SUPPLEMENTAL MATERIALS

Overfeeding saturated fat increases LDL aggregation susceptibility while overfeeding unsaturated fat decreases proteoglycan-binding of lipoproteins

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Supplemental Table I. Dietary sources of the extra 1000 kcal in UNSAT, SAT, and CARB groups, and the percent of the extra kcal as saturated fat (SFA), monounsaturated fat (MUFA), polyunsaturated fat (PUFA) and carbohydrates (CARB).

Group	**UNSAT**	**SAT**	**CARB**
Extra 1000 kcal from	36 g Olive oil	30 g Coconut oil	2.8 dL Orange juice
	266 g Pesto	40 g Butter	4.3 dL Sugar-sweetened beverage
	54 g Pecan nuts	100 g Blue cheese (40% fat)	200 g Candy
	20 g Butter		
SFA%	21	76	-
MUFA%	57	21	-
PUFA%	22	3	-
CARB%	-	-	100

Supplemental Table II. Characteristics of participants for whom the LDL proteome was relatively quantified individually.

Group	UNSAT	SAT	p-value†
Number (Female/male)	3/2	4/1	0.490
Age (years)	48.6 ± 12	49.2 ± 11.7	0.938
BMI (kg/m^2)	28.2 ± 5.3	24.8 ± 3.6	0.279
LDL-C (mmol/l)	3.3 ± 0.6	3.1 ± 1.2	0.677
Serum SFA%			
Baseline	35.1 ± 0.7	34.0 ± 0.6	**0.021**
Change (log2FC)	-0.01 ± 0.05	+0.11 ± 0.06	**0.008**
LDL-aggregation, EC50			
Baseline	4.1 ± 1.2	4.2 ± 1.3	0.819
Change (log2FC)	+0.28 ± 0.23	-0.93 ± 0.33	**<0.001**
PG-binding (nM/well)			
Baseline	2.9 ± 0.6	2.0 ± 0.7	0.079
Change (log2FC)	-0.38 ± 0.19	+0.22 ± 0.42	**0.028**
oxLDL (mU/L)			
Baseline	47.4 ± 8.3	38.3 ± 14	0.256
Change (log2FC)	-0.32 ± 0.37	+0.18 ± 0.20	**0.039**

Data are shown as mean ± standard deviation. Individual proteomics was quantified post hoc for five participants in the UNSAT and SAT groups. The participants for quantification were selected based on i) groupwise similarity with respect to baseline characteristics (sex, age, BMI, LDL-cholesterol) and ii) representing the changes in the primary outcomes (LDL aggregation, PG-binding, oxLDL) as observed in the whole group. The groups were compared using Chi-square test for categorical variables and unpaired Student's t-test for continuous variables. †p-value for comparison between groups. FC, fold change; LDL-C, low-density lipoprotein cholesterol; oxLDL, oxidized low-density lipoprotein; PG, proteoglycan; SFA%, percentage of saturated fatty acids of total fatty acids.

Supplemental Table III. Major resource table.

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
N/A				

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Parent - Male					
Parent - Female					

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Lot # (preferred but not required)	Persistent ID / URL
N/A					

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL
N/A			

Cultured Cells

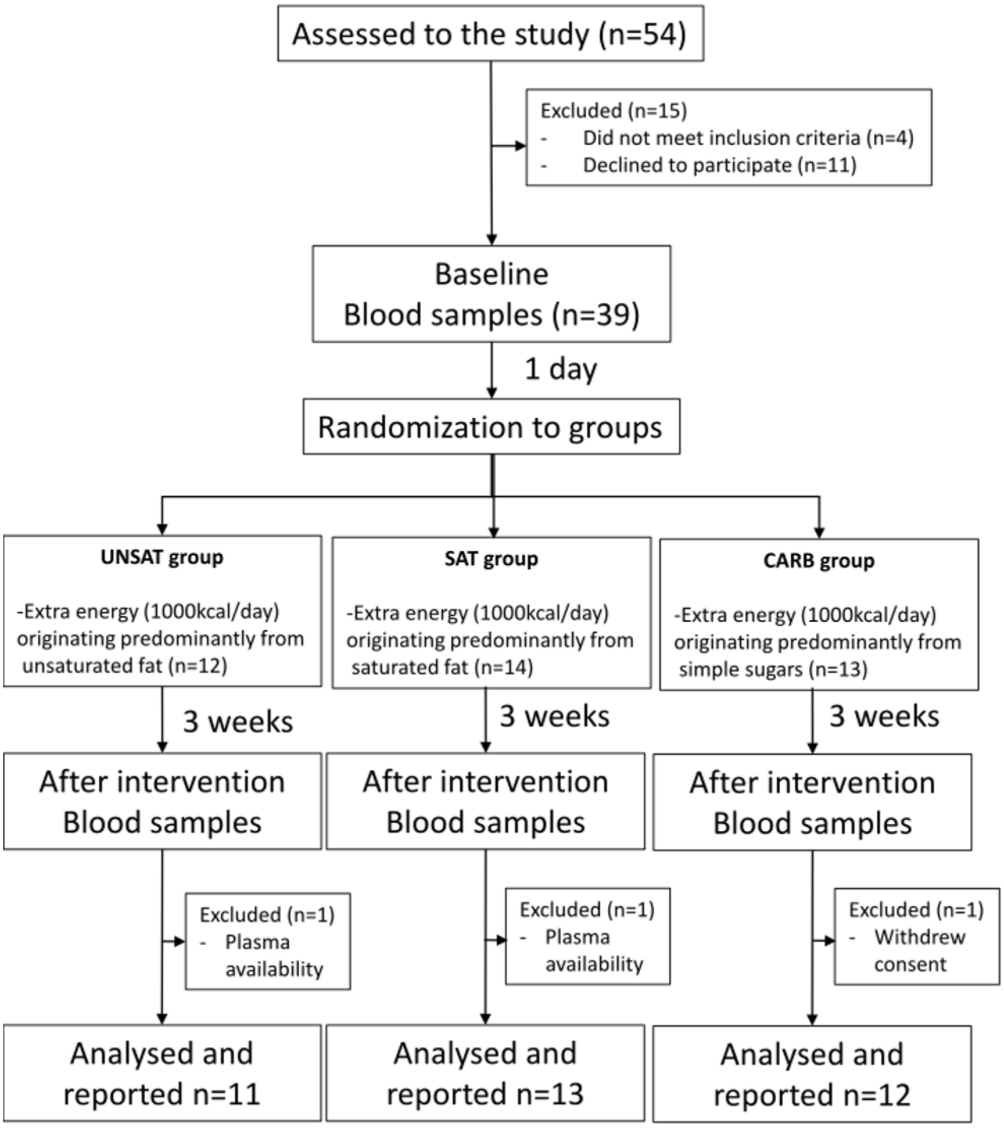
Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
N/A			

Data & Code Availability

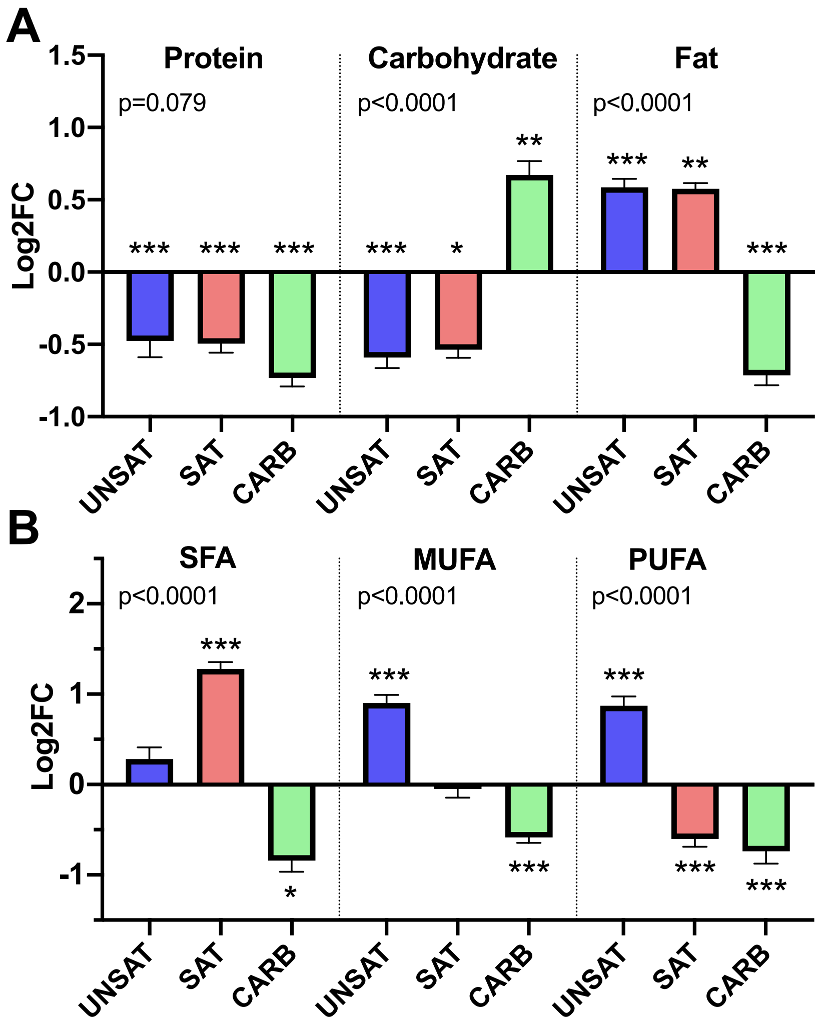
Description	Source / Repository	Persistent ID / URL
Data described in the manuscript, code book, and analytic code will be made available upon request.		

Other

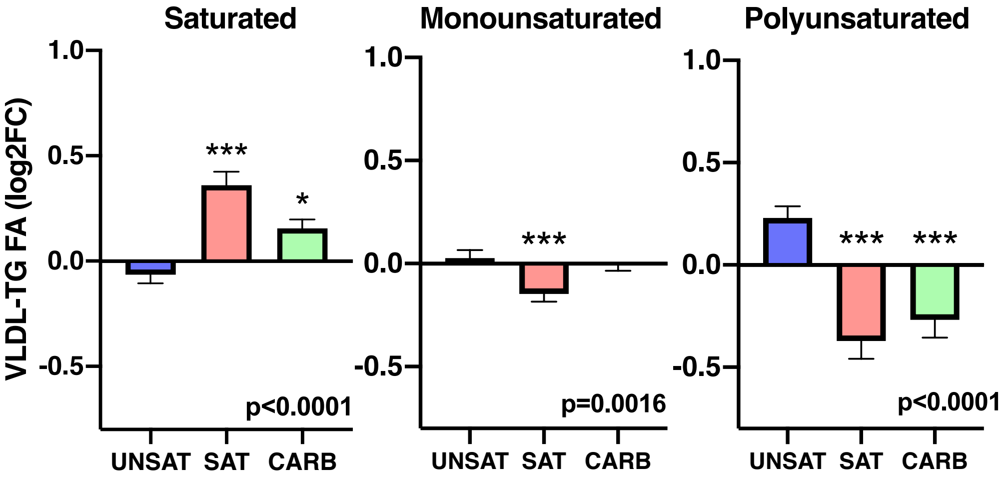
Description	Source / Repository	Persistent ID / URL
N/A		



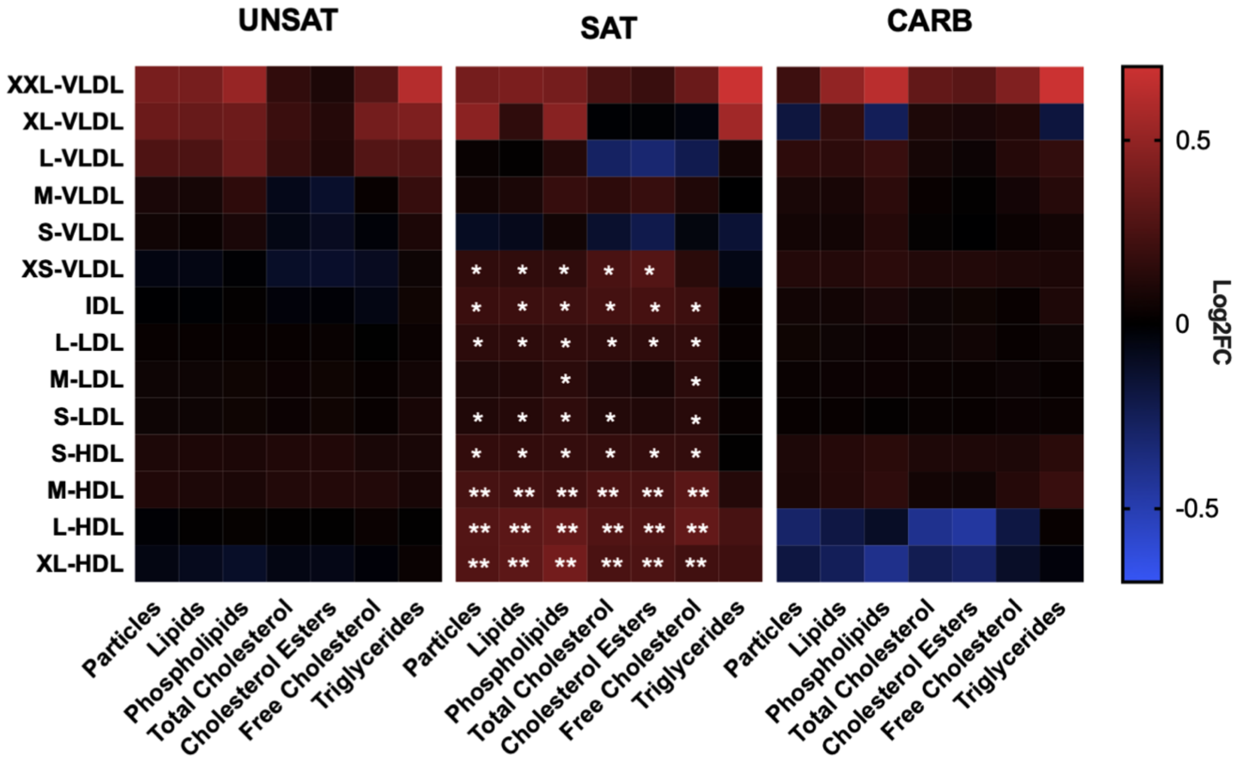
Supplemental Figure I. Study flow chart. 54 participants were assessed for the study, of whom 39 were found eligible. For the present study, plasma samples from 36 participants were available. kcal, kilocalorie; n, number.



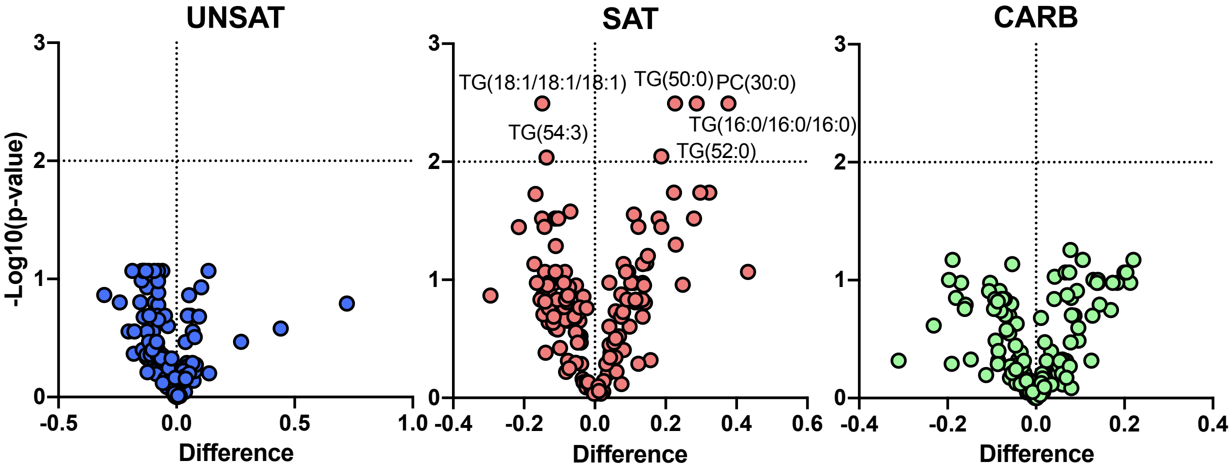
Supplemental Figure II. Difference in the diet of the study participants in the UNSAT, SAT and CARB group between baseline and after the 3-week intervention. Dietary information of the participants was collected from food diaries at the baseline and at the end of the 3-week intervention. Data are presented as mean ± standard error of mean of the log2 fold change values. (A) Change in the energy percentage derived from protein, carbohydrates, and fat in the UNSAT, SAT and CARB groups. (B) Change in the percentage of fat derived from SFA, MUFA and PUFA in the same groups. Two-way ANOVA with repeated measures was used to test differences between groups during interventions. P-values (at the top) are reported for the effect of interaction term (Time × Group). Asterisks represent p-values for within-group changes calculated by Šidak's post hoc test. FC, fold change; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.



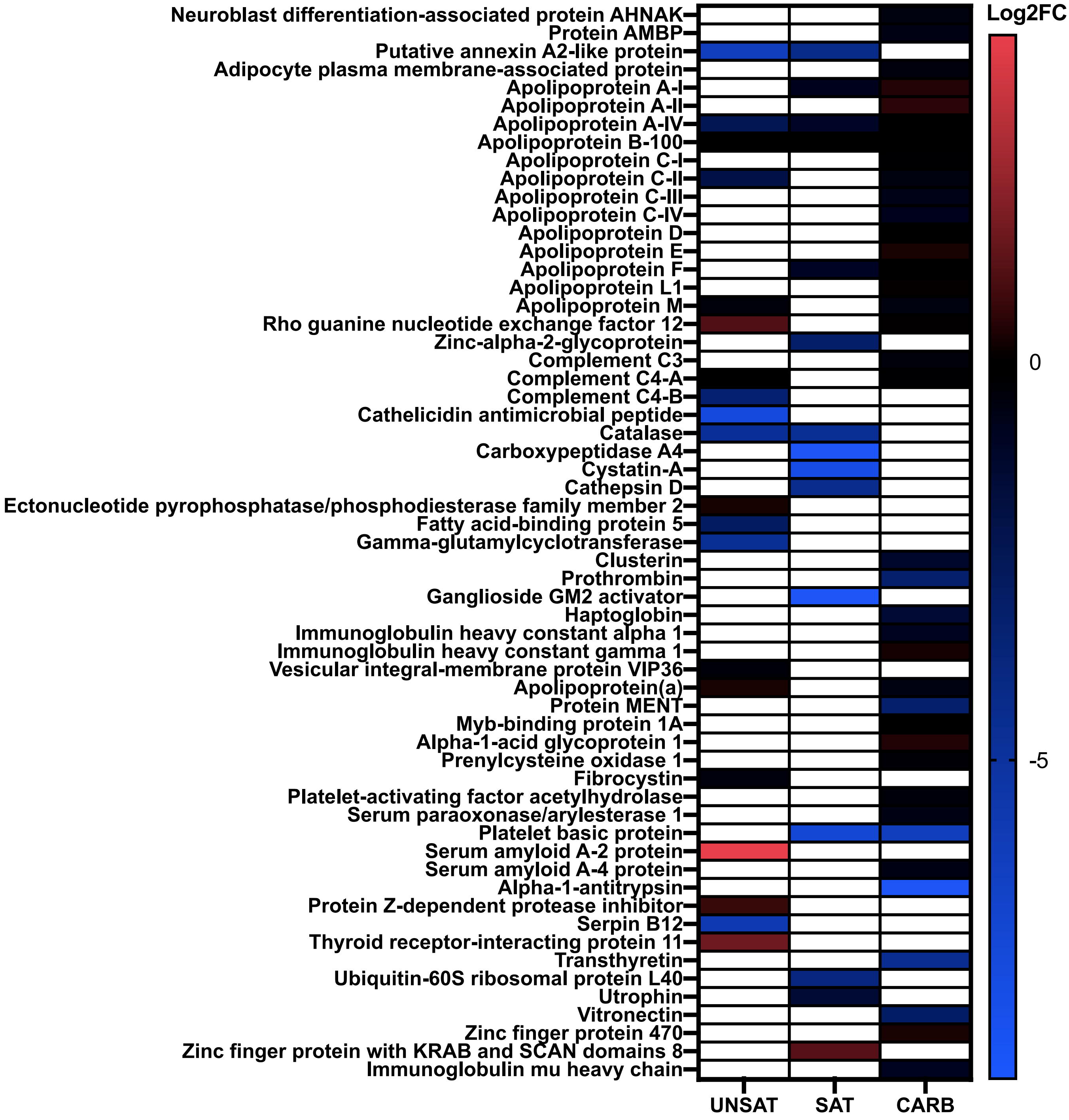
Supplemental Figure III. Change in VLDL-TG fatty acids after overfeeding unsaturated fat (UNSAT), saturated fat (SAT) or simple sugars (CARB) for 3 weeks. The fatty acid composition of VLDL-TGs was determined from samples collected at the baseline and at the end of the intervention as a measure of dietary compliance. The amount of saturated fatty acids was calculated as the sum of C14:0, C16:0, and C18:0 and that of the monounsaturated as the sum of C16:1ω-7, 18:1ω-9. Polyunsaturated fatty acids comprise of 18:2ω-6. The changes are presented as mean ± standard error of mean of the log2 values of fold changes. A positive value indicates an increase and a negative value a decrease in the amount of the fatty acids. Two-way ANOVA with Šidak's post hoc test was used to compare the changes from baseline between the groups. P-values (at the bottom) are reported for the effect of interaction term (Time × Group). Asterisks represent p-values for within-group changes calculated by Šidak's post hoc test. *, p<0.05, **, p<0.01, ***, p<0.001. UNSAT, n=11; SAT, n=13; CARB, n=12. FA, fatty acid; FC, fold change; TG, triacylglycerol.



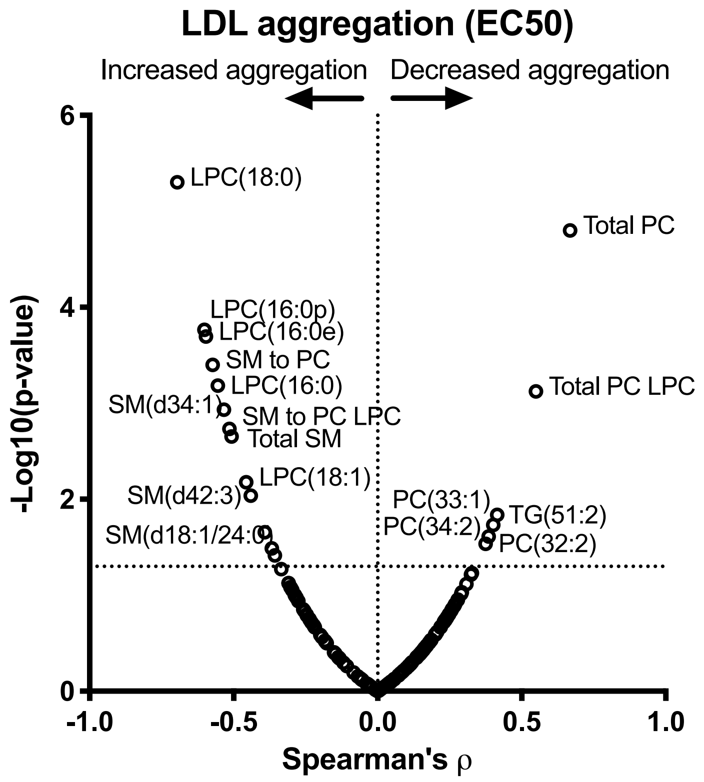
Supplemental Figure IV. Change in the lipoproteins subclasses in plasma during the 3-week overfeeding of unsaturated fat (UNSAT), saturated fat (SAT), and simple sugars (CARB). Quantitative NMR metabolomics was used to determine the concentrations lipoprotein subclasses and their components at baseline and after the 3-week overfeeding of UNSAT, SAT, and CARB diets. The log transformed metabolite concentrations were compared using paired two-sided Student's t-test with false discovery rate correction method described by Benjamini and Hochberg. Accepted FDR was set 1%. An increase in a metabolite is shown with red, a decrease with blue. *, $p<0.05$, **, $p<0.01$. UNSAT, n=11; SAT, n=13; CARB, n=12. FC, fold change; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; L, large; LDL, low-density lipoprotein, M, medium; S, small; VLDL, very low-density lipoprotein; XL, very large; XS, very small.



Supplemental Figure V. Change in LDL lipidome during the 3-week overfeeding of unsaturated fat (UNSAT), saturated fat (SAT), and simple carbs (CARB). LDL lipid composition was determined using liquid chromatography-mass spectrometry and percentage concentrations of both core and surface lipids were calculated. The log transformed lipid proportions were compared using paired two-sided Student's t-test with false discovery rate (FDR) correction method described by Benjamini and Hochberg. Accepted FDR was set 1%. Two outliers, one in the UNSAT group and one in the SAT group, were excluded. A positive difference indicates an increase and a negative difference a decrease in the lipid. UNSAT, n=10; SAT, n=12; CARB, n=12. TG=triacylglycerol, PC=phosphatidylcholine.



Supplemental Figure VI. Proteomics of pooled groups before and after dietary intervention. Each pool was measured 3 times, averages of the relative intensities were normalized to ApoB-100. Changes within groups are presented as log2 fold changes. Blue shading indicates a decrease after intervention, red increase. White cells indicate the protein was not identified in this group. FC, fold change.



Supplemental Figure VII. Relationships between LDL lipidome and LDL aggregation susceptibility at baseline. The associations for lipid proportions and aggregation susceptibility (EC50) were analyzed using Spearman's correlation coefficient analysis. Two outliers, one in the UNSAT group and one in the SAT group, were excluded from analysis. UNSAT, n=10; SAT, n=12; CARB, n=12. LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SM, sphingomyelin.