small-VLDL; XS-VLDL, extra small-VLDL; IDL, intermediate density lipoprotein; L-LDL, large low density lipoprotein; M-LDL, medium-LDL; S-LDL, small-LDL; XL-HDL, extra-large high density lipoprotein; L-HDL, large-HDL; M-HDL, medium-HDL; S-HDL, small-HDL

Table S4. Safety assessment analyses.

	Placebo	**Simvaststin**	**Ezetimibe**	**Simvastatin + Ezetimibe**	***P* value**
Alanine aminotransferase (ALT); % variation from baseline	1.68±7.26	-2.81±11.90	-6.43±11.2	21.74±16.0	NS
Gamma-glutamyltransferas (GT); % variation from baseline	5.46±13.7	-21.0±11.2	0.84±8.38	8.88±19.7	NS
Creatine Phosphokinase (CK), % variation from baseline	17.5± 14.7	22.5± 14.8	27.6± 17.5	-0.7± 12.6	NS

Data show mean ± SEM. Multi-Way ANOVA followed by LSD-test. NS: not significant.

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