# Biomedical consequences of elevated cholesterol-containing lipoproteins and apolipoproteins on cardiovascular and non-cardiovascular outcomes

Blood lipoproteins and apolipoprotein

A F Schmidt et. al.

# Contents

Bi	ibliography	14
3	Supplementary results	12
2	Tables	9
1	Figures	3

### Figures



**Supplementary Figure 1:** Spearman's pairwise correlation between the phenotypic NMR measured blood lipids (*left*: based on a n=14,834 UCLEB[1] sample) and between the genetic association with these blood lipids (*right*: based on a n=33,029 meta-analysis of UCLEB and Kettunen[2]); the margin order was based on hierarchical clustering of the Euclidean distance.



**Supplementary Figure 2:** Mendelian randomization estimates of the total effects of a one SD increase in cholesterol-containing lipoprotein and apolipoprotein concentration. With independent replication data from the GLGC GWAS [3], and technical replication using *cis* MR analysis of Apo-A1 and Apo-B concentrations.

	-0.25	0.3		0.22	0.54	0.36	0.62	-0.21	—	CHD (logOR)
		0.09		0.08		0.12		-0.1	—	HF (logOR)
									—	AF (logOR)
									—	Any stroke (logOR)
									—	Any ischemic stroke (logOR)
					-1.03				—	Large artery stroke (logOR)
									—	Cardioembolic stroke (logOR)
				0.15					—	Small vessel stroke (logOR)
		-0.01	-0.04				-0.01	-0.01	—	cIMT (mm)
	-0.14							-0.27	—	Carotid plaque (logOR)
		0.03			0.09				—	Glucose (mmol/l)
			-0.54	-0.1	0.71		-0.28	-0.11	—	HbA1c (mmol/mol)
	-0.05	0.12		0.05	0.2	0.11			—	T2DM (logOR)
	0.08	0.17	1.2	0.16	0.67	0.12	0.59	0.14	—	CRP (mg/L)
	-0.4	0.47		0.45		0.81		-0.45	—	SBP (mmHg)
	-0.15	0.27		0.2				-0.17	—	DBP (mmHg)
		0.48							—	PBL (logOR)
					0.05				—	BUN (mg/dl)
							0.01		—	eGFR (SD of log(eGFR))
			-0.27						—	CKD (logOR)
			-0.96		-1.61		-0.44	-0.11	—	IBD (logOR)
			-0.95		-1.21		-0.64	-0.14	—	UC (logOR)
		0.19			-1.39				—	CD (logOR)
					•				—	Arthritis (logOR)
							0.25		—	ALS (logOR)
					•	-0.42			—	MS (logOR)
	-0.02		-0.16		-0.29			0.03	—	Alzheimer (logOR)
					0.82				-	Parkinson (logOR)
		•			-2.14	-0.86		0.32	—	Pancreatic cancer (logOR)
			0.71		•		0.39		-	Colon cancer (logOR)
			-1.14	0.18	•				—	Rectal cancer (logOR)
	•			•	•	•			-	Melanoma (logOR)
			0.68					0.16	—	Lung cancer (logOR)
Groups	HDL-C (NMR)	TG (NMR)	TC (NMR)	VLDL-C (NMR)	IDL-C (NMR)	Rem-chol (NMR)	Apo-B (NMR)	Apo-A1 (NMR)	dir x – log (n)	- 5 - 0 5

**Supplementary Figure 3:** Multivariable Mendelian randomization estimates of the direct pathway effect of one SD change in blood lipid, conditional on LDL-C.

	0.37	0.33	0.48	0.19	0.45	0.37	0.54	0.7	—	CHD (logOR)
		0.08	0.06	0.1	0.04		0.06	0.16	-	HF (logOR)
								0.22	-	AF (logOR)
			0.05		0.05		0.05		-	Any stroke (logOR)
			0.05		0.04		0.06		-	Any ischemic stroke (logOR)
	0.12								-	Large artery stroke (logOR)
									-	Cardioembolic stroke (logOR)
									-	Small vessel stroke (logOR)
	0.02		0.02	-0.01	0.02		0.01		-	cIMT (mm)
	0.24	0.17	0.29	0.13	0.34	0.26	0.3	•	-	Carotid plaque (logOR)
						•			-	Glucose (mmol/l)
	-0.09	-0.25	-0.11	-0.18	-0.12		-0.13		-	HbA1c (mmol/mol)
	-0.06	•		0.04	-0.07		-0.06	0.15	-	T2DM (logOR)
	-0.21	0.14	-0.11		-0.06		0.06		-	CRP (mg/L)
	0.38	0.42	0.71	0.53	0.49	0.52	0.39	2.09	-	SBP (mmHg)
				0.12				0.76	-	DBP (mmHg)
		0.61		•					-	PBL (logOR)
		-0.01							-	BUN (mg/dl)
								-0.01	-	eGFR (SD of log(eGFR))
					-0.08			•	-	CKD (logOR)
	0.14		0.2		0.12				-	IBD (logOR)
	0.22						•	•	-	UC (logOR)
		0.16	0.13		0.13				-	CD (logOR)
		•	-0.11			•	-0.13	•	-	Arthritis (logOR)
	0.09	0.12	0.11	•		•			-	ALS (logOR)
		•		•	•			•	-	MS (logOR)
	0.05	•	0.02	•	0.02		0.04	•	-	Alzheimer (logOR)
		•			-0.07		•	0.48	-	Parkinson (logOR)
	•	•			•	-0.35		-1.34	-	Pancreatic cancer (logOR)
	•	•	0.12	•	0.12	•	0.14	•	-	Colon cancer (logOR)
	0.16	•	•	0.39	0.17	•	•	•	-	Rectal cancer (logOR)
	-0.07	•		•	•	•	•	•	-	Melanoma (logOR)
	-0.25	•	-0.21	•	-0.17	•	-0.14	•	-	Lung cancer (logOR)
Groups	LDL-C (NMR)	TG (NMR)	TC (NMR)	VLDL-C (NMR)	IDL-C (NMR)	Rem-chol (NMR)	Apo-B (NMR)	Apo-A1 (NMR)	dir x – log <sub>a</sub> (n)	

**Supplementary Figure 4:** Multivariable Mendelian randomization estimates of the direct pathway effect of one SD change in blood lipid, conditional on HDL-C.

	0.24	-0.17	0.28		0.24	0.37	0.44	-0.24	-	CHD (logOR)
									-	HF (logOR)
									-	AF (logOR)
						•	•		-	Any stroke (logOR)
									-	Any ischemic stroke (logOR)
						0.3			-	Large artery stroke (logOR)
									-	Cardioembolic stroke (logOR)
	-0.15				-0.19		-0.31		-	Small vessel stroke (logOR)
	0.01		0.02		0.02	0.02	0.03		-	cIMT (mm)
	0.2		0.37		0.28	0.44	0.33		-	Carotid plaque (logOR)
		-0.05	-0.01	0.11		-0.03			-	Glucose (mmol/l)
		-0.33				-0.19			-	HbA1c (mmol/mol)
	-0.09	-0.04	-0.08	0.27	-0.07	-0.14	-0.12	-0.09	-	T2DM (logOR)
	-0.15	0.13	-0.14		-0.1	-0.19	-0.19	0.06	-	CRP (mg/L)
	0.22	-0.25			0.24	0.62			-	SBP (mmHg)
	-0.11	-0.24	-0.19		-0.15		-0.22		-	DBP (mmHg)
		0.29	0.5						-	PBL (logOR)
		-0.01				-0.01			-	BUN (mg/dl)
									-	eGFR (SD of log(eGFR))
									-	CKD (logOR)
	0.15		0.2		0.18		0.14		-	IBD (logOR)
	0.14								-	UC (logOR)
					0.11				-	CD (logOR)
							•		-	Arthritis (logOR)
								0.09	-	ALS (logOR)
	•	•							-	MS (logOR)
	0.05	-0.02	0.02		0.03		0.04		-	Alzheimer (logOR)
						0.21			-	Parkinson (logOR)
	0.29		0.39		0.27				-	Pancreatic cancer (logOR)
		•		0.57					-	Colon cancer (logOR)
	0.18						0.28		-	Rectal cancer (logOR)
	-0.1		-0.1			-0.25			-	Melanoma (logOR)
	-0.26		-0.2		-0.18	-0.4			-	Lung cancer (logOR)
Groups	LDL-C (NMR)	HDL-C (NMR)	TC (NMR)	VLDL-C (NMR)	IDL-C (NMR)	Rem-chol (NMR)	Apo-B (NMR)	Apo-A1 (NMR)	dir x – log "(n)	

**Supplementary Figure 5:** Multivariable Mendelian randomization estimates of the direct pathway effect of one SD change in blood lipid, conditional on Triglycerides.

	0.35	-0.27	0.14		0.19			1.33		—	CHD (logOR)
			0.06	0.27	0.1		0.08	0.63		—	HF (logOR)
				0.23		0.15	0.07		0.26	—	AF (logOR)
			•							—	Any stroke (logOR)
										—	Any ischemic stroke (logOR)
	0.12				-0.31		-0.28			—	Large artery stroke (logOR)
								0.96		—	Cardioembolic stroke (logOR)
						•				—	Small vessel stroke (logOR)
	0.02		-0.01			0.02		0.04		—	cIMT (mm)
	0.34	-0.13		0.88	0.28		0.17	1.02		—	Carotid plaque (logOR)
		-0.03				0.07				—	Glucose (mmol/l)
			-0.15					•		—	HbA1c (mmol/mol)
	-0.1	•	0.07		0.11	-0.16	•			—	T2DM (logOR)
	-0.24	0.2	0.32	0.89	0.16	0.29	0.1	0.7	-0.74	—	CRP (mg/L)
	0.28	-0.24	0.3		0.39		0.39	•		—	SBP (mmHg)
		-0.16	•	-0.73	-0.18		•	•		—	DBP (mmHg)
		0.41	0.71		•			•		—	PBL (logOR)
		-0.01	•		•	0.02	•			—	BUN (mg/dl)
		•			•	-0.01		•	-0.01	—	eGFR (SD of log(eGFR))
		•	•							—	CKD (logOR)
	0.12	•		-1.06	•	-0.78		-1.04		—	IBD (logOR)
	0.16	•		-1.3	•	-0.96		-0.91		—	UC (logOR)
		•	0.21	-0.97	•	-0.63		-1.36		—	CD (logOR)
		•	•		•		•	•		—	Arthritis (logOR)
		•	0.13		-0.24		-0.18	•		—	ALS (logOR)
			•					1.99		-	MS (logOR)
	0.05	-0.03	-0.03		-0.04		•	-0.33		—	Alzheimer (logOR)
			•	1.14	0.2	0.59	0.21			-	Parkinson (logOR)
					-1.1		-0.62	•		—	Pancreatic cancer (logOR)
							•			-	Colon cancer (logOR)
	0.25			-1.4						—	Rectal cancer (logOR)
	-0.1									—	Melanoma (logOR)
	-0.26									-	Lung cancer (logOR)
Groups	LDL-C (NMR)	HDL-C (NMR)	TG (NMR)	TC (NMR)	VLDL-C (NMR)	IDL-C (NMR)	Rem-chol (NMR)	Apo-B (NMR)	Apo-A1 (NMR)	dir x — Ind. (n)	

**Supplementary Figure 6:** Multivariable Mendelian randomization estimates of the direct pathway effect of one SD change in blood lipid, (fully) conditional on LDL-C, HDL-C, and Triglycerides.

### 2 Tables

Supplementary Table 1: Mendelian randomization analysis synopsis.

#### Mendelian randomization steps

#### Filter variants on:

- F-statistic larger than 24,
- For *cis*-MR:
  - F-statistic larger than 15,
  - Within a 50 kbp upstream and downstream window around the gene,
- Minor allele frequency of at least 0.01,
- Pairwise r-squared less than 0.10.

#### (MV)MR modelling:

- Inverse variance weighted (IVW),
- MR-Egger,
- · Corrected for residual LD using a UKB reference panel,
- Removal of variants with large leverage or heterogeneity statistics,
- Rucker framework, selecting the MR-Egger results if  $Q_{\rm IVW} Q_{\rm Egger}$  is larger than 3.84.

#### Prioritization:

- For each exposure and outcome pair:
  - Determine the *total effect* using univariable MR,
    Determine the *direct effects* conditioning on I)
    LDL-C, II) HDL-C, III) TG, and IV) LDL-C+HDL-C+TG
    using multivariable MVMR,
    - \* Skip duplicate exposure models, e.g., when LDL-C is the exposure of interest do not run an MVMR model including LDL-C twice.
  - An association between an exposure and outcome pair is considered *prioritized* when it is significant and directionally concordant in at least 60% of the combined univariable and multivariable models.

Blood lipid	Standard deviation
LDL-C	0.646
HDL-C	0.416
TG	0.613
VLDL-C	0.282
IDL-C	0.232
Rem-chol	0.461
Chol	1.238
Аро-В	0.238
Apo-A1	0.276

**Supplementary Table 2:** The standard deviation of NMR measured blood lipids in mmol/L (total cholesterol, triglycerides, lipoproteins) or g/L (apolipoproteins)

Exposure	Median	Quantile: 0.15	Quantile: 0.85	Conditional model
Apo-A1	15.75	15.62	16.05	LDL-C
HDL-C	18.36	17.84	19.28	LDL-C
Аро-В	11.02	9.11	11.54	LDL-C
VLDL-C	7.88	7.80	8.02	LDL-C
IDL-C	6.41	5.98	6.93	LDL-C
Rem-chol	6.15	6.05	6.46	LDL-C
TG	13.34	12.88	13.79	LDL-C
ТС	14.17	12.57	14.82	LDL-C
Apo-A1	15.60	15.52	15.81	HDL-C
Аро-В	18.24	17.40	19.00	HDL-C
LDL-C	25.85	23.58	26.17	HDL-C
VLDL-C	7.49	7.45	7.60	HDL-C
IDL-C	24.86	24.20	25.51	HDL-C
Rem-chol	7.40	7.40	7.65	HDL-C
TG	12.27	11.90	13.42	HDL-C
ТС	29.57	28.92	29.95	HDL-C
Apo-A1	16.45	16.26	16.78	TG
HDL-C	14.54	14.37	14.86	TG
Аро-В	18.11	16.85	19.30	TG
LDL-C	19.85	16.94	20.80	TG
VLDL-C	3.13	3.07	3.13	TG
IDL-C	18.96	16.92	19.54	TG
Rem-chol	6.61	6.58	6.75	TG
ТС	18.12	16.67	18.64	TG
Apo-A1	3.16	3.01	3.24	LDL-C, HDL-C & TG
HDL-C	11.11	10.94	11.31	LDL-C & TG
Аро-В	2.65	2.61	2.75	LDL-C, HDL-C & TG
LDL-C	16.71	14.95	17.83	HDL-C & TG
VLDL-C	2.74	2.72	2.83	LDL-C, HDL-C & TG
IDL-C	5.49	5.42	5.74	LDL-C, HDL-C & TG
Rem-chol	3.15	3.13	3.29	LDL-C, HDL-C & TG
TG	8.61	8.46	9.37	LDL-C & HDL-C
тс	2.81	2.79	2.93	LDL-C, HDL-C & TG

**Supplementary Table 3:** Conditional F-statistics for the considered multivariable Mendelian randomiation models

### **3** Supplementary results

### NMR GWAS participants characteristics

Genetic instruments for cholesterol-containing lipoprotein and apolipoprotein concentrations were sourced from a meta-analysis of Kettunen et al, and the UCLEB consortium. The former included data from Finnish, Estonian, Dutch and German cohorts, with a median age of 46.9 years, median BMI of 26.35 kg/m<sup>2</sup> and 54% women enrolled, see [2]. UCLEB consisted of UK-based cohorts with a mean age of 58.6 years, mean BMI of 26.4 kg/m<sup>2</sup>, and 44% women included [1]. Please see the source publications (referenced in the main manuscript) for the subject characteristics of the GWAS outcome data.

# Validation of the LDL-C, HDL-C and TG MR effects on disease incidence and biomarkers

The univariable MR effects of LDL-C, HDL-C, and TG were validated using independent replication data from GLGC based on clinical chemistry measurements (Supplementary Figure 2), showing strong agreement both in terms of effect direction and significance.

### Univariable MR of Apo-B and Apo-A1 concentration

The MR analyses described for lipid fractions used instruments selected from across the genome (genome-wide MR). In these analyses, horizontal pleiotropy was addressed analytically (see methods). As described by Schmidt *et al.* 2020 [4] MR analyses of protein concentration, such as Apo-B and Apo-A1, may be protected further against horizontal pleiotropy by selecting instrument from a cis window around the protein encoding gene. Here we compared results from genome-wide MR to that of cis-MR and explored agreement (Supplementary Figure 2, and Supplementary Data 8-11).

Higher Apo-B concentration was positively associated with the risk of CHD, (ischemic) stroke, CD, Alzheimer's disease, and furthermore increased cIMT, carotid plaque and SBP. Conversely, higher genetically instrumented Apo-B concentration was associated with lower HbA1c concentration as well as with pancreatic cancer risk. Aside from a directionally discordant effect on HF and arthritis, results from both cis and genome-wide approached agreed in both effect direction and magnitude, providing empirical support for analytical correction of horizontal pleiotropy. For HF and arthritis, we made further comparisons against the LDL-C, IDL-C and VLDL-C (the carriers Apo-B) effects, providing support for the observed genome-wide Apo-B effect where higher concentrations increased HF risk and lowered arthritis risk (Supplementary Figure 2). Due to the modest number of cis variants for Apo-A1 (2 APOA1 variants compared to 8 for APOB; Tables S10-S13) agreement between both analytical methods was limited. Instead, we focussed on the genome-wide MR results, where we expect the larger number of variant leads to better control of horizontal pleiotropy. The genome-wide MR results suggest that higher ApoA-1 concentration decreases the risk of CHD, and T2DM, decreases carotid plaque size, DBP, while increasing CRP concentrations (Supplementary Figure 2).

### **Bibliography**

- [1] Tina Shah et al. "Population genomics of cardiometabolic traits: design of the University College London-London School of Hygiene and Tropical Medicine-Edinburgh-Bristol (UCLEB) Consortium." In: *PloS one* 8 (8 2013), e71345. ISSN: 1932-6203. DOI: 10.1371/journal.pone.0071345. epublish.
- [2] Johannes Kettunen et al. "Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA." In: *Nature communications* 7 (Mar. 2016), p. 11122. ISSN: 2041-1723. DOI: 10.1038/ncomms11122. epublish.
- [3] Cristen J Willer et al. "Discovery and refinement of loci associated with lipid levels." In: *Nature genetics* 45 (11 Nov. 2013), pp. 1274–1283. ISSN: 1546-1718. DOI: 10.1038/ng.2797.
- [4] Amand F. Schmidt et al. "Genetic drug target validation using Mendelian randomisation." In: *Nature communications* 11 (1 June 2020), p. 3255. ISSN: 2041-1723. DOI: 10.1038/s41467-020-16969-0. epublish.