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Defective FXR-FGF15 signaling and bile acid homeostasis in cystic fibrosis mice can be restored by the laxative polyethylene glycol

Bertolini, Anna; van de Peppel, Ivo P.; Doktorova-Demmin, Marcela; Bodewes, Frank A. J. A.; de Jonge, Hugo; Bijvelds, Marcel; Verkade, Henkjan J.; Jonker, Johan W.

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1 **Defective FXR-FGF15 signaling and bile acid homeostasis in cystic fibrosis mice**
2 **can be restored by the laxative polyethylene glycol**

3

4 **Running title:** Laxative restores FXR-FGF15 signaling in CF mice

5

6 **Authors:**

7 1. Anna Bertolini^{1,2}

8 2. Ivo P. van de Peppel^{1,2}

9 3. Marcela Doktorova-Demmin¹

10 4. Frank A. J. A. Bodewes²

11 5. Hugo de Jonge³

12 6. Marcel Bijvelds³

13 7. Henkjan J. Verkade^{1,2}

14 8. Johan W. Jonker^{1,4}

15

16 ¹ Section of Molecular Metabolism and Nutrition, Laboratory of Pediatrics, University of
17 Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The
18 Netherlands.

19 ² Pediatric Gastroenterology and Hepatology, University of Groningen, University Medical
20 Center, Hanzeplein 1, 9713 GZ, Groningen, The Netherlands.

21 ³ Gastroenterology & Hepatology, Erasmus MC-University Medical Center Rotterdam, The
22 Netherlands.

23 ⁴ To whom correspondence should be addressed.

24

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26

27 **Author contributions:** AB, IvdP and MD performed experiments, analyzed and
28 interpreted data. HJV, JWJ, FAJAB, MD and IPvdP designed the experiments. HJV, JWJ,
29 FAJAB, HdJ and MB supervised research and interpreted data. AB, IPvdP, HJV and JWJ
30 wrote the manuscript.

31

32 **Corresponding author:**

33 Prof. dr. J. W. Jonker

34 Section of Molecular Metabolism and Nutrition, Laboratory of Pediatrics, University of
35 Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 GZ, Groningen, The
36 Netherlands.

37 Telephone: +31-503611261

38 Email: j.w.jonker@umcg.nl

39

40 **ABSTRACT**

41 The gastrointestinal phenotype of cystic fibrosis (CF) features intestinal bile acid (BA)
42 malabsorption, impaired intestinal farnesoid X receptor (FXR) activation and consequently
43 reduced fibroblast growth factor 19 (FGF19, FGF15 in mice) production. The osmotic
44 laxative polyethylene glycol (PEG) has been shown to decrease intestinal mucus
45 accumulation in CF mice and could, by doing so, improve BA reabsorption. Here we
46 determined the effect of PEG on BA excretion and FXR-FGF15 signaling in CF mice. Male
47 *Cftr*^{-/-tm1Unc} (CF) and wild type (WT) littermates were administered PEG 4000 in drinking
48 water and fed either chow or a semisynthetic diet. PEG was withdrawn for three days
49 before termination. Fecal BA excretion was measured at PEG dosages of 37 g/L (100%)
50 and 0 g/L (0%). Ileal FXR activation was assessed by gene expression of its downstream
51 targets *Fgf15* and *Shp*. In CF mice, PEG withdrawal increased fecal BA excretion on either
52 diet as compared to full PEG dosage (chow, 2-fold, $p=0.06$; semisynthetic, 4.4-fold,
53 $p=0.007$). PEG withdrawal did not affect fecal BA excretion in WT mice on either diet. After
54 PEG withdrawal, gene expression levels of intestinal FXR target genes *Fgf15* and *Shp*
55 were decreased in CF mice, but unaffected in WT littermates. PEG did not affect the gene
56 expression of the main intestinal BA transporter ASBT. PEG treatment ameliorates
57 intestinal BA malabsorption in CF mice and restores intestinal FXR-FGF15 signaling,
58 independently from *Asbt* gene expression. These findings highlight the potential of PEG in
59 the prevention and treatment of the gastrointestinal phenotype of CF.

60

61 **New & Noteworthy:** A gastrointestinal feature of cystic fibrosis is bile acid malabsorption
62 and consequent impairment of FXR-FGF15 signaling. FXR-FGF15 signaling regulates
63 various metabolic processes and could be implicated in metabolic and gastrointestinal
64 complications of cystic fibrosis, such as diabetes and liver disease. In cystic fibrosis mice,

65 treatment with the osmotic laxative polyethylene glycol is associated with decreased fecal
66 bile acid loss and restoration of FXR-FGF15 signaling.

67

68 **Keywords:** cystic fibrosis, bile acids, FXR, FGF15, polyethylene glycol

69

70 INTRODUCTION

71 Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the
72 *CFTR* gene. CFTR functions as an ion channel to regulate chloride and bicarbonate
73 transport and water volume on epithelial surfaces (25). In CF, reduced CFTR function in
74 the epithelia of mucin-producing organs leads to the accumulation of viscous mucus,
75 which promotes obstruction, infection and inflammation (12). Although the main cause of
76 death in CF is lung disease (25), metabolic and gastrointestinal manifestations are
77 becoming more frequent due to increased life expectancy thanks to improved treatment of
78 pulmonary complications. The most prominent metabolic complication is CF-related
79 diabetes mellitus (CFRD), affecting one third of patients (16). The CF gastrointestinal
80 phenotype is characterized by obstruction, microbial dysbiosis and inflammation (21).
81 Gastrointestinal complications include meconium ileus in the first days of life, as well as
82 malnutrition in infancy. Exocrine pancreatic insufficiency and various degrees of CF-
83 related liver disease (CFLD) mostly ensue during childhood. As patients age, abdominal
84 pain, constipation and the more severe distal intestinal obstruction syndrome (DIOS)
85 further decrease their quality of life (25). Impairment of gut health affects numerous
86 processes in the body (34). In CF, intestinal dysbiosis and subsequent chronic low-grade
87 inflammation are linked to gastrointestinal malignancies, CFLD, CFRD, osteoporosis, and
88 increased cardiovascular risk (19). Improving gut health in CF may thus improve several
89 complications of this multiorgan disease.

90 The gastrointestinal phenotype of CF is further characterized by increased fecal loss of
91 bile acids (BA) in both patients (24) and CF mouse models (3, 4, 6, 11, 36). BAs are
92 synthesized by the liver and secreted into the duodenum, where they aid in fat absorption.
93 Under physiological conditions, ~95% of secreted BAs are reabsorbed by the small
94 intestine, mostly via the apical sodium-dependent bile acid transporter (ASBT, SLC10A2),
95 to be returned to the liver and thereby complete the enterohepatic circulation (18). In CF,

96 intestinal reabsorption of BAs is impaired, resulting in increased fecal BA loss (3, 4, 6, 11,
97 24, 36). Besides their role in fat absorption, BAs exert important metabolic effects, mainly
98 via the BA-sensing farnesoid X receptor (FXR) and its target fibroblast growth factor 19
99 (FGF19 in humans, FGF15 in mice) (18). Upon reabsorption, BAs activate FXR in ileal
100 enterocytes, resulting in FGF15/19 production. FGF19 travels to the liver via portal blood
101 to exert negative feedback on BA synthesis (18). In CF, BA malabsorption and possibly
102 other mechanisms result in defective FXR-FGF19 signaling, as suggested by reduced ileal
103 *Fgf15* mRNA levels in mice (8) and reduced serum FGF19 in patients (28). In patients,
104 reduced FGF15/19 levels are associated with high fasting plasma glucose and type 2
105 diabetes (10). In lean mice, *Fgf15* deficiency resulted in glucose intolerance and
106 diminished hepatic glycogen storage (17). Additionally, FGF19 administration protects
107 against sclerosing cholangitis (38) and steatosis (39), lesions similar to those observed in
108 CFLD. Impaired FXR-FGF19 signaling may therefore be implicated in the development
109 and/or progression of CF complications such as CFLD and CFRD. Thus, restoring BA
110 homeostasis in CF is an attractive avenue to improve CF complications.

111 The mechanism underlying BA malabsorption in CF is unclear, however two
112 hypotheses prevail. Firstly, the thickened intestinal mucus layer could impair the
113 translocation of BAs from the lumen to the epithelium for their reabsorption. Secondly,
114 intestinal dysbiosis could promote bacterial BA deconjugation and thereby decrease BA
115 reabsorption, as ASBT preferentially transports conjugated rather than deconjugated BAs
116 (13). Moreover, CF-mediated changes in ASBT expression or functionality could be
117 involved. Some of the factors mentioned in these hypotheses were improved in CF mice
118 upon treatment with the osmotic laxative polyethylene glycol (PEG) (22). PEG is routinely
119 administered to mice lacking *Cftr* expression to prevent development of lethal intestinal
120 obstruction (7). PEG decreased mucus accumulation in the small intestine, intestinal
121 bacterial load, and the expression of certain inflammatory genes (22). We therefore

122 hypothesized that PEG treatment could improve the reabsorption of BAs in CF. In this
123 study, we aimed to determine the effect of PEG treatment on BA malabsorption and FXR
124 signaling in CF mice. Our results indicate that indeed PEG treatment is associated with
125 decreased fecal BA loss, as well as increased FXR-FGF15 signaling.
126

127 **METHODS**

128

129 *Animals*

130 Male *Cftr*^{-/-} (*Cftr*^{tm1UNC} on a >99% C57BL/6 background, CF) mice (n=15) and wild-type
131 (WT) littermates (n=15) aged 8-20 weeks obtained from an in-house breeding colony were
132 housed individually under conventional (non-specific pathogen-free) housing conditions in
133 a light- and temperature-controlled facility (12-hour light-dark cycles, 21°C) with *ad libitum*
134 access to water and food. Two diets were used to account for outcome dependency on
135 dietary factors. The mice received either chow [RM3 (E) FG, Special Diet Services,
136 England; composition by proximate analysis: fat 4.3% (cholesterol 0.05%), protein 22.4%,
137 fiber 4.2% (of which 25% cellulose, 57% hemicellulose, 9% pectin, and 9% lignin),
138 nitrogen-free extract 51.2%), or a semisynthetic diet (No. 4063.02, AB diets, The
139 Netherlands; composition: fat 5.2% (cholesterol 0.01%), protein 17.3%, fiber (100%
140 cellulose) 10.5%, nitrogen-free extract 55.7%]. Animal experiments were approved by the
141 Ethics Committee for Animal Experiments of the University of Groningen. All experiments
142 were performed in accordance with relevant guidelines and regulations (including
143 laboratory and biosafety regulations).

144

145 *Experimental procedures*

146 PEG (polyethylene glycol 4000 with electrolytes, Ipsen Farmaceutica, The Netherlands,
147 containing, in g/l: 32 PEG 4000, 0.73 NaCl, 0.375 KCl, 0.84 NaHCO₃, and 2.85 Na₂SO₄,
148 tot. 37g/l) was administered via drinking water in decreasing concentrations. All mice,
149 irrespective of their genotype, were administered PEG (37 g/l water) since weaning to
150 prevent the intestinal obstruction often observed in these CF mice (7). On day 0, PEG
151 dosage was decreased by 50% (18.5 g/l water) to determine the PEG-dependency of CF
152 mice. On day 7, PEG treatment was stopped for three days until termination. Fecal pellets
153 were collected over a 24-hour period before decreasing PEG dosage (day 0, 100% PEG)

154 and daily from day 8 to 10 (0% PEG). This procedure was followed for both groups, the
 155 one receiving chow (CF n=5, WT n=4) and the other receiving semisynthetic diet (CF n=3,
 156 WT n=5). Additionally, a separate group of mice (CF n=7, WT n=6) fed semisynthetic diet
 157 was administered PEG at full dosage (37 g/L water) until termination and was included for
 158 ileal gene expression only. Mice were anesthetized with isoflurane and euthanized by
 159 cervical dislocation. Terminal blood samples were collected in EDTA-coated tubes.
 160 Tissues were collected and immediately frozen in liquid nitrogen.

161

162 *Analytical methods*

163 *Neutral sterol (NS) and bile acid (BA) analyses.* NS and BAs were extracted and
 164 measured by gas chromatography (GC) as previously described (32). Total amounts were
 165 calculated as the sum of the individual species. BA species included: α -muricholic acid, β -
 166 muricholic acid, chenodeoxycholic acid, cholic acid, deoxycholic acid, hyodeoxycholic acid,
 167 ω -muricholic acid and ursodeoxycholic acid. NS species included: cholesterol, coprostanol
 168 and dihydrocholesterol.

169 *Gene expression analysis.* The small intestine was divided into three segments of equal
 170 length. Total RNA was isolated from mid-sections of the most distal of the three segments
 171 (ileum) with TRI-Reagent (Sigma, St. Louis, MO, USA) and quantified by NanoDrop
 172 (NanoDrop Technologies, Wilmington, DE, USA). Primers were designed using Primer-
 173 BLAST and optimized for use with Hi-ROX SensiMixTM SYBR Green master mix (Bioline,
 174 Taunton, MA, USA). Primers used are listed in **Table 1**. Real-time qPCR analyses were
 175 performed on a StepOnePlusTM Real-Time PCR system (Applied Biosystems, Foster City,
 176 CA, USA). Gene expression levels were normalized to 36B4 (*Rplp0*).

Gene	Forward primer 5'---3'	Reverse primer 3'---5'
<i>Fgf15</i>	GCC ATC AAG GAC GTC AGC A	CTT CCT CCG AGT AGC GAA TCA G
<i>Shp</i>	AAG GGC ACG ATC CTC TTC AA	CTG TTG CAG GTG TGC GAT GT

<i>Asbt</i>	ACC ACT TGC TCC ACA CTG CTT	CCC GAG TCA ACC CAC ATC TT
<i>Gata4</i>	GAG ATG CGC CCC ATC AAG	GAC ACA GTA CTG AAT GTC TGG GAC AT
<i>Rplp0</i>	CTG TTG GCC AAT AAG GTG CC	GGA GGT CTT CTC GGG TCC TA

177 **Table 1** - qPCR primer sequences used in this study.

178

179 *Statistical analyses.* GraphPad Prism v6.0 for Macintosh (GraphPad Software, La Jolla,
180 CA, USA) was used for data analyses. We analyzed data using a mixed-model ANOVA
181 with genotype as between-subjects factor, and PEG treatment as within-subjects factor
182 using SPSS v25.0 for Windows IBM SPSS Statistics for Windows, Version 25.0 (IBM,
183 Armonk, NY). Statistical differences were subsequently tested using the Student's T-test
184 for unpaired data and the paired T-test for paired data. For correlation analyses,
185 Spearman's rank correlation coefficient was used. Alpha was set at 0.05. In figures 1-4,
186 data concerning 100% PEG dosage refers to 24-hour feces collected on day 0. Data
187 concerning 0% PEG dosage represents the average of 24-hour feces collected on days 8,
188 9 and 10.

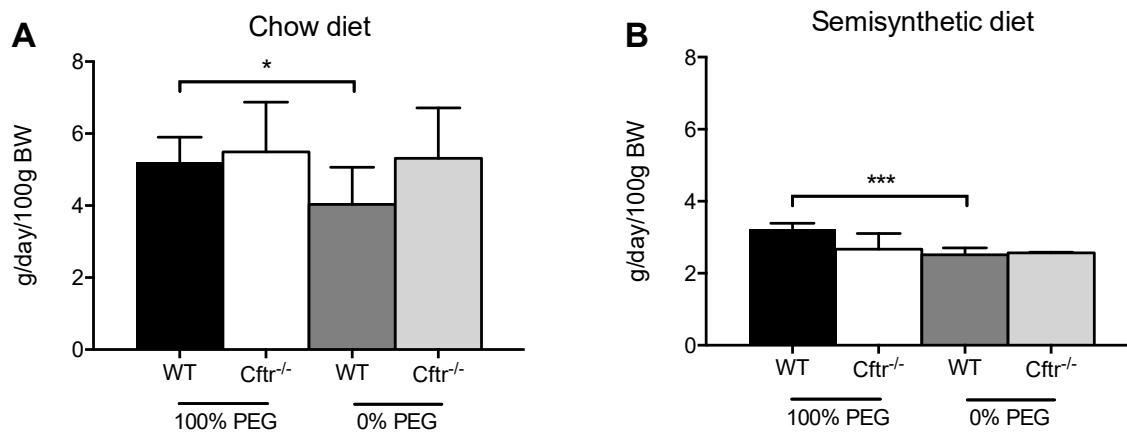
189

190 **RESULTS**

191

192 **PEG treatment ameliorates bile acid malabsorption in CF mice**

193 To investigate the effect of PEG on BA malabsorption in CF mice, PEG was reduced
194 stepwise until complete withdrawal. All mice survived without signs of bowel obstruction or
195 overt diarrhea. The body weight of CF mice tended to be lower than that of WT, however
196 statistical significance was not reached (data not shown). The fecal output was higher in
197 mice fed chow compared to mice fed the semisynthetic diet (**Fig. 1A vs. 1B**), despite
198 similar food intake (data not shown). PEG withdrawal decreased the fecal output in WT
199 mice on either diet (**Fig. 1A,B**), but not in CF mice.



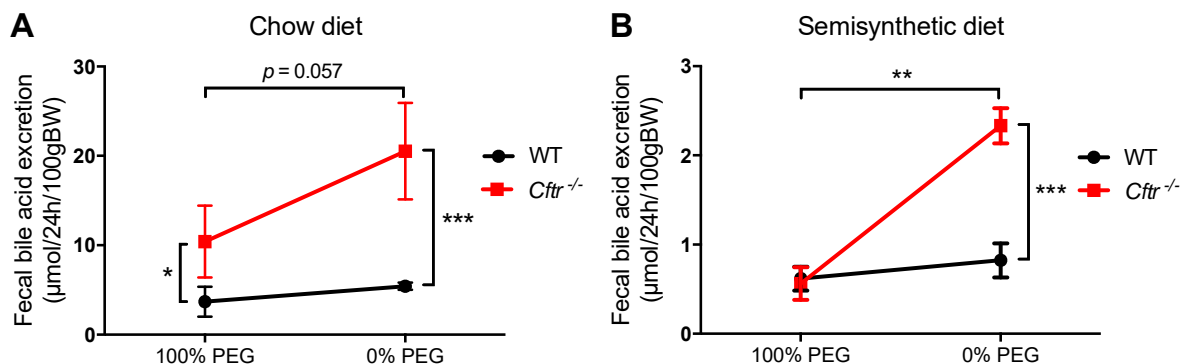
200

201 **Figure 1.** Effect of PEG on fecal output in WT and CF mice maintained on (A) chow and
202 (B) semisynthetic diet. Data refers to dry fecal weight and was normalized to body weight.
203 Data are presented as mean±SD, n=3-5. Data of WT mice was compared with that of CF
204 mice by Student's T test. Within-individual mouse changes in fecal output with 100% or 0%
205 PEG treatments were compared by paired T test. PEG: polyethylene glycol.

206

207 PEG withdrawal increased fecal BA excretion by two-fold in CF mice receiving a chow
208 diet (**Fig. 2A**). In contrast, PEG withdrawal exerted little effect on the fecal BA excretion in
209 WT mice (**Fig. 2A**).

210 In CF mice, there is high variability in the absolute amount of fecal BAs observed in
 211 previous studies (3, 4, 6, 11, 36), which might be related to the diet, genetic background or
 212 environmental factors. In a previous study, fecal BA excretion was lower in rats fed a
 213 semisynthetic diet compared to chow (14). To investigate dependency of the outcome on
 214 diet, we also performed the same experiment with a semisynthetic diet, which has a
 215 different fiber content and composition. Compared to the groups maintained on chow,
 216 mice receiving semisynthetic diet showed a 5-to-10-fold lower fecal excretion of BAs (**Fig.**
 217 **2A vs. 2B**). With PEG, fecal BA excretion was similar between CF and WT mice on a
 218 semisynthetic diet (**Fig. 2B**), whereas in those fed chow this was different between the
 219 genotypes (**Fig. 2A**). In CF mice fed a semisynthetic diet, PEG withdrawal increased fecal
 220 BA excretion by about 4-fold (**Fig. 2B**). As observed on chow, PEG did not affect fecal BA
 221 excretion in WT mice (**Fig. 2B**). These findings indicate that PEG improves BA
 222 malabsorption in CF mice, on either diet.

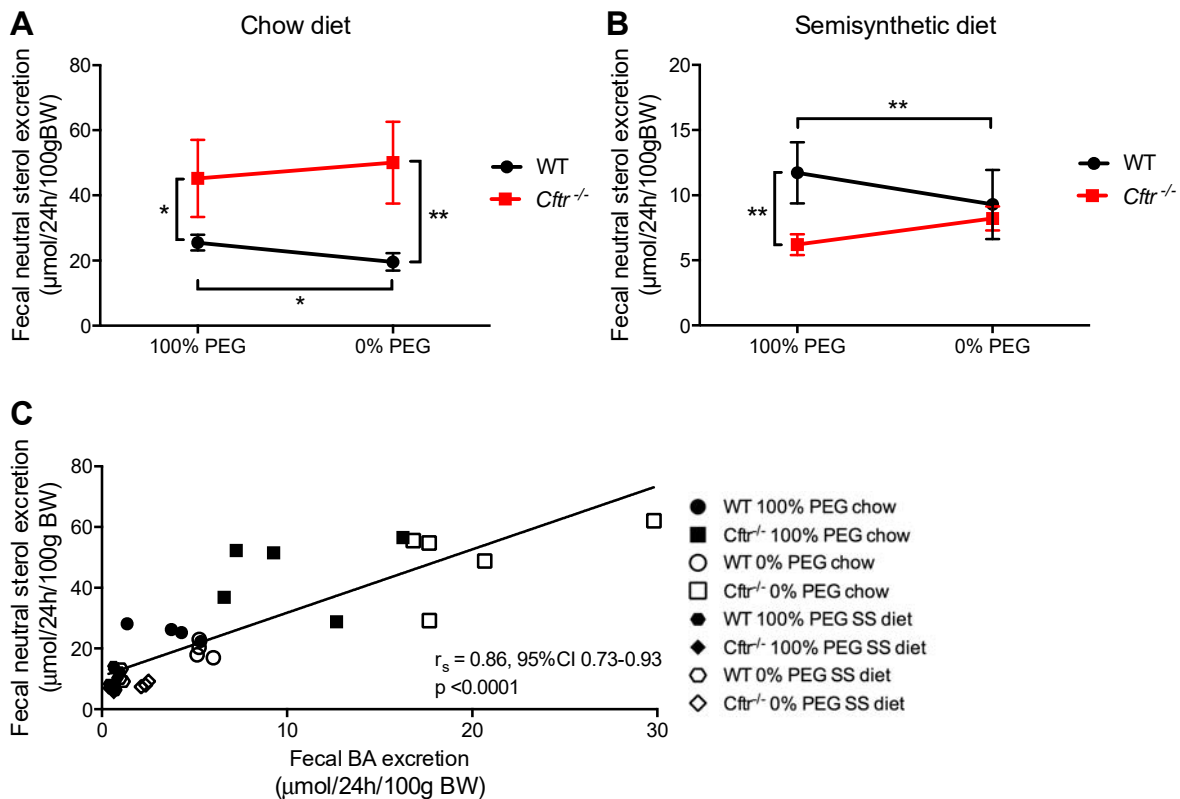


223
 224 **Figure 2.** Effect of PEG on fecal BA excretion in WT and CF mice maintained on (A) chow
 225 and (B) semisynthetic diet. Fecal BA excretion was determined by gas chromatography
 226 and normalized to body weight. Data are presented as mean±SD, n=3-5. Data of WT mice
 227 was compared with that of CF mice by Student's T test. Potential changes in fecal BA
 228 excretion in individual animals, as a result of PEG withdrawal, were assessed by a paired
 229 T test.

230

231 PEG treatment does not affect fecal neutral sterol excretion

232 Since BAs are essential for intestinal absorption of fat, including cholesterol, fecal
233 neutral sterol (NS) excretion was determined (**Fig. 3**). This was lower in mice receiving
234 semisynthetic diet as compared to chow (**Fig. 3A vs. 3B**). In WT mice on either diet, PEG
235 withdrawal was associated with a decrease in fecal NS excretion (**Fig. 3A,B**). Fecal NS
236 excretion was higher in CF as compared to WT mice fed chow, independent of PEG
237 treatment (**Fig. 3A**). Upon semisynthetic diet, fecal NS excretion was similar between CF
238 and WT mice and was unaffected by PEG in CF mice (**Fig. 3B**). We found a positive
239 relationship between fecal BA and NS excretion (**Fig. 3C**). Interestingly, coprostanol, a
240 cholesterol metabolite formed by intestinal microbial conversion, was only found in 1 out of
241 8 mice fed a semisynthetic diet, whereas it was found in all mice of either genotype fed
242 chow (data not shown).



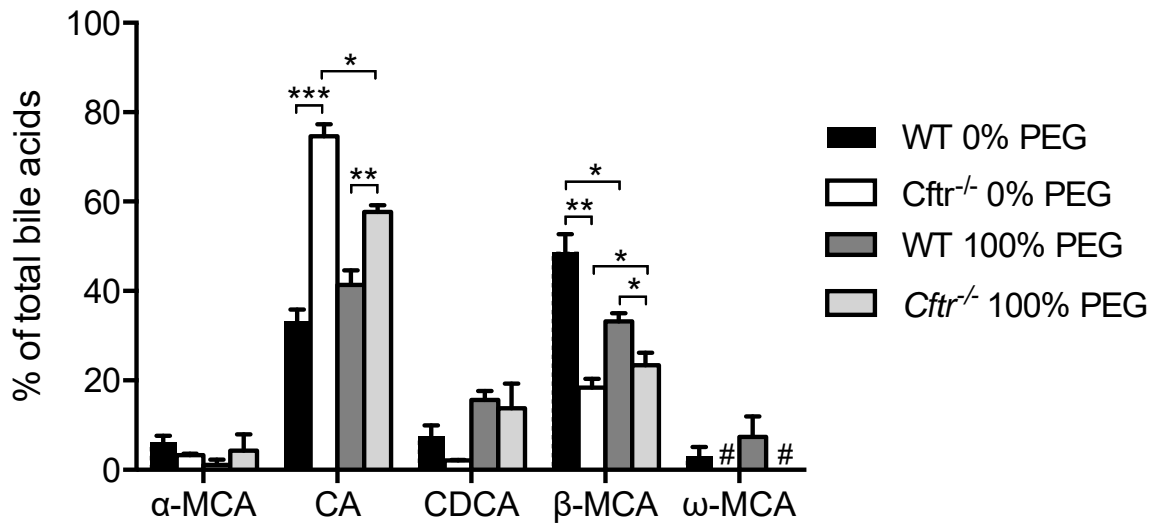
243

244 **Figure 3.** Effect of PEG and diet on fecal neutral sterol (NS) excretion in WT and CF mice
245 maintained on (A) chow and (B) semisynthetic diet. Fecal NS excretion was determined by
246 gas chromatography and normalized to body weight. Data is presented as mean \pm SD, n=3-
247 5. Data of WT mice was compared with that of CF mice by Student's T test. Within-
248 individual mouse changes in fecal NS excretion while receiving 100% or 0% PEG
249 treatment were compared by paired T test. (C) Correlation plot between fecal NS excretion
250 and fecal BA excretion, including data from Fig. 2A,B and Fig. 3A,B. For correlation
251 analyses, Spearman's rank correlation coefficient was used. PEG, polyethylene glycol.

252

253 **PEG treatment partly normalizes the fecal BA composition in CF mice**

254 The fecal BA composition is altered in CF patients and mice, in whom the contribution
255 of the primary BA cholic acid (CA) is high and that of deoxycholate (DCA) is generally low
256 (4, 33, 36). We also found that the contribution of CA to the fecal BA composition was
257 substantially higher in untreated CF as compared to WT mice (**Fig. 4**), and this difference
258 in CA contribution among the two genotypes was reduced by PEG treatment (**Fig. 4**). PEG
259 treatment decreased the CA contribution in CF mice (**Fig. 4**). The contribution of the
260 primary BA chenodeoxycholic acid (CDCA), a potent FXR activator, to the fecal BA
261 composition, tended to be lower in untreated CF as compared to WT mice, and tended to
262 be increased by PEG treatment in CF mice (**Fig. 4**). The contribution of β -muricholic acid
263 (β -MCA) to the fecal BA composition was decreased in untreated CF as compared to WT
264 mice, and was increased by PEG in CF mice (**Fig. 4**). Together, these findings indicate
265 that PEG partially restored imbalances in the fecal BA composition in CF mice. In contrast
266 with previous studies in CF and WT mice fed a liquid diet (4, 36), no fecal deoxycholic acid
267 (DCA) was detected.



268

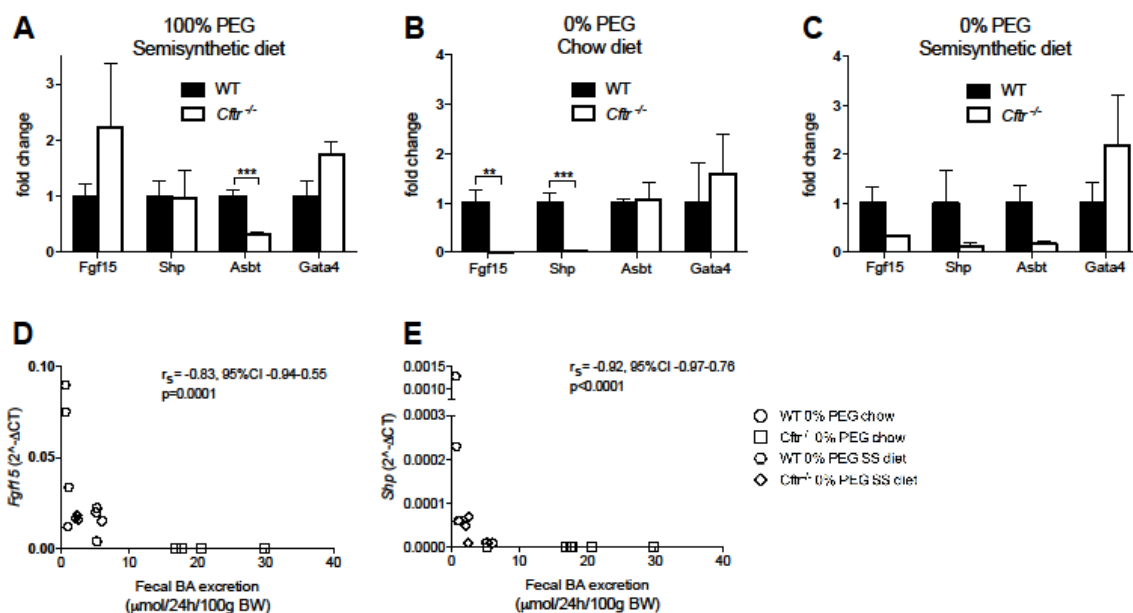
269 **Figure 4.** Effect of PEG on the fecal BA composition in mice fed semisynthetic diet. Data
 270 is shown as percentages of total fecal bile acids. Individual BA species were detected by
 271 gas chromatography. Bile acid species include: α-MCA, α-muricholic acid; CA, cholic acid;
 272 CDCA, chenodeoxycholic acid; β-MCA, β-muricholic acid; ω-MCA, ω-muricholic acid. n=3-
 273 5. Data of WT mice was compared with that of CF mice by Student's T test. Within-
 274 individual mouse changes in fecal BA composition while receiving 100% or 0% PEG
 275 treatment were compared by paired T test. PEG, polyethylene glycol.

276

277 **PEG treatment restores FXR-FGF15 signaling in CF mice**

278 To investigate the effect of decreased fecal BA excretion on FXR signaling, we
 279 measured ileal gene expression levels of its downstream targets, *Fgf15* and small
 280 heterodimer partner (*Shp*, *NR0B2*) in the ileum, where BA reabsorption is most
 281 pronounced. With PEG treatment, *Fgf15* and *Shp* mRNA levels were similar between CF
 282 and WT mice fed a semisynthetic diet (**Fig. 5A**). In contrast, after PEG withdrawal, both
 283 *Fgf15* and *Shp* expression were suppressed in CF compared to WT mice. This
 284 suppression was stronger in mice receiving chow (**Fig. 5B,C**). In WT mice, PEG treatment
 285 did not affect *Fgf15* or *Shp* gene expression. We found a strong inverse correlation

286 between fecal BA excretion and *Fgf15* expression and between fecal BA excretion and
 287 *Shp* expression, indicating that increased fecal BA excretion was associated with lower
 288 gene expression of the FXR target genes *Fgf15* and *Shp* (Fig. 5D,E). No correlation was
 289 observed between CDCA levels and *Fgf15* gene expression (data not shown).
 290 Interestingly, PEG had no major effect on the expression of the main intestinal BA
 291 transporter, *Asbt*. However, without PEG treatment, its expression tended to be lower in
 292 CF mice fed semisynthetic diet as compared to WT mice (Fig. 5A,C). The transcription
 293 factor *Gata4*, known to repress expression of *Asbt* (27), was unchanged in CF as
 294 compared to WT mice on both diets (Fig. 5A-C). Accordingly, we found no correlation
 295 between *Asbt* and *Gata4* gene expression (data not shown). Additionally, no correlation
 296 was found between *Asbt* and *Shp* (data not shown). Together, these findings indicate that
 297 improvement of BA malabsorption in CF mice by PEG treatment is associated with
 298 restored FXR-FGF15 signaling independent of *Asbt* expression.



299
 300 **Figure 5.** Effect of PEG on ileal gene expression in WT and CF mice (A) on 100% PEG
 301 treatment with semisynthetic diet, n=3-5 (B) on 0% PEG with chow, n=4-5 and (C) on
 302 100% PEG with semisynthetic diet, n=6-7. Primers used are listed in Table 1. Data are

303 normalized to the housekeeping gene *Rplp0* (36B4) and are expressed relative to WT
304 values. Data are shown as mean \pm SE. (D) Correlation plot between fecal BA excretion
305 and *Fgf15* and (E) Correlation plot between fecal BA excretion and *Shp*. For correlation
306 analyses, Spearman's rank correlation coefficient was used. PEG, polyethylene glycol;
307 *Fgf15*, fibroblast growth-factor 15; *Shp*, small heterodimer partner; *Asbt*, apical sodium-
308 dependent bile acid transporter; *Gata4*, GATA-binding factor 4.
309

310 **DISCUSSION**

311 In this study we show that PEG treatment completely prevented BA malabsorption in
312 CF mice fed a semisynthetic diet, whereas this was partially prevented on a chow diet. In
313 concomitance with improved BA absorption, FXR-FGF15 signaling was restored in CF
314 mice fed a semi-synthetic diet by PEG treatment.

315 There are several mechanisms that can explain the decrease in fecal BA loss by PEG
316 treatment. In CF, mucins remain abnormally aggregated, adhere strongly and accumulate
317 on the epithelium (30). Such a thickened mucus layer could impair BA reabsorption by
318 acting as a poorly penetrable barrier. PEG has previously been shown to reduce mucus
319 accumulation in the intestine of CF mice (22) and could have therefore facilitated BA
320 reabsorption in our study. Decreased intestinal transit time was proposed as underlying
321 mechanism (22). We, however, did not assess the effect of PEG on mucus accumulation
322 in intestinal crypts in the current study.

323 Decreased ASBT-mediated BA reuptake in CF could also be responsible for BA
324 malabsorption. This, however, was not supported by our data. Previous studies have
325 shown changes in *Asbt* expression in CF mouse models, either decreased or increased
326 expression (2, 8, 20). In the current study, expression tended to be lower in CF mice upon
327 semisynthetic diet and was unchanged upon a chow diet, suggesting that dietary factors
328 may influence *Asbt* expression. Intestinal FXR activation has been shown to inhibit *Asbt*
329 expression via *Shp* (23). However, here, as well as in a previous study (8), *Asbt*
330 expression in CF mice tended to be reduced concomitantly with reduced *Shp*, suggesting
331 that the regulation of *Asbt* expression by FXR-SHP may not be pivotal in CF. *Asbt*
332 expression is also affected by gut microbiota, which represses expression via the
333 transcription factor *Gata4* (26). We found no correlation between *Asbt* and *Gata4*
334 expression. These findings suggest that other factors besides FXR and GATA4 regulate
335 *Asbt* expression in CF. Whereas PEG treatment decreased fecal BA loss and restored

336 FXR-FGF15 signaling in CF mice, the ileal expression of *Asbt* was still decreased upon
337 PEG treatment, indicating that the effects of PEG on BA homeostasis were not mediated
338 by changes in *Asbt* expression. We cannot exclude, however, that ASBT protein function
339 is compromised in CF and partially restored by PEG.

340

341 Impaired FXR-FGF15 signaling in untreated CF mice is reflected in the fecal BA
342 composition, where an increased contribution of CA observed by us and others (4, 33, 36)
343 reflects increased hepatic BA synthesis, likely due to lack of inhibition by FGF15 signaling.
344 PEG treatment was associated with restoration of FXR-FGF15 signaling in CF mice. Our
345 finding that PEG reduced the contribution of CA to the fecal BA pool in CF mice could
346 reflect the increased FXR-FGF15 signaling observed upon PEG treatment. The strong
347 correlation between fecal BA excretion and *Fgf15* and *Shp* expression suggests that FXR-
348 FGF15 signaling was restored by improved BA reabsorption.

349 PEG could also have affected FXR-FGF15 signaling in CF by affecting the gut microbial
350 composition (37). Microbiota-induced changes in the BA pool composition can modulate
351 FXR stimulation, as microbiota-dependent BAs such as the secondary BA deoxycholic
352 acid (DCA) are FXR agonists (31). Small intestinal bacterial overgrowth (SIBO) has been
353 reported in CF mice fed a liquid diet (22), therefore increased BA deconjugation could be
354 expected. Since ASBT preferentially transports conjugated rather than deconjugated BAs
355 (13), greater fecal BA loss could be expected in CF mice with SIBO. PEG was shown to
356 decrease SIBO in CF mice (22) and to decrease secondary BAs such as DCA in WT rats
357 (37). Although in previous studies DCA was found in small amounts in the feces of WT and
358 CF mice (4, 5), we could not detect any DCA or coprostanol (both microbial metabolites)
359 upon semisynthetic diet, suggesting that the catabolic activity of the gut microbiota was
360 decreased. This could be due to the fact that, although the semisynthetic diet contains
361 cellulose, refined cellulose is digested poorly by the microbiota compared to cellulose

362 derived from dietary fiber, at least in humans (32). Furthermore, no correlation between
363 fecal CDCA levels and Fgf15 gene expression was found, suggesting that the changes in
364 FXR activation were not due to increased activation by CDCA. Together, these findings
365 suggest that restoration of FXR-FGF15 signaling in CF mice occurred as a consequence
366 of improved BA reabsorption upon PEG treatment, rather than microbiota-dependent
367 changes in the BA composition that could have heightened FXR stimulation.

368

369 In line with previous observations (14), we found that fecal BA excretion in both
370 genotypes was up to 10-fold higher in mice receiving chow as compared to a
371 semisynthetic diet. The macronutrient composition, including fat, was similar across the
372 two diets used, although more simple rather than complex carbohydrates were found in
373 the semisynthetic diet. The fiber content and composition, however, differed greatly. By
374 proximate analysis, the semisynthetic diet contained 10.5% of fiber, consisting exclusively
375 of cellulose. Chow contained 4.2% of fiber, composed of cellulose (25%), hemicellulose
376 (57%), pectin (9%) and lignin (9%). *In vitro* binding of BAs by dietary fiber has been
377 demonstrated. Cellulose, the sole fiber in the semisynthetic diet, does not bind BAs,
378 whereas other fibers such as pectin and lignin do, to varying extents (35). Therefore, the
379 higher fecal BA excretion observed in chow-fed mice could be due to the presence of BA-
380 binding fibers such as pectin and lignin in chow. Whereas we found an up to 10-fold
381 increase in fecal BA excretion upon chow compared to semisynthetic diet, other studies
382 reported 2-to-5-fold increases in fecal labelled cholate excretion upon chow compared to
383 semisynthetic diet (14, 29). Besides the lack of BA-binding fiber, another mechanism that
384 could contribute to the decreased fecal BA excretion upon semisynthetic diet compared to
385 chow is a decrease in the microbial catabolic activity in the intestine upon feeding a
386 semisynthetic diet. Our data show that upon semisynthetic diet there was a decrease in

387 coprostanol and complete lack of the secondary bile acid deoxycholic acid, suggesting that
388 the microbial catabolic activity was decreased.

389 Compared to semisynthetic diet, besides increased fecal loss of BAs upon chow, we
390 also observed increased loss of fecal NS upon chow. This could be due to the higher
391 cholesterol content in chow (0.05%) compared to semisynthetic diet (0.01%), to decreased
392 cholesterol absorption upon chow due to increased fecal BA loss, or to binding of
393 cholesterol by dietary fiber along with BAs. As for binding of BAs, binding of cholesterol by
394 cellulose was reported as negligible (15). The strong correlation between fecal BA and NS
395 excretion could reflect all mechanisms. However, since in CF mice PEG treatment did not
396 affect fecal NS to the extent it affected fecal BA excretion, this suggest that the effect of
397 cholesterol binding by dietary fiber and difference in cholesterol content in the diet
398 contributes more to this correlation.

399

400 Our study shows that, in CF mice, the osmotic laxative PEG is associated with
401 decreased BA malabsorption and restoration of FXR-FGF15 signaling, independently from
402 *Asbt* expression. PEG is the most commonly prescribed and most effective osmotic
403 laxative for constipation (1) and, as constipation is common in CF and its incidence
404 increases with age (9), CF patients are already frequently prescribed PEG. PEG is virtually
405 free of important side effects at standard dosage (27). Besides its indication for
406 constipation in CF, based on the evidence provided in CF mice so far, PEG could also be
407 useful for reducing SIBO and the consequences of gut dysbiosis and inflammation in CF
408 (22). Our study shows that FXR-FGF15 signaling can be restored by PEG in CF. Given the
409 metabolic implications of FXR-FGF19/15 signaling, it remains to be established whether
410 this could improve CF-related complications such as cystic fibrosis-related diabetes
411 (CFRD) and cystic fibrosis-related liver disease (CFLD).

412

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420

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