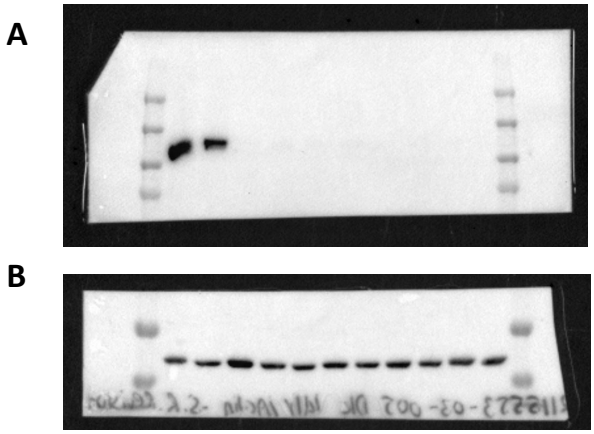
The impact of a humanized bile acid composition on atherosclerosis development in hypercholesterolaemic Cyp2c70 knockout mice

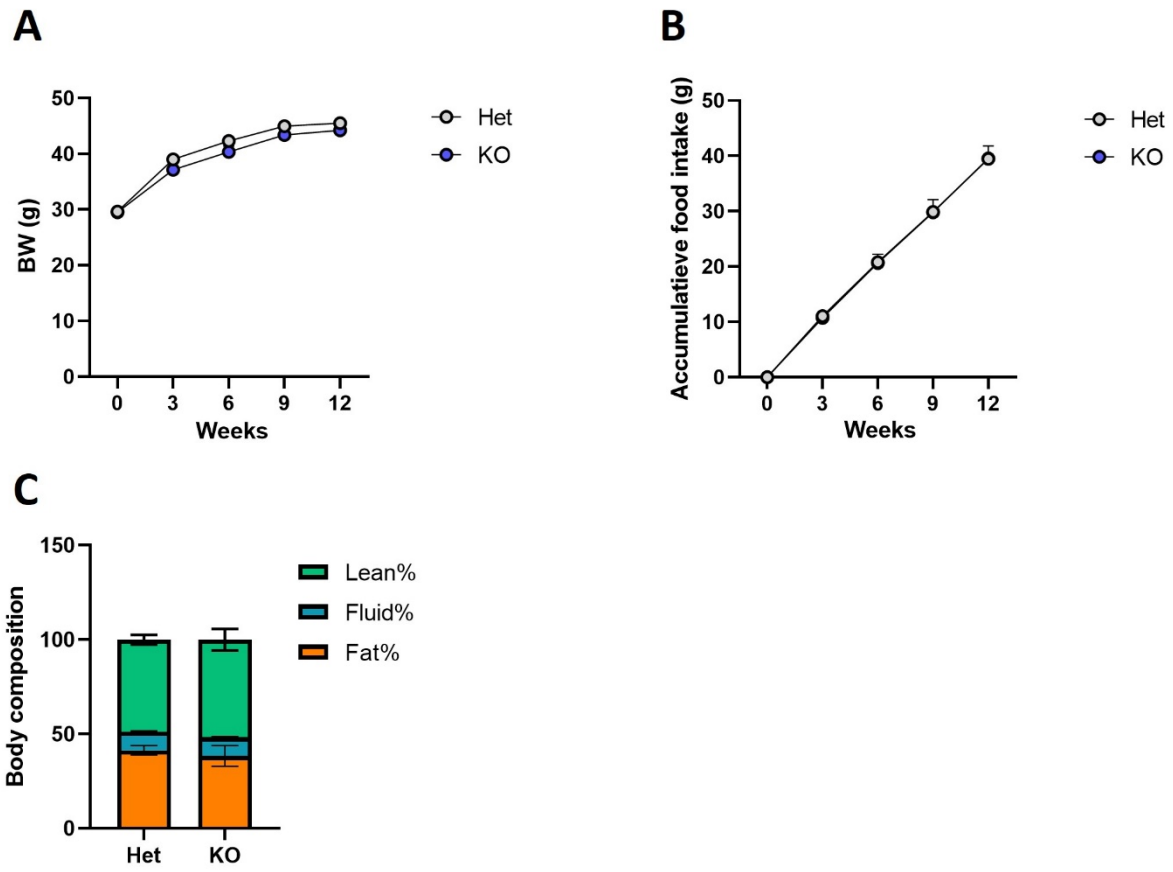
Tess Yntema[1], Tim R. Eijgenraam[1,2], Niels J. Kloosterhuis[1], Rick Havinga[1], Mirjam H. Koster[1], Milaine V. Hovingh[1], Jan Freark de Boer[1,3], Debby P. Y. Koonen[1#], Folkert Kuipers[1,4#]

#Equal contribution

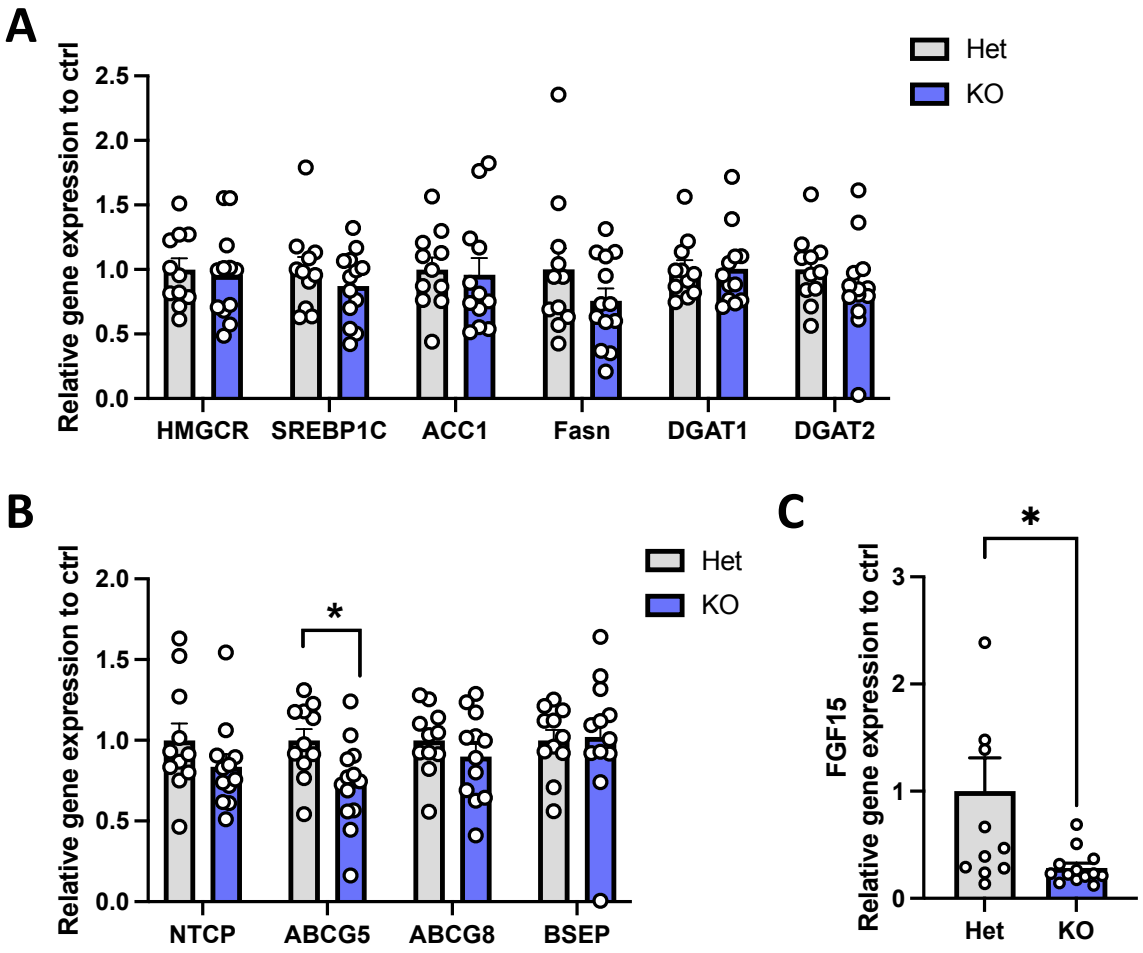
Supplementary Information



Supplemental Figure S1. Full-length Western Blots accompanying Fig. 1B. (A) LDLR and **(B)** β-actin.



Supplemental Figure S2. No effect of humanized BA pool on body weight, food intake and body composition. (A) Body weight and **(B)** accumulative food intake measurements during 12 weeks of WD feeding in *Cyp2c70*$^{-/-}$ and *Cyp2c70*$^{+/-}$ mice. **(C)** Body composition after 12 weeks of WD feeding. Data represent mean ± SEM (n=11-13 per group).



Supplemental Figure S3. The humanized BA pool of hypercholesterolemic WD-fed *Cyp2c70*$^{-/-}$ mice does not affect expression of hepatic lipogenesis genes. Hepatic mRNA expression levels of **(A)** lipogenic genes *Hmgcr*, *Srebf1*, *Acaca*, *Fasn*, *Dgat1*, and *Dgat2* and **(B)** bile acid and cholesterol transport genes *Ntcp*, *Abcg5*, *Abcg8*, and *Bsep* (n=11-13 per group) in *Cyp2c70*$^{-/-}$ and *Cyp2c70*$^{+/-}$ mice after 12 weeks of WD feeding. **(C)** *Fgf15* mRNA expression in ileum of *Cyp2c70*$^{-/-}$ and *Cyp2c70*$^{+/-}$ mice after 12 weeks of WD feeding. Gene expression is normalized to cyclophilin A and depicted as fold change compared to the control group. Data represent mean ± SEM (n=10-13 per group). *p<0.05 as determined by Student's T-test (**B**) and Mann-Whitney U test (**C**).

Table S1. Primer sequences (5'-3') used for qPCR in this study.

Target	Forward	Reverse	Reference
Ppia	TTCCTCCTTTCACAGAATTATTCCA	CCGCCAGTGCCATTATGG	1
Cyp7a1	AGCAACTAAACAACCTGCCAGTAC TA	GTCCGGATATTCAAGGATGCA	2
Cyp8b1	AAGGCTGGCTTCCTGAGCTT	AACAGCTCATCGGCCTCATC	In-house designed
Hmgcr	AGCTTGCCCGAATTGTATGTG	TCTGTTGTGAACCATGTGACTTC	3
Srebf1	TTACTCGAGCCTGCCTTCAG	TAGATGGTGGCTGCTGAGTG	In-house designed
Acaca	TGTCCACCCAAGCATTTCTTC	CATCCAACACCAGTTCAGTATACGT	In-house designed
Fasn	AGATCCTGGAACGAGAACACGAT	GAGACGTGTCACTCCTGGACTTG	4
Dgat1	AATCATCTGCTTCCCAGCAGCTGTG GCC	CACCACAGGTTGACATCCCGGTAG GAATAAAG	5
Dgat2	GGAAAAACAGCTGCAGGTCATCTC AGTACTAC	CACAGCTATCAGCCAGCAGTCTGT GCAGAAG	5
Ntcp	TGTACCCTACGTCCTCAAG	AGGAGGTAGCCAGTAAGTG	In-house designed
Abcg5	CAGCTTAGGTGTCCTGCATGTGTC	CAAGGAGACATCTTTGAGGATTTG CC	5
Abcg8	TCAGAGAGACCCTGGCTTTCATTG	GCAGCTCGGCGATTACGTCTTC	5
Bsep	GAGTGAGGCCAAAGTACAGG	GACTGTTGATAGGCGATGGG	In-house designed
Fgf15	GACTGCGAGGAGGACCAAAA	ACGTCCTTGATGGCAATCGT	In-house designed

References

1. Bieghs, V. *et al*. Trapping of oxidized LDL in lysosomes of Kupffer cells is a trigger for hepatic inflammation. *Liver Int* **33**, 1056–61 (2013).
2. Taylor, AM. *et al.* Cyclodextrin mediates rapid changes in lipid balance in Npc1-/- mice without carrying cholesterol through the bloodstream. *J Lipid Res* **53**, 2331–42 (2012).
3. Rodriguez-Agudo, D. *et al*. ER stress increases StarD5 expression by stabilizing its mRNA and leads to relocalization of its protein from the nucleus to the membranes. *J Lipid Res* **53**, 2708–15 (2012).
4. Wang, KY. *et al*. Histamine regulation in glucose and lipid metabolism via histamine receptors: model for nonalcoholic steatohepatitis in mice. *Am J Pathol* **177**, 713-23 (2010).
5. Yu H. *et al*. GPR146 Deficiency Protects against Hypercholesterolemia and Atherosclerosis. *Cell* **179**, 1276–1288 (2019).