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Supplemental Material

Exposure of *Ldlr*^{-/-} Mice to a PFAS Mixture and Outcomes Related to Circulating Lipids, Bile Acid Excretion, and the Intestinal Transporter ASBT

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Figure S1. Fat weight in *Ldlr*^{-/-} mice exposed to the PFAS mixture. Male and female *Ldlr*^{-/-} mice were exposed to vehicle water or the PFAS mixture for 7 weeks. Fat weight was measured (n=10 mice per treatment group) via EchoMRI after 2, 4, and 7 weeks of PFAS exposure. Data given as a percentage of total body weight. Two-Way ANOVA was utilized to analyze both main effects (sex, PFAS) as well as the interaction between sex and PFAS (p<0.05). The Holm-Sidak post-hoc test was used for multiple comparisons. With past consultation from biostatisticians, a significant interaction term supersedes the main effects and can make their meaning unclear. We therefore have not included the main effects p-values for any result with a significant interaction. Box plots represent the median values with upper and lower quartiles; whiskers extend to the 1st and 99th percentiles. Bold p-values represent p<0.05; italicized p-values represent p<0.10. Data is reported in Excel Table S18.

Figure S2. Additional circulating cytokines in *Ldlr*^{-/-} mice exposed to the PFAS mixture. Circulating cytokine protein levels for (A) IL-10, (B) IL-1 β , (C) IFN- γ , (D) IFN- α , and (E) IFN- β were measured from plasma collected at euthanasia from mice of each treatment group (n=5). Two-Way ANOVA was utilized to analyze both main effects (sex, PFAS) as well as the interaction between sex and PFAS (p<0.05). The Holm-Sidak post-hoc test was used for multiple comparisons. Box plots represent the median values with upper and lower quartiles; whiskers extend to the 1st and 99th percentiles. Bold p-values represent p<0.05; italicized p-values represent p<0.10. Data is reported in Excel Table S19.

Figure S3. Additional hepatic bile acid transporters after exposure to the PFAS mixture. Total hepatic RNA from n=10 mice from each treatment group was isolated and expression levels of the transporters (A) *Abcc2*, (B) *Slc51β*, (C) *Slc10a2*, and (D) *Ephx1* were determined by RT-PCR. GAPDH was used as a housekeeping gene. Hepatic NTCP protein levels were analyzed via western blot for: female⁺vehicle (n=10), female⁺PFAS (n=10), male⁺vehicle (n=10), and male⁺PFAS (n=9). E) Western blot analysis of NTCP protein in the liver. F) Quantification of band intensity for NTCP protein relative to β-actin. Non-normally distributed data was Log10 transformed prior to statistical analysis. Two-Way ANOVA was utilized to analyze both main effects (sex, PFAS) as well as the interaction between sex and PFAS (p<0.05). The Holm-Sidak post-hoc test was used for multiple comparisons. With past consultation from biostatisticians, a significant interaction term supersedes the main effects and can make their meaning unclear. We therefore have not included the main effects p-values for any result with a significant interaction. Box plots represent the median values with upper and lower quartiles; whiskers extend to the 1st and 99th percentiles. Bold p-values represent p<0.05; italicized p-values represent p<0.10. Data reported in Excel Table S20 and S21.

Figure S4. Sex hormones in female mice after exposure to the PFAS mixture. Circulating hormone levels for (A) FSH, (B) progesterone, and (C) LH were measured from plasma collected at euthanasia from female control mice (n=8) and female PFAS-exposed mice (n=8). Statistical significance for FSH was determined by t-test (p<0.05). Statistical significance for progesterone and LH was determined by Mann-Whitney Rank Sum Test (p<0.05). Box plots represent the median values with upper and lower quartiles; whiskers extend to the 1st and 99th percentiles. Data reported in Excel Table S22.

Table S1. Clinton-Cybulsky Diet (Research Diets; New Brunswick, NJ, USA).

Table S2. Primer sequences used in RT-PCR.

Table S3. Correlations between circulating PFAS and cholesterol subfractions after exposure of *Ldlr*^{-/-} mice to the PFAS mixture.

Table S4. Differentially expressed genes in the ileum after exposure of male *Ldlr*^{-/-} mice to the PFAS mixture.

Table S5. Expression of genes related to the acute inflammatory response and lipid metabolism after exposure of male *Ldlr*^{-/-} mice to the PFAS mixture.

Table S6. Modulation of hepatic nuclear receptor signaling after exposure of *Ldlr*^{-/-} mice to the PFAS mixture.

Additional File- Excel Document