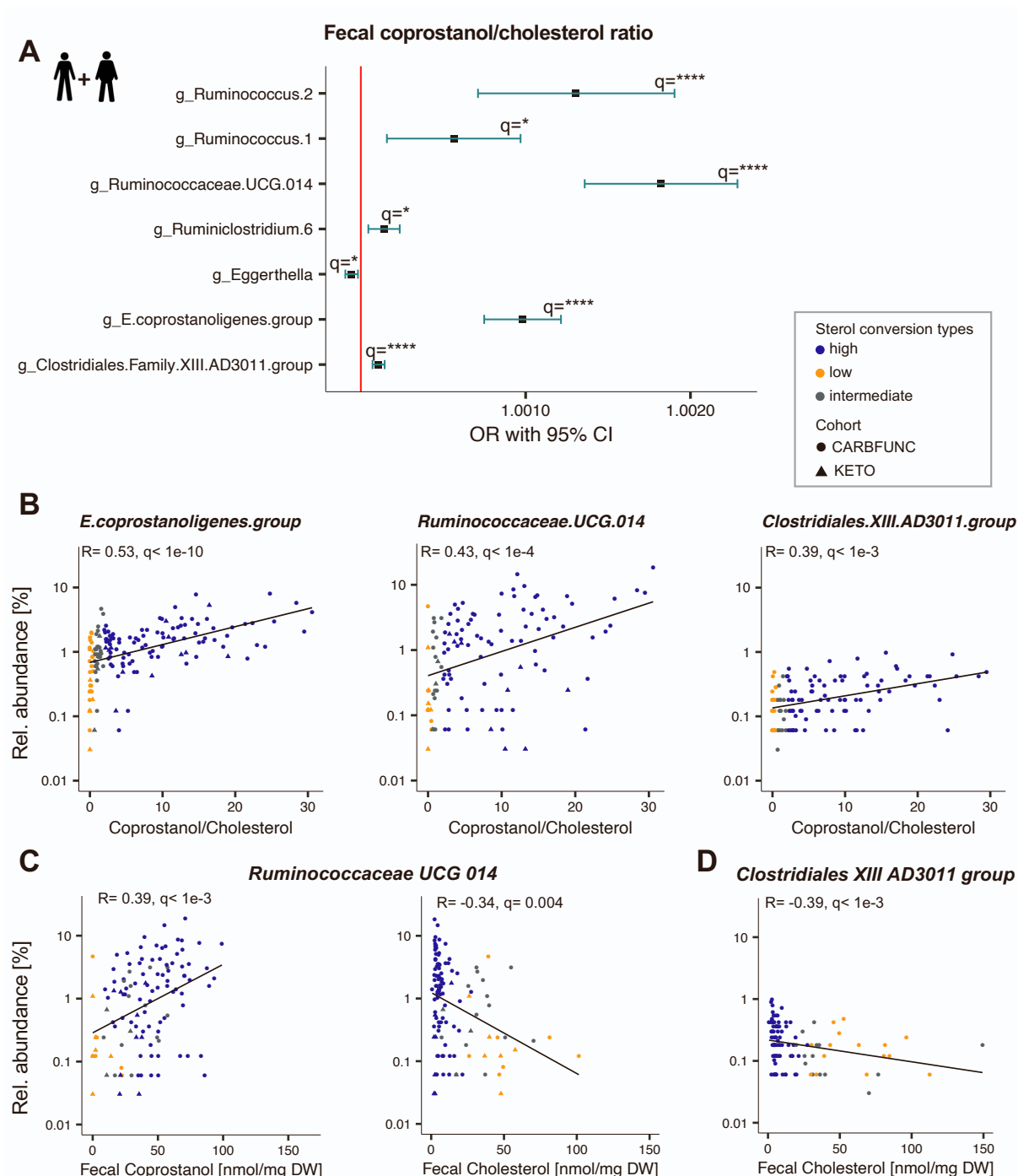


## **Supplemental information**

### **High-fat diet impact on intestinal cholesterol conversion by the microbiota and serum cholesterol levels**

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**Figure S1. Associations of fecal taxonomic microbiota composition with the fecal coprostanol/cholesterol ratio, Related to Figure 2.**

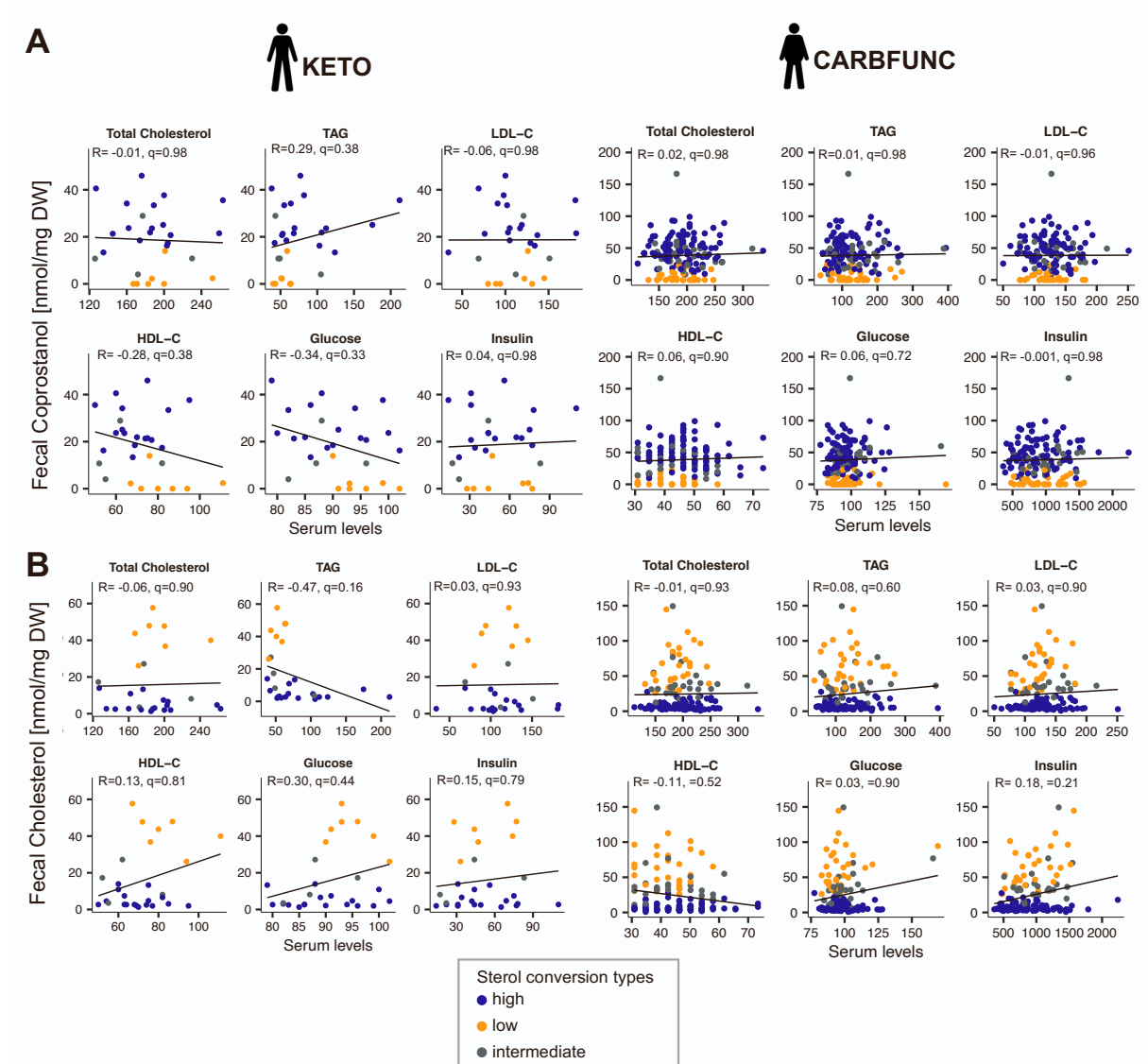
(A) Positive and negative associations of bacterial taxa with the fecal coprostanol/cholesterol ratio, as identified by the generalized linear mixed model (GLMM) for the combined dataset of KETO ( $n = 23$ ) and CARBFUNC ( $n = 145$ ) study participants. For the GLMM input, zero values were replaced with a pseudocount and cohort and gender added as random and fixed effects (see Methods for details).

(B) Significant associations with the fecal coprostanol/cholesterol ratio, as supported by Spearman's rank correlation analysis, of *Eubacterium coprostanoligenes.group* ( $n = 158$ ), *Ruminococcaceae.UGC.014* ( $n = 95$ ) and *Clostridiales.XIII.AD3011* ( $n = 111$ , only detected in CARBFUNC samples).

(C) Across both cohorts combined, *Ruminococcaceae.UGC.014* ( $n = 95$ ) was positively correlated with fecal coprostanol and negatively correlated with fecal cholesterol concentrations.

(D) *Clostridiales.XIII.AD3011* ( $n = 111$ ) was only detected in CARBFUNC samples and negatively correlated with fecal cholesterol concentrations.

For all analyses, p-values were Benjamini-Hochberg (BH) corrected:  $q > 0.1$  ns,  $q < 0.1$  \*,  $q < 0.05$  \*\*,  $q < 0.01$  \*\*\*,  $q < 0.001$  \*\*\*\*

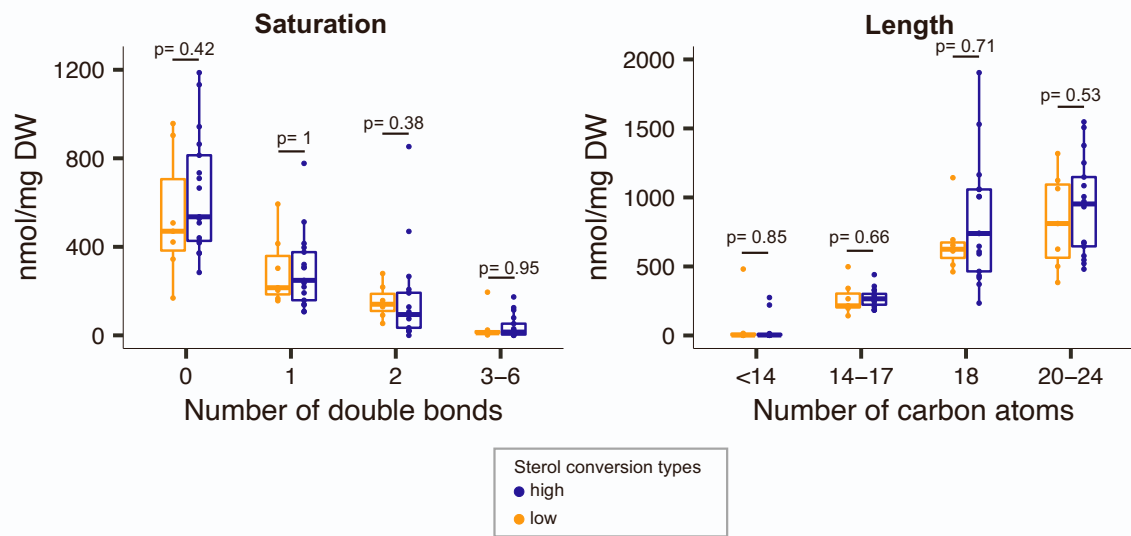


**Figure S2. No associations between fecal cholesterol and coprostanol concentrations and serum lipid levels in both cohorts, Related to Figure 4.** Neither fecal cholesterol nor coprostanol levels were significantly correlated with serum lipids in the KETO or CARBFUNC cohorts. All serum lipids and blood glucose were measured as mg/dL and insulin levels as pmol/L. Spearman's rank correlation with Benjamini-Hochberg (BH) correction.  $n_{\text{KETO}} = 28$ ,  $n_{\text{CARBFUNC}} = 145$ ; Abbreviations: TAG, triacylglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.



KETO

## Fecal Fatty Acids



**Figure S3. Similar fecal fatty acid profiles in cholesterol high and low-converters from the KETO cohort before the LCHF diet intervention, Related to Figure 5.** Wilcoxon rank-sum test,  $p > 0.05$  ns,  $n(\text{low-converter}) = 7$ ,  $n(\text{high-converter}) = 17$ . Data are represented as mean  $\pm$  SD.

**Table S1. Overview of KETO and CARBFUNC study cohort parameters, Related to Figures 1 and 4.** Table shows mean values +/- standard deviations (sd). Continuous variables were compared with the Wilcoxon rank-sum test, categorical variables with the Chi-Square test.  $p > 0.05$  ns,  $p < 0.05$  \*,  $p < 0.01$  \*\*,  $p < 0.001$  \*\*\*; Abbreviations: TAG, triacylglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; DW, dry weight.

	KETO (n=28) mean $\pm$ sd	CARBFUNC (n=145) mean $\pm$ sd	p	Significance thresholds
Sex (female vs male)	20 vs 8	72 vs 73	0.041	*
Age [years]	37 $\pm$ 11.73	43 $\pm$ 8.32	0.023	***
BMI	23.89 $\pm$ 3.14	36.86 $\pm$ 4.62	< 1e-22	***
Weight [kg]	70.23 $\pm$ 11.51	112.16 $\pm$ 18.05	< 1e-20	***
<u>Serum metabolites [mg/dL]</u>				
Total cholesterol	186.48 $\pm$ 34.73	193.91 $\pm$ 36.64	0.32	ns
TAG	78.57 $\pm$ 39.08	130.46 $\pm$ 60.93	< 1e-7	***
HDL-C	71.26 $\pm$ 14.29	44.80 $\pm$ 9.07	< 1e-13	***
LDL-C	110.86 $\pm$ 31.33	126.74 $\pm$ 31.6	0.03	*
TAG/HDL-C	1.13 $\pm$ 0.82	3.19 $\pm$ 2.03	< 1e-11	***
LDL-C/HDL-C	1.60 $\pm$ 0.60	2.98 $\pm$ 1.07	< 1e-10	***
Glucose	90.79 $\pm$ 6.60	97.54 $\pm$ 12.67	0.002	***
Insulin [pmol/L]	50.39 $\pm$ 23.91	921.51 $\pm$ 332.29	< 1e-16	***
<u>Fecal metabolites [nmol/mg DW]</u>				
Coprostanol	18.71 $\pm$ 13.44	38.75 $\pm$ 27.05	< 1e-3	***
Cholesterol	15.76 $\pm$ 17.54	24.46 $\pm$ 29.13	0.054	ns
Coprostanol/Cholesterol	5.50 $\pm$ 5.49	6.38 $\pm$ 7.31	0.62	ns
Sitosterol	4.62 $\pm$ 3.99	6.21 $\pm$ 6.89	0.64	ns
Stigmastanol	10.39 $\pm$ 6.71	9.75 $\pm$ 6.44	0.54	ns
Campesterol	1.43 $\pm$ 1.51	2.49 $\pm$ 3.33	0.07	ns
Acetate	223.09 $\pm$ 183.82	158.35 $\pm$ 110.33	0.086	ns
Propionate	82.47 $\pm$ 67.7	62.64 $\pm$ 47.37	0.19	ns
Butyrate	62.85 $\pm$ 49.25	58.2 $\pm$ 51.68	0.57	ns
Iso-Butyrate	10.3 $\pm$ 6.65	8.19 $\pm$ 6.59	0.015	*