Supplemental Information

Biophysical Characterization of Human Lipoproteins, their Subclasses and their Lipids by ^{1}H NMR Spectroscopy

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Tables

Supplemental Table S1. ^{1}H-NMR resonances of lipids isolated in $CDCl_3$/methanol-d4.[a]

Chemical shift ppm]	Compound	Chemical group	Position
0.6949	C	C**H**$_3$	C-18
0.6974	CE	C**H**$_3$	C-18
0.8635	C / CE	C**H**$_3$	C-26, C-27
0.8681	C / CE	C**H**$_3$	C-26, C-27
0.8747	C / CE / FA	C**H**$_3$	C-26, C-27 / FA-CH_3
0.8790	C / CE / FA	C**H**$_3$	C-26, C-27 / FA-CH_3
0.8871	FA	C**H**$_3$	FA-CH_3
0.8996	FA	C**H**$_3$	FA-CH_3
0.9110	FA	C**H**$_3$	FA-CH_3
0.9229	C / CE	C**H**$_3$	C-21
0.9337	C / CE	C**H**$_3$	C-21
0.9438	C / CE	C**H**, C**H**$_2$	C-9, C-14, C-22
0.9547	C / CE	C**H**, C**H**$_2$	C-9, C-14, C-22
0.9667	C / CE	C**H**, C**H**$_2$	C-9, C-14, C-22
0.9733	C / CE	C**H**, C**H**$_2$	C-9, C-14, C-22
0.9813	C / CE	C**H**, C**H**$_2$	C-9, C-14, C-22
0.9920	C / CE	C**H**, C**H**$_2$	C-9, C-14, C-22
1.0013	C / CE	C**H**, C**H**$_2$	C-9, C-14, C-22
1.0090	C / CE	C**H**, C**H**$_2$	C-9, C-14, C-22
1.0125	C / CE	C**H**, C**H**$_2$	C-9, C-14, C-22
1.0177	C	C**H**$_3$	C-19
1.0236	C / CE	CH, C**H**$_2$	C-9, C-14, C-22
1.0295	C / CE	C**H**, C**H**$_2$	C-9, C-14, C-22
1.0406	CE	C**H**$_3$	C-19
1.0523	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.0616	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.0711	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.0816	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.0920	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.1021	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.1104	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.1199	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24

1.1286	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.1349	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.1452	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.1535	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.1677	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.1770	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.1873	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.1996	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.2090	C /CE	C**H**, C**H**$_2$	C-1, C-12, C-15, C-17, C-23, C-24
1.2674	FA / C / CE	C**H**, C**H**$_2$	FA-CH_2 / C-16, C-20, C-22, C-23
1.2808	FA / C / CE	C**H**, C**H**$_2$	FA-CH_2 / C-16, C-20, C-22, C-23
1.2929	FA / C / CE	C**H**, C**H**$_2$	FA-CH_2 / C-16, C-20, C-22, C-23
1.3070	FA / C / CE	C**H**, C**H**$_2$	FA-CH_2 / C-16, C-20, C-22, C-23
1.3202	FA / C / CE	C**H**, C**H**$_2$	FA-CH_2 / C-16, C-20, C-22, C-23
1.3427	C / CE / FA	C**H**, C**H**$_2$	C-16, C-20, C-22, C-23 / FA-CH_2
1.3528	C / CE / FA	C**H**, C**H**$_2$	C-16, C-20, C-22, C-23 / FA-CH_2
1.3654	C / CE / FA	C**H**, C**H**$_2$	C-16, C-20, C-22, C-23 / FA-CH_2
1.3777	C / CE / FA	C**H**, C**H**$_2$	C-16, C-20, C-22, C-23 / FA-CH_2
1.4052	C / CE / FA	C**H**, C**H**$_2$	C-16, C-20, C-22, C-23 / FA-CH_2
1.4471	C / CE	C**H**, C**H**$_2$	C-7, C-8, C-11, C-15, C-25
1.4516	C / CE	C**H**, C**H**$_2$	C-7, C-8, C-11, C-15, C-25
1.4638	C / CE	C**H**, C**H**$_2$	C-7, C-8, C-11, C-15, C-25
1.4818	C / CE	C**H**, C**H**$_2$	C-7, C-8, C-11, C-15, C-25
1.4910	C / CE	C**H**, C**H**$_2$	C-7, C-8, C-11, C-15, C-25
1.5148	C / CE	C**H**, C**H**$_2$	C-7, C-8, C-11, C-15, C-25
1.5262	C / CE	C**H**, C**H**$_2$	C-7, C-8, C-11, C-15, C-25
1.5368	C / CE	C**H**, C**H**$_2$	C-7, C-8, C-11, C-15, C-25
1.5478	C / CE	C**H**, C**H**$_2$	C-7, C-8, C-11, C-15, C-25
1.5599	C / CE	C**H**, C**H**$_2$	C-7, C-8, C-11, C-15, C-25
1.5797	C / CE / FA	C**H**$_2$ / C**H**$_2$-CH_2-COO	C-7, C-8, C-11, C-15, C-25 / FA-CH_2
1.5849	C / CE / FA	C**H**$_2$ / C**H**$_2$-CH_2-COO	C-7, C-8, C-11, C-15, C-25 / FA-CH_2
1.6049	C / CE / FA	C**H**$_2$ / C**H**$_2$-CH_2-COO	C-7, C-8, C-11, C-15, C-25 / FA-CH_2
1.6233	FA	C**H**$_2$-CH_2-COO	FA-CH_2
1.6352	FA	C**H**$_2$-CH_2-COO	FA-CH_2
1.67-1.78	FA	CH=CH-CH_2-C**H**$_2$-CH_2-COO	FA-CH_2
1.8171	C / CE	C**H**$_2$	C-1, C-2, C-16
1.8278	C / CE	C**H**$_2$	C-1, C-2, C-16
1.8337	C / CE	C**H**$_2$	C-1, C-2, C-16
1.8444	C / CE	C**H**$_2$	C-1, C-2, C-16
1.8547	C / CE	C**H**$_2$	C-1, C-2, C-16
1.8672	C / CE	C**H**$_2$	C-1, C-2, C-16
1.8750	C / CE	C**H**$_2$	C-1, C-2, C-16
1.8804	CE	C**H**$_2$	C-2
1.8967	CE	C**H**$_2$	C-2
1.9028	CE	C**H**$_2$	C-2
1.9152	CE	C**H**$_2$	C-2
1.9644	C / CE	C**H**$_2$	C-7, C-12
1.9734	C / CE	C**H**$_2$	C-7, C-12

1.9807	C / CE	C**H**$_2$	C-7, C-12
2.0034	C / CE / FA	C**H**$_2$ / C**H**$_2$-CH=CH	C-7, C-12 / FA-CH$_2$
2.0192	C / CE / FA	C**H**$_2$ / C**H**$_2$-CH=CH	C-7, C-12 / FA-CH$_2$
2.0262	C / CE / FA	C**H**$_2$ / C**H**$_2$-CH=CH	C-7, C-12 / FA-CH$_2$
2.0407	C / CE / FA	C**H**$_2$ / C**H**$_2$-CH=CH	C-7, C-12 / FA-CH$_2$
2.0673	FA	CH=CH-C**H**$_2$-CH$_2$-CH$_2$-COO	FA-CH$_2$
2.0770	FA	CH=CH-C**H**$_2$-CH$_2$-CH$_2$-COO	FA-CH$_2$
2.0993	FA	CH=CH-C**H**$_2$-CH$_2$-CH$_2$-COO	FA-CH$_2$
2.1266	FA	CH=CH-C**H**$_2$-CH$_2$-CH$_2$-COO	FA-CH$_2$
2.1383	FA	CH=CH-C**H**$_2$-CH$_2$-CH$_2$-COO	FA-CH$_2$
2.1662	FA	CH=CH-C**H**$_2$-CH$_2$-CH$_2$-COO	FA-CH$_2$
2.1798	FA	CH=CH-C**H**$_2$-CH$_2$-CH$_2$-COO	FA-CH$_2$
2.1923	FA	CH=CH-C**H**$_2$-CH$_2$-CH$_2$-COO	FA-CH$_2$
2.2028	C	C**H**$_2$	C-4
2.2262	C	C**H**$_2$	C-4
2.2388	C	C**H**$_2$	C-4
2.2431	C	C**H**$_2$	C-4
2.2471	C	C**H**$_2$	C-4
2.2505	C	C**H**$_2$	C-4
2.2542	C	C**H**$_2$	C-4
2.2602	C / CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.2620	C / CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.2716	C / CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.2776	C / CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.2826	C / CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.2950	CE / FA	C**H**$_2$-COO	FA-CH$_2$
2.3072	CE / FA	C**H**$_2$-COO	FA-CH$_2$
2.3188	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3275	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3295	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3323	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3386	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3417	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3450	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3496	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3518	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3574	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3586	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.3620	CE / FA	C**H**$_2$ / C**H**$_2$-COO	C-4, FA-CH$_2$
2.4125	FA	CH=CH-C**H**$_2$-C**H**$_2$-COO	FA-CH$_2$
2.7688	FA	CH=CH-C**H**$_2$-CH=CH	FA-CH$_2$
2.7800	FA	CH=CH-C**H**$_2$-CH=CH	FA-CH$_2$
2.7916	FA	CH=CH-C**H**$_2$-CH=CH	FA-CH$_2$
2.8147	FA	CH=CH-(C**H**$_2$-CH=CH-)$_x$	FA-CH$_2$
2.8231	FA	CH=CH-(C**H**$_2$-CH=CH-)$_x$	FA-CH$_2$
2.8341	FA	CH=CH-(C**H**$_2$-CH=CH-)$_x$	FA-CH$_2$
2.8411	FA	CH=CH-(C**H**$_2$-CH=CH-)$_x$	FA-CH$_2$
2.8514	FA	CH=CH-(C**H**$_2$-CH=CH-)$_x$	FA-CH$_2$

2.8607	FA	CH=CH-(C**H**$_2$-CH=CH-)$_x$	FA-CH$_2$
2.8682	FA	CH=CH-(C**H**$_2$-CH=CH-)$_x$	FA-CH$_2$
3.0919	PE	C**H**$_2$	PE-2'
3.1026	PE	C**H**$_2$	PE-2'
3.1099	PE	C**H**$_2$	PE-2'
3.2140	SM	N^+(C**H**$_3$)$_3$	SM-CH$_3$
3.2246	PC	N^+(C**H**$_3$)$_3$	PC-CH$_3$
3.4402	C / MeOH	C**H**OH	C-3
3.4485	C / MeOH	C**H**OH	C-3
3.4590	C / MeOH	C**H**OH	C-3
3.4669	C / MeOH	C**H**OH	C-3
3.4748	C / MeOH	C**H**OH	C-3
3.4853	C / MeOH	C**H**OH	C-3
3.4936	C / MeOH	C**H**OH	C-3
3.5974	Choline	C**H**$_2$	PC-2'
3.6038	Choline	C**H**$_2$	PC-2'
3.6125	Choline	C**H**$_2$	PC-2'
3.7616	PI	C**H**	PI-6'
3.7772	PI	C**H**	PI-6'
3.7938	PI	C**H**	PI-6'
3.8684	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.8727	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.8791	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.8847	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.8907	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.8963	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.9015	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.9118	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.9174	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.9251	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.9316	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.9389	HG	C**H**, C**H**$_2$	PLA-1^d,1^u, CAR-1',2',3', SPH-2,3^u, PI,1'
3.9932	HG	C**H**$_2$	PLA-3, PC-3, PE-3
4.0038	HG	C**H**$_2$	PLA-3, PC-3, PE-3
4.0139	HG	C**H**$_2$	PLA-3, PC-3, PE-3
4.1467	HG	C**H**$_2$	PI-1^u, PC-1^u, PE-1^u, PI-1', TG-1^u, DG-1^u, SPH-3^d
4.1579	HG	C**H**$_2$	PI-1^u, PC-1^u, PE-1^u, PI-1', TG-1^u, DG-1^u, SPH-3^d
4.1672	HG	C**H**$_2$	PI-1^u, PC-1^u, PE-1^u, PI-1', TG-1^u, DG-1^u, SPH-3^d
4.1778	HG	C**H**$_2$	PI-1^u, PC-1^u, PE-1^u, PI-1', TG-1^u, DG-1^u, SPH-3^d
4.1871	HG	C**H**$_2$	PI-1^u, PC-1^u, PE-1^u, PI-1', TG-1^u, DG-1^u, SPH-3^d
4.2511	Choline	C**H**$_2$	PC-1'
4.3243	TG	C**H**$_2$	TG-1^d
4.3310	TG	C**H**$_2$	TG-1^d
4.3439	TG / DG	C**H**$_2$	TG-1^d, DG-1^d
4.3508	TG / DG	C**H**$_2$	TG-1^d, DG-1^d
4.3615	PLA	-CH$_2$-C**H**=CH-O-	PLA-b
4.3713	PLA	-CH$_2$-C**H**=CH-O-	PLA-b
4.4156	HG	C**H**$_2$	PC-1^d, PE-1^d, PI-1^d

4.4206	HG	C**H**$_2$	PC-1^d, PE-1^d, PI-1^d
4.4354	HG	C**H**$_2$	PC-1^d, PE-1^d, PI-1^d
4.4407	HG	C**H**$_2$	PC-1^d, PE-1^d, PI-1^d
4.5716	CE / MeOH	C**H**OR	C-3
4.5788	CE / MeOH	C**H**OR	C-3
4.5898	CE / MeOH	C**H**OR	C-3
4.5993	CE / MeOH	C**H**OR	C-3
4.6078	CE / MeOH	C**H**OR	C-3
4.6169	CE / MeOH	C**H**OR	C-3
4.6260	CE / MeOH	C**H**OR	C-3
5.2225	HG	C**H**	PC-2, PE-2, PI-2
5.2279	HG	C**H**	PC-2, PE-2, PI-2
5.2324	HG	C**H**	PC-2, PE-2, PI-2
5.2374	HG	C**H**	PC-2, PE-2, PI-2
5.2431	HG	C**H**	PC-2, PE-2, PI-2
5.2485	HG	C**H**	PC-2, PE-2, PI-2
5.2521	HG	C**H**	PC-2, PE-2, PI-2
5.2577	HG	C**H**	PC-2, PE-2, PI-2
5.2764	TG	C**H**	TG-2
5.2845	TG	C**H**	TG-2
5.2925	TG	C**H**	TG-2
5.3086	FA	C**H**=C**H**	FA-CH
5.3205	FA	C**H**=C**H**	FA-CH
5.3268	FA	C**H**=C**H**	FA-CH
5.3389	C / FA	C**H**, C**H**=C**H**	C-6, FA-CH
5.3448	C / FA	C**H**, C**H**=C**H**	C-6, FA-CH
5.3494	C / FA	C**H**, C**H**=C**H**	C-6, FA-CH
5.3543	C / FA	C**H**, C**H**=C**H**	C-6, FA-CH
5.3625	FA	C**H**=C**H**	FA-CH
5.3680	FA	C**H**=C**H**	FA-CH
5.3780	CE / FA	C**H**, C**H**=C**H**	C-6, FA-CH
5.3848	CE / FA	C**H**, C**H**=C**H**	C-6, FA-CH
5.3923	CE / FA	C**H**, C**H**=C**H**	C-6, FA-CH
5.4049	FA	C**H**=C**H**	FA-CH
5.4423	SM	-CH$_2$-CH=C**H**-CHOH-	SPH-a
5.4550	SM	-CH$_2$-CH=C**H**-CHOH-	SPH-a
5.4683	SM	-CH$_2$-CH=C**H**-CHOH-	SPH-a
5.6887	SM	-CH$_2$-C**H**=CH-CHOH-	SPH-b
5.7011	SM	-CH$_2$-C**H**=CH-CHOH-	SPH-b
5.7129	SM	-CH$_2$-C**H**=CH-CHOH-	SPH-b
5.7259	SM	-CH$_2$-C**H**=CH-CHOH-	SPH-b
5.7383	SM	-CH$_2$-C**H**=CH-CHOH-	SPH-b
5.9181	PLA	-CH$_2$-CH=C**H**-O-	PLA-a
5.9279	PLA	-CH$_2$-CH=C**H**-O-	PLA-a

[a]Samples were contained in a mixture of $CDCl_3$/methanol-d4 (2:1, by volume). In addition, 0.03 vol% tetramethylsilane (TMS) and 14.11 mM pyrazine was added as reference. The spectra were recorded at 293 K at 600.12 MHz and referenced to internal TMS. For obtaining approximate chemical shifts relative to DSS in D_2O a value of 0.074 ppm has to be added to the given values (see Materials

and Methods). However, the size of the particles also determine the chemical shifts. In serum at high DSS concentrations (32 mM) the mean position of the cholesterol C18 signal is 0.722 ppm. With the above correction the mean size dependent shift would be -0.05 ppm. C, cholesterol; CAR, cardiolipin; CE, cholesterol ester; DG; diacylglycerol; FA, fatty acid; HG, head group; MeOH, methanol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PLA, plasmalogen; SM, sphingomyelin; SPH, sphingosine; TG, triacylglycerol. The CH protons of the sphingosine and plasmalogene double bonds are designated as a (next to head group) and b (next to alkyl chain). The diastereotopic CH_2 protons with two separated signals are designated as u (upfield) and d (downfield). Note, that the signal positions of the resolved multiplet components are given.

Supplemental Table S2: T_1 relaxations times of internal standards and selected functional groups of lipids.[a]

	Pyrazine -(C**H**)$_4$	TMS -(C**H**$_3$)$_4$	DSS -(C**H**$_3$)$_3$	Choline -N(C**H**$_3$)$_3$	Fatty acid -(CH=CH) -C**H**$_2$- (CH=CH)-	Fatty acid -C**H**$_2$-	Fatty acid -C**H**$_3$	Cholesterol -C^{18}**H**$_3$
Lipid extracts								
δ [ppm]	8.636	0.00	-	3.202	2.779	1.260	0.883	0.688
T_1 [s]	6.85	5.65	-	0.82	1.54	1.26	3.16	0.85
Serum								
δ [ppm]	8.642	-	0.00[b]	3.247	2.759	1.306	0.893	0.67
T_1 [s]	9.66	-	3.95	0.39	0.47	0.43	0.55	0.48
HDL								
δ [ppm]	-	-	-	3.209	2.697	1.220	0.829	0.644
T_1 [s]	-	-	-	0.53	0.59	0.56	0.76	0.64

[a]The protons observed are given in bold letters. 600 MHz spectra of lipid extracts were measured at 293 K, of serum and HDL at 310 K. The serum and the HDL sample were from different patients.

[b]Note that the DSS signal shifts with its concentration, at 0.1 mM DSS it is located at -0.029 ppm.

Supplemental Table S3. T_1 relaxations times of internal standards at different temperatures in human blood serum.[a]

T [K]	Pyrazine -(CH)$_4$	Dioxane -(CH)$_4$	DSS -(CH$_3$)$_3$
	T_1 [s]		
283	5.44	3.30	2.23
293	6.92	4.11	278
303	8.55	4.97	3.54
310	9.66	5.63	3.95
313	10.20	5.91	3.98
323	12.36	7.02	4.81

[a] T_1 relaxation times were measured at 600 MHz. The protons observed are given in bold letters.

Supplemental Table S4. NMR signals usable for a quantitative analysis of lipids in NMR spectra of extracts[a]

Mean position [ppm]	Integration range [ppm]	Group	Application
0.641	0.550 - 0.732	C, CE (C-18)	Total cholesterol (C_M)
0.841	0.732 - 0.949	C, CE (C-21, C-26, C-27), FA (-C<u>H</u>$_3$)	CH$_3$-groups of cholesterol, fatty acids of CE, TG, PL, SL, PLA, and of side chains of SL and PLA.
1.011	0.996 - 1.026	C (C-19)	Cholesterol (C_M)
1.042	1.026 - 1.057	CE (C-19)	Cholesterol esters (C_M)
1.320	1.216 - 1.424	C, CE (C-16, C-20, C-22, C-23), FA (-C<u>H</u>$_2$-)	Estimation of mean fatty acid chain length
1.663	1.552 - 1.672	C, CE (C-7, C-8, C-11, C-15, C-25), FA (-C<u>H</u>$_2$-CH$_2$-COO-)	Total fatty acid residues (C_M)
2.092	1.949 - 2.235	C (C-4), FA (-C<u>H</u>$_2$-CH=CH-)	Double bounds of fatty acid residues (control)
2.339	2.236 - 2.389	C, CE (C-4) / FA (-C<u>H</u>$_2$-COO-)	Total fatty acid residues (control)
2.761	2.720 - 2.801	FA (-CH=CH-C<u>H</u>$_2$-CH=CH-)	Estimation of polyunsaturated fatty acid ratio (diunsaturated:polyunsaturated)
2.823	2.721 - 2.926	FA (-CH=CH-(C<u>H</u>$_2$-CH=CH-)$_x$), FA (-CH=CH-C<u>H</u>$_2$-CH=CH-)	Estimation of polyunsaturated fatty acid ratio (diunsaturated:polyunsaturated)

2.864	2.801 - 2.927	FA (-CH=CH-(C<u>H</u>$_2$-CH=CH-)$_x$)	Estimation of polyunsaturated fatty acid ratio (diunsaturated:polyunsaturated)
3.102	3.070 - 3.133	PE (PE-2')	Ethanolamine head group, concentration of PE (C_M)
3.224	3.194 - 3.255	SM (-Choline), PC (-Choline), MeOH-^{13}C-Satellite	Choline head group of mainly PC and SM (C_M)
3.468	3.422 - 3.513	MeOH- ^{13}C-Satellite, C (C-3)	Used for quantification and correction of MeOH- ^{13}C-Satellite
3.598	3.567 - 3.629	PC (PC-2'), PI (PI-4')	PC and PI (control)
3.778	3.737 - 3.819	PI (PI-6')	Inositol head group of PI (C_M)
4.171	4.141 - 4.200	PI (PI-1^u, PI-1'), PC (PC-1^u), PE (PE-1^u), TG (TG-1^u), SPH (SPH-3d)	PI, PC, PE, TG and SPH (control)
4.256	4.217 - 4.295	PC (PC-1')	Choline head group (PC/SM) (control)
4.341	4.302 - 4.380	TG (TG-1^d)	Triacylglyceride (TG)
5.233	5.200 - 5.266	PC (PC-2), PE (PE-2), PI (PI-2)	PC, PE and PI (control)
5.283	5.266 - 5.300	TG (TG-2)	TG (control)
5.296	5.042 - 5.551	C, CE (C-1), FA (-C<u>H</u>=C<u>H</u>-), PC (PC-2), PE (PE-2), PI (PI-2)	Estimation of fatty acid saturation degree
5.466	5.436 - 5.496	SL (SPH-a)	Sphingosine (control)
5.720	5.682 - 5.758	SL (SPH-b)	Sphingosine (C_M)
5.921	5.887 - 5.955	PLA (PLA-a)	Plasmalogene (C_M)

[a]The diastereotopic CH_2 protons with two separated signals are designated as u (upfield) and d (downfield). The CH protons of the shingosine and plasmalogene double bonds are designated as a (next to head group) and b (next to alkyl chain). (C_M) useful for calculation of the molar concentration. C = Total Cholesterol, CE = Cholesterol ester, FA = Fatty acid, TG = Triacylglyceride, PC = Phosphatidylcholine, PE = Phosphatidylethanolamine, PI = Phosphatidylinositol, PLA = Plasmalogene, SL = Sphingolipids, SM = Sphingomyelin, SPH = Sphingosine.

Supplemental Table S5. Distribution of fatty acid species in different lipoprotein samples[a]

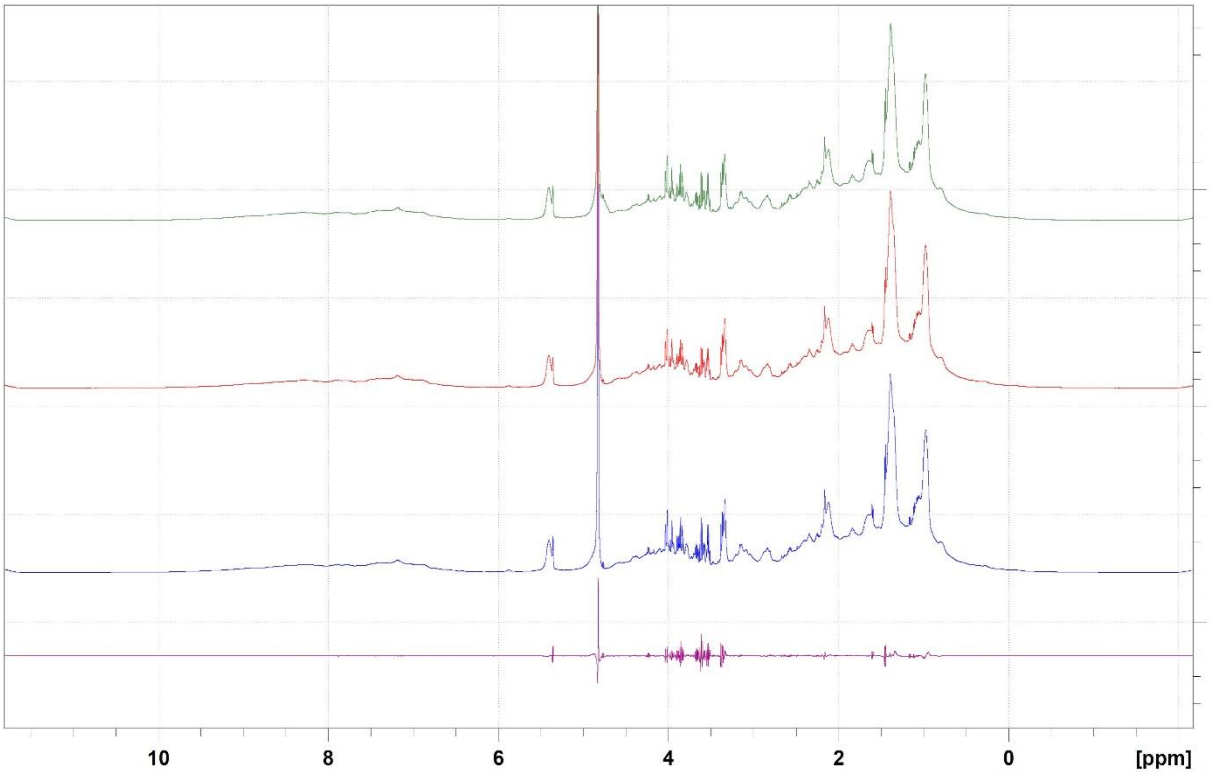
Fatty acid	Serum	VLDL	IDL	LDL	HDL2	HDL3
			Relative molar concentration [%]			
16:0	24.0	33.1	29.6	21.9	27.3	33.3

18:0	7.7	7.2	11.5	8.8	9.1	11.6
20:0	0.0	0.0	2.7	1.8	2.1	0.7
22:0	0.0	0.0	1.6	2.9	3.0	1.6
24:0	0.0	0.0	0.0	3.9	4.0	2.1
18:1	16.1	27.1	11.4	6.8	4.8	1.9
24:1	8.0	1.4	2.3	5.6	2.6	0.2
18:2	31.9	22.8	30.3	37.0	34.4	33.6
20:3	3.0	2.6	2.8	2.0	0.2	0.4
20:4	5.1	2.5	2.8	2.7	1.0	2.5
20:5	2.8	1.9	2.2	2.6	4.7	6.4
22:5	0.6	0.9	2.9	4.1	7.2	5.7
22:6	0.8	0.5	0.0	0.0	0.1	0.0

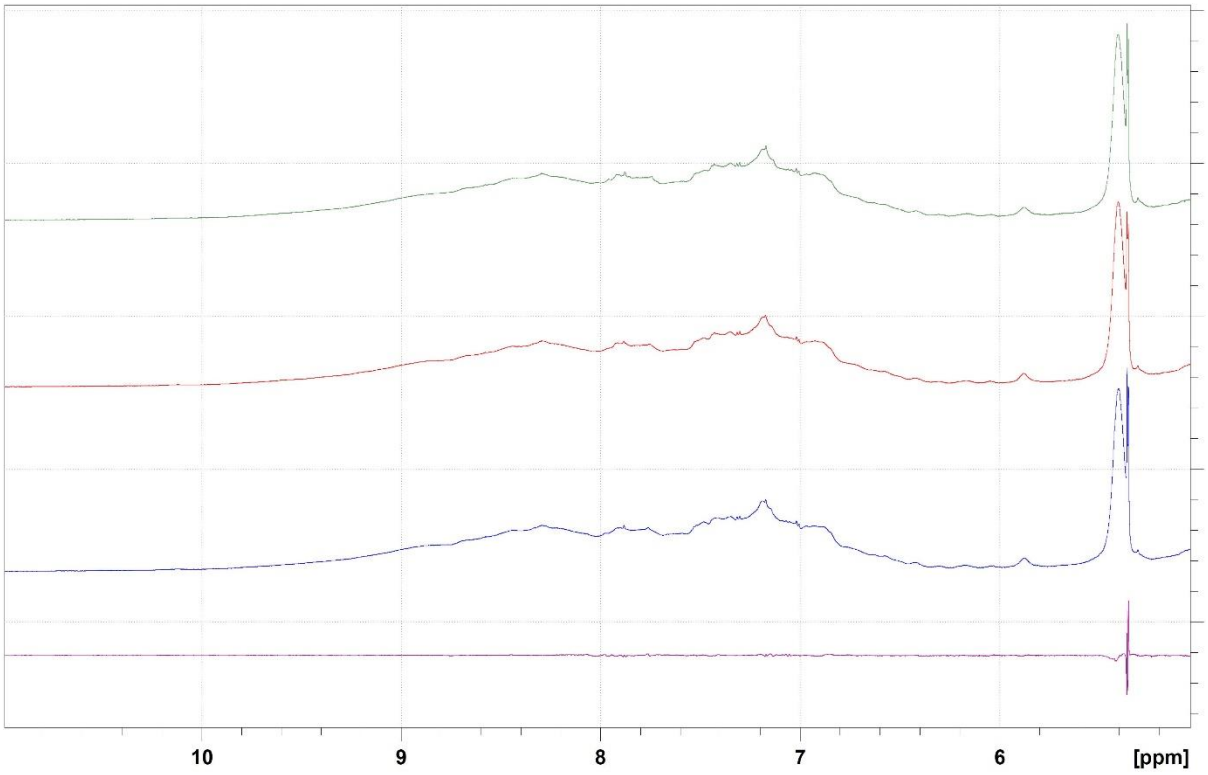
[a]The distribution was obtained by a multidimensional of the NMR spectra of lipoprotein extracts assuming a continuous length distribution.

Figures

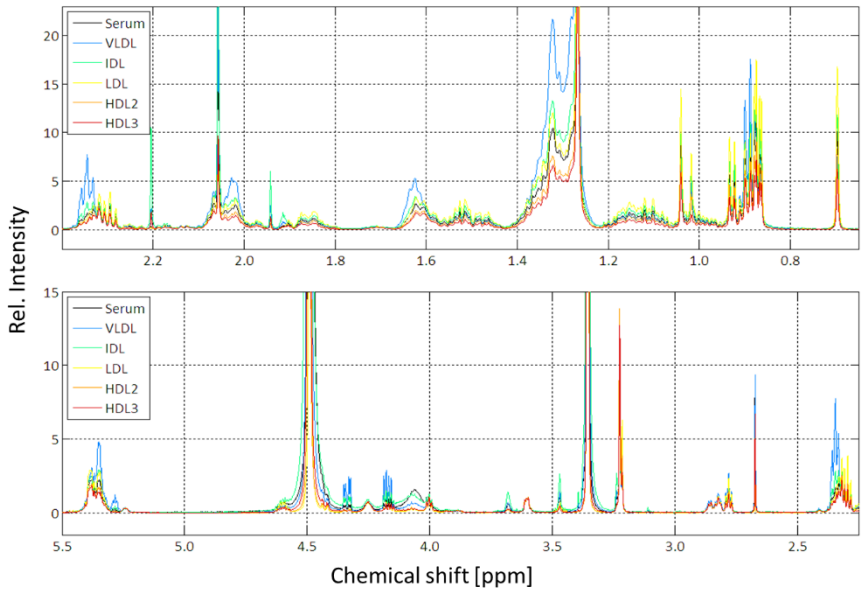
Supplemental Figure S1. Effect of freezing and thawing on ^{1}H NMR spectra of human blood serum. 600 MHz 1D-NOESY spectra of human blood serum. (from top to bottom) fresh sample, sample after freezing, storing at 253 K for 5 h and thawing, sample after four freezing-thawing cycles, difference between first and last spectrum.



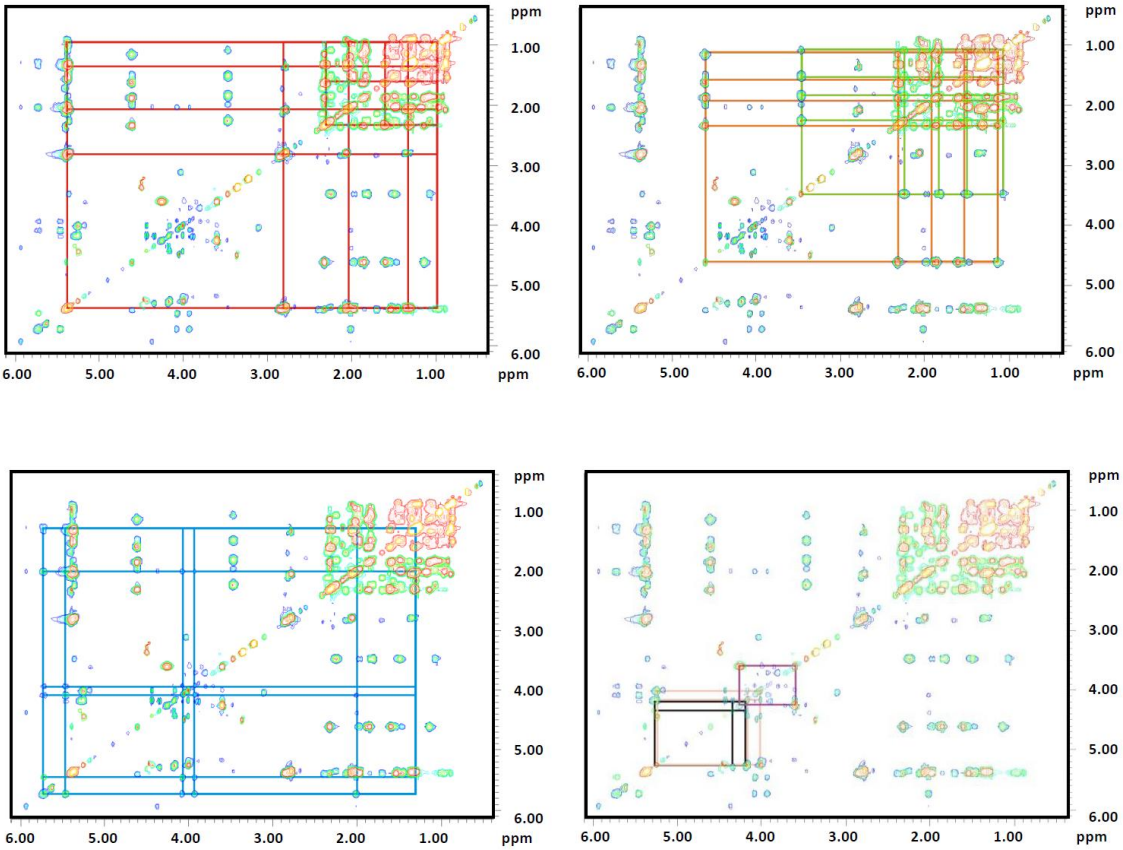
Supplemental Figure S2. Effect of freezing and thawing on ^{1}H NMR spectra of the proteins of human blood serum. 600 MHz 1D-NOESY spectra of human blood serum, downfield part of the spectra shown in Fig. S1 scaled up 6-times. (from top to bottom) fresh sample, sample after freezing, storing at 253 K for 5 h and thawing, sample after four freezing-thawing cycles, difference between first and last spectrum.



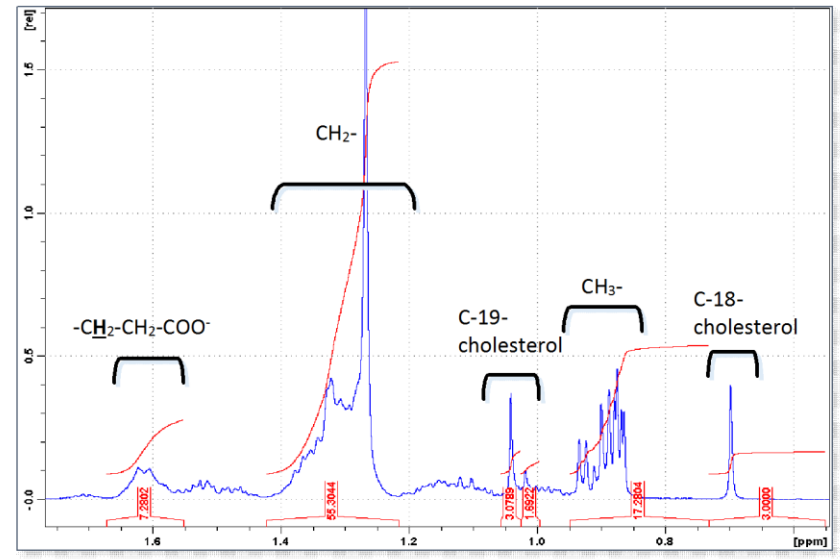
Supplemental Figure S3. Overlay of the 600 MHz ^{1}H NMR spectra of chloroform-methanol extracts of different lipoprotein fractions. The different lipoprotein fractions obtained by analytical ultracentrifugation and shown in Fig. 2 were extracted with perdeutero chloroform and perdeutero methanol (2:1) as described in Materials and Methods. 600 MHz ^{1}H NMR spectra, 293 K, reference TMS.

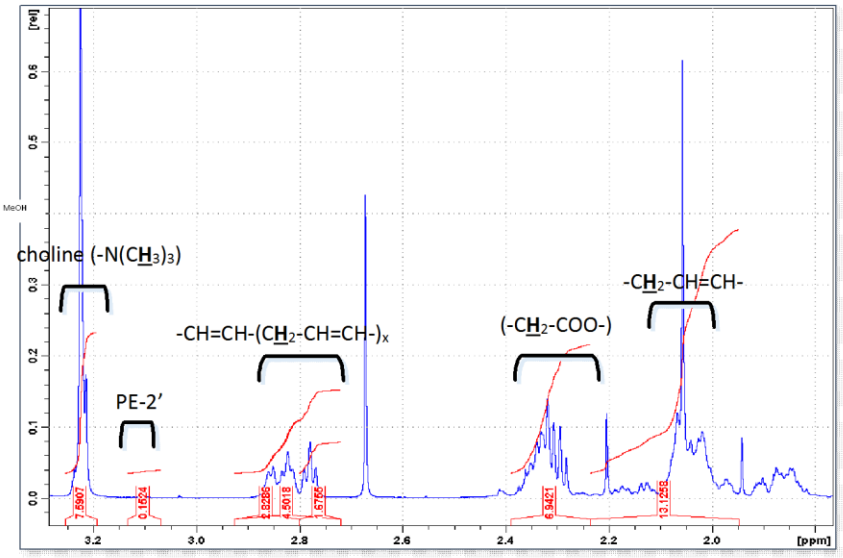


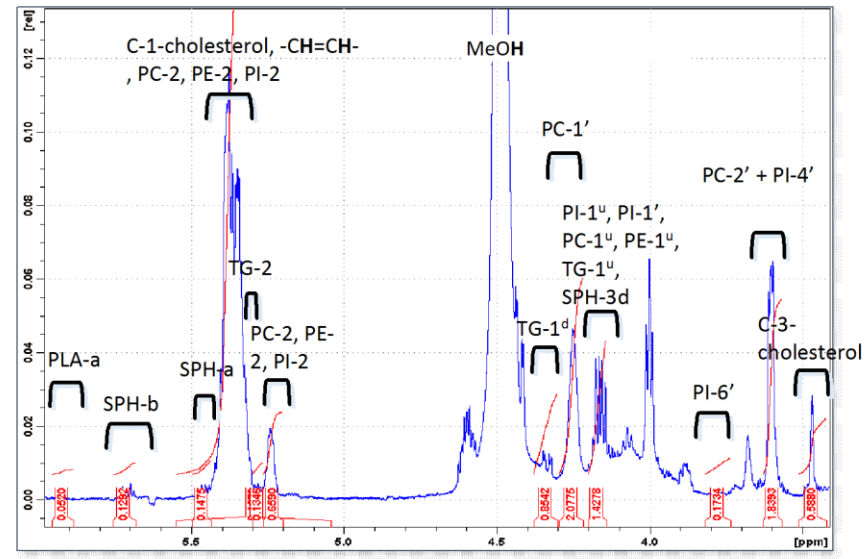
Supplemental Figure S4: Identification of lipid components of LDL extracts. 600 MHz ^{1}H NMR spectra of LDL extracted as described in Materials and Methods. Temperature 293 K, mixing time 60 ms. The signals of deutero methanol and chloroform were reduced by suitable processing. (Top, left) signals of fatty acids. (Top, right) signals of cholesterol and cholesterol esters. (Bottom, left) signals of sphingosine. (Bottom, right) signals of choline, glycerol in phopsphoglycerides and triacylglycerols. For assignments see Supplemental Table S1.



Supplemental Figure S5. ^{1}H NMR spectra of a chloroform-methanol extract of HDL3. HDL3 was obtained by analytical ultracentrifugation and extracted with perdeutero chloroform and perdeutero methanol (2:1) as described in Materials and Methods. 600 MHz ^{1}H NMR spectra, 293 K, reference TMS. The integration ranges of Table S4 are indicated.


CH2-
-CH2-CH2-COO-
C-19-cholesterol
CH3-
C-18-cholesterol
[rel]
[ppm]


MeOH
choline (-N(CH3)3)
-CH=CH-(CH2-CH=CH-)x
PE-2'
(-CH2-COO-)
-CH2-CH=CH-
[rel]
[ppm]


C-1-cholesterol, -CH=CH- , PC-2, PE-2, PI-2
MeOH
PC-1'
PC-2' + PI-4'
PI-1u, PI-1', PC-1u, PE-1u, TG-1u, SPH-3d
TG-2
TG-1d
PC-2, PE-2, PI-2
C-3-cholesterol
PLA-a
SPH-a
SPH-b
PI-6'
[rel]
[ppm]

Supplemental Figure S6: CH_3 group and cholesterol signal quantification. (A) ^{1}H-NMR spectra of neat cholesterol in $CDCl_3$/MeOD with line broadenings LB adapted to the line width of the C-18H_3 resonance of cholesterol of native LDL at 323 K and 293 K. (B) Spectra obtained after subtracting the artificial cholesterol spectra (A) from the CH_2/CH_3 region of LDL.