Metabolic cages

Indirect calorimetry and activity measurements were performed with a Comprehensive Laboratory Animal Monitoring system (CLAMS) open-circuit Oxymax (Columbus Instruments). Mice were individually housed in cages with a known O_2 concentration and flow rate. After 1 day of acclimation, measurements were performed for 4 days.

Body composition

Body composition was assessed using time domain NMR (Minispec Analyst AD; Bruker Optics).

Pair-feeding experiment

All mice were housed individually and provided with normal chow in a food dispenser. Pre-weighed food was replaced daily, and food intake measurements of Cyp8b1$^{-/-}$ mice were determined by weighing the food dispenser every 24h. A pairfed group of wild-type (WT) mice was given the same daily amount of diet consumed by Cyp8b1$^{-/-}$ mice. One-third of the total diet was given to the paired group at 8 AM and the remaining two-thirds at 6 PM daily.

Lipid extraction and analysis of FAEs and MAGs

Lipid extraction and analysis were performed as previously described [1, 2]. Frozen tissue of mucosal intestine and mediobasal hypothalamus was blade-homogenized in 1.0 mL of methanol solution containing the internal standards, [$^2H_5$] 2-arachidonylglycerol (2-AG), [$^2H_4$]-AEA, [$^2H_4$]-OEA (Cayman Chemical, Ann Arbor, MI, USA). Lipids were extracted with chloroform (2 mL) and washed with water (1 mL). Organic (lower) phases were collected and separated by open-bed silica gel column chromatography as previously described [3]. Eluate was gently dried under

Higuchi S, *et al. Gut* 2020; 69:1620–1628. doi: 10.1136/gutjnl-2019-319693

25 N_2 stream (99.998% pure) and resuspended in 0.2 mL of methanol:chloroform (9:1), with 1 μL
26 injection for ultra-performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS)
27 analysis.

28 Data acquisition was performed using an Acquity I Class UPLC with in-line connection to
29 a Xevo TQ-S Micro Triple Quadrapole Mass Spectrometer (Waters Corporation, Milford, MA,
30 USA) and accompanying electrospray ionization (ESI) sample delivery. Lipids were separated
31 using an Acquity UPLC BEH C_{18} column (2.1 x 50 mm i.d., 1.7 μm, Waters) and inline guard
32 column (UPLC BEH C_{18} VanGuard PreColumn; 2.1 x 5 mm i.d.; 1.7 μm, Waters). Lipids were
33 eluted by a gradient of water and methanol (containing 0.25% acetic acid, 5 mM ammonium
34 acetate) at a flow rate of 0.4 mL/min and gradient: 80% methanol 0.5 min, 80% to 100% methanol
35 0.5 to 2.5 mins, 100% methanol 2.5 to 3.0 mins, 100% to 80% methanol 3.0 to 3.1 mins, and 80%
36 methanol 3.1 to 4.5 mins. Column was maintained at 40°C and samples were kept at 10°C in
37 sample manager. MS detection was in positive ion mode with capillary voltage maintained at 1.10
38 kV and Argon (99.998%) was used as collision gas. Cone voltages and collision energies for
39 respective analytes: 2-AG = 30v, 12v; DHG = 34v, 14v; AEA = 30v, 14v; OEA = 28v, 16v; DHEA
40 = 30v, 16v; [$^2H_5$]-2-AG = 25v, 44v; [$^2H_4$]-AEA = 26v, 16v; [$^2H_4$]-OEA = 48v, 14v. Lipids were
41 quantified using a stable isotope dilution method detecting proton or sodium adducts of the
42 molecular ions $[M + H/Na]^+$ in multiple reaction monitoring (MRM) mode. For many MAGs, acyl
43 migration from sn-2 to sn-1 glycerol positions is known to occur; for these analytes, the sum of
44 these isoforms is presented. Tissue processing and LCMS analyses for experiments occurred
45 independently of other experiments. Extracted ion chromatograms for MRM transitions were used
46 to quantify analytes: 2-AG (*m/z* = 379.3 > 287.3), 2-DG (*m/z* = 403.3 > 311.2), AEA (*m/z* = 348.3
47 > 62.0), OEA (*m/z* = 326.4 > 62.1), DHEA, (*m/z* = 372.3 > 62.0), 19:2 MAG (*m/z* = 386.4 > 277.2),
48 with [$^2H_5$]-2-AG (*m/z* = 384.3 > 93.4), [$^2H_4$]-AEA (*m/z* = 352.4 > 66.1), and [$^2H_4$]-OEA (*m/z* = 330.4
49 > 66.0) as internal standards. Controls included one "blank" sample that was processed and

Higuchi S, *et al. Gut* 2020; 69:1620–1628. doi: 10.1136/gutjnl-2019-319693

50 analyzed in the same manner as all samples, except no tissue was included. This control revealed
51 no detectable endocannabinoids and related lipids included in our analysis.
52

53 **Behavioral analysis**

54 Animals were placed into feeding chambers 5 days prior to recording for acclimation. We
55 gave Research diet (D12079B) for meal patterning experiment. Feeding behaviors were assessed
56 starting 1 h prior to dark cycle (1,700 h) over a 24 h period. Data were processed using TSE
57 Phenomaster software.
58

59 **Fecal lipid extraction and measurement**

60 Mice were housed individually for 1 or 3 days during ad libitum feeding. Feces for 1 or 3
61 days were collected from each cage. Collected feces were dried in an incubator for 16h at 42 ℃
62 and 100 mg of feces were homogenized with 1 ml of 1M NaCl. One ml of homogenized solution
63 was added into 6 ml of chloroform:methanol (2:1). Chloroform layers were collected after
64 centrifugation, then evaporated under N_2 flow until dry. One ml of 2% Triton X-100 in chloroform
65 was added and evaporated again under N_2 flow. One ml of ddH_2O was added and vortexed until
66 sample dissolved. Fecal free fatty acid (FFA) content was assessed using a colorimetric assay
67 (Wako; HR Series NEFA-HA(2)). We measured free fatty acids because they make up the vast
68 majority of fats in feces, whereas intact triglycerides make up less than 1% of the fat in the feces[4].
69

70 **Realtime PCR**

71 For gastrointestinal tissues, mucosa and the underlying muscle tissue were separated by
72 gently scraping the mucosa from the small intestine, cecum and large intestine. Tissue RNA was
73 extracted using Trizol (Thermo Fisher Scientific). The cDNA was synthesized from RNA using a

Higuchi S, *et al. Gut* 2020; 69:1620–1628. doi: 10.1136/gutjnl-2019-319693

74 High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative realtime PCR
75 was performed using Taqman reagents (Bio Rad) and b-actin was used as housekeeping gene
76 for normalization. The sequences of the sense and antisense primers used for amplification were
77 as follows: GPR119 forward, TCCAGAGAGGACCAGAGAAAGC; GPR119 reverse,
78 GCAGCGTCTTAGCCATCGA; *β-actin* forward, CCTGAGCGCAAGTACTCTGTGT; *β-actin*
79 reverse, GCTGATCCACATCTGCTGGAA; Pparα forward, TGGTGGACCTCCGGCA; Pparα
80 reverse, TCTTCTTGATGACCTGCACGA; Abca1 forward, AAAACCGCAGACATCCTTCAG;
81 Abca1 reverse, CATACCGAAACTCGTTCACCC; Acox1 forward,
82 CGATCCAGACTTCCAACATGAG; Acox1 reverse, CCATGGTGGCACTCTTCTTAACA; Apoc3
83 forward, GGCTGGATGGACAATCACTT; Apob reverse, GTGGCTTCTGTCACTGCTAAAG;
84 Col1a forward, GACGCCATCAAGGTCTACTG; Col1a reverse, ACGGGAATCCATCGGTCA;
85 Cpt1a forward, CACCAACGGGCTCATCTTCT; Cpt1a reverse,
86 CCTTCTATCGAATTTGCTCTGGTT; Dpt forward, GGTGGCTACGGGTACCCATA; Dpt reverse,
87 GTCAGAGCCTTCCTTCTTGC.
88
89 **Gastric emptying**

90 Liquid gastric emptying was assessed by using an acetaminophen absorption test [5].
91 Mice were fasted 5h and given a lipid meal (Ensure:Corn Oil (17:3); Ensure, 1.48 kcal/ml, 28.5%
92 kcal from fat, Abbot. Corn oil, Fisher Science) at 10 ml/kg by oral gavage, followed 1h later with
93 oral gavage of 1% acetaminophen solution (Sigma) at a dose of 100 mg/kg. Blood samples (50
94 μL) were collected 15-min after acetaminophen gavage. Plasma acetaminophen levels were
95 measured by using an enzymatic spectrophotometric assay (Sekisui Diagnostics). For gut
96 hormone receptor antagonist experiments, a single dose of the GLP-1 receptor antagonist
97 Exendin-9 (0.3 mg/kg, BACHEM) or the Y2 receptor antagonist BIIE0246 (2 mg/kg, Tocris) was
98 given by intraperitoneal injection, 30 min before lipid meal gavage [6, 7]. For Arvanil (Tocris)

Higuchi S, *et al. Gut* 2020; 69:1620–1628. doi: 10.1136/gutjnl-2019-319693

99 experiments, a single dose of 2.5 mg/Kg Arvanil [8] was injected intraperitoneally, 1h before lipid
100 meal gavage.

101 Solid gastric emptying was also measured following ingestion of normal chow diet. Mice
102 were fasted for 16-h with free access to water, then allowed access to chow diet for 1-h. Mice
103 were food-deprived again for 2-h prior to euthanasia. Food intake during the 1-h feeding period
104 was measured using a food dispenser. Food content in the stomach was measured at euthanasia.
105 Solid gastric emptying was calculated by the following formula: solid gastric emptying (%) = {1-
106 (food content in stomach/food intake)}x100. For CA (Sigma), chenodeoxycholic acid (CDCA)
107 (Sigma) and α-muricholic acid (α-MCA) (Cayman) treatment, mice were orally gavaged with 50
108 mg/kg of these BAs in 1.5% $NaHCO_3$ for 4 consecutive days at 6 PM.

109

110 **Surgery and Duodenal Infusion**

111 Three days before duodenal catheter implantation surgery, normal chow was removed
112 and replaced with a liquid diet (Ensure). Under isoflurane anesthesia, an infusion catheter (0.12”
113 I.D.x0.025” O.D. silastic tubing, Dow Corning) was inserted into the duodenum of mice. On the
114 test day (4 days after the surgery), mice were fasted for 5-h and then received 200 µl of olive oil
115 as bolus through the intestinal infusion cannula. Systemic blood samples were collected at 0, 5,
116 60 and 120-min after olive oil infusion for gut hormones measurement.

117

118 **Hormone assays**

119 One hundred µL of blood samples were collected and mixed with 1% DPP-4 inhibitor
120 (Milipore) and 5% aprotinin (Fisher) and kept on ice for hormone assay in plasma. Ileal mucosal
121 tissues were scraped and suspended in RIPA buffer containing 1% DPP-4 inhibitor and 5%

Higuchi S, *et al. Gut* 2020; 69:1620–1628. doi: 10.1136/gutjnl-2019-319693

122 aprotinin for hormone assay. Plasma and ileal GLP-1 were measured by using immunoassay
123 (total GLP-1 assay kit; Mesoscale Discovery). Plasma glucose-dependent insulinotropic
124 polypeptide (GIP), plasma and ileal PYY were measured by using the Milliplex gut hormone panel
125 (Millipore). Plasma ghrelin was measured by using immunoassay (Active Ghrelin ELISA, Kamiya
126 Biomedical Company). Plasma growth differentiation factor 15 (GDF15) was measure by using
127 immunoassay (Mouse/Rat GDF15 Quantikine ELISA Kit, R&D Systems).
128

129 **Tracer studies**

130 Mice were fasted during the daytime for 5-h then injected with poloxamer-407 (1 g/kg, i.p.).
131 A radiolabeled lipid preparation containing 1.25 μCi each of ^{3}H-2-oleoylglycerol and ^{14}C-oleic acid
132 (American Radiolabeled Chemicals) in 100 μl of olive oil was given to each mouse by oral gavage.
133 Blood samples were collected at 0, 1, 2, 4, 8, 12 and 24-h after gavage. Ionizing radiation in
134 plasma samples was analyzed using a scintillation counter (Tricarb 2910TR; PerkinElmer), to
135 determine lipid absorption.
136

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158

159 **Supplementary Figure Legends**

160 Supplementary Figure 1. Indirect calorimetry measurement. (A)Respiratory exchange ratio (RER),
161 (B) volume of oxygen consumed (VO_2), (C) heat production (D) and locomotor activity for 24h.
162 (n=5 for each group).

163

Higuchi S*, et al. Gut* 2020; 69:1620–1628. doi: 10.1136/gutjnl-2019-319693

164 Supplementary Figure 2. Jejunal Ppar-α and target gene expression after solid gastric
165 experiment. (A) gene expression in mucosal tissue, (B) gene expression in muscle tissue. (WT;
166 n=5, Cyp8b1$^{-/-}$; n=6).

167

168 Supplementary Figure 3. Meal patterning analysis. (A) meal size, (B) meal duration, (C) frequency
169 and (D) post meal interval. (WT; n=5, Cyp8b1$^{-/-}$; n=6). ** $p<0.01$ (Student's *t*-test).

170

171 Supplementary Figure 4. Fatty acid ethanolamides and cannabinoids in jejunal intestine after fat-
172 free diet intake. Effect of 16-h food deprivation (fast) and refeeding (refed; 30-min after gavage
173 with a liquified preparation of the fat-free diet) on the levels of OEA (A), AEA (B), DHEA (C), 2-
174 AG (D) and DHG (E) in jejunum (WT; n=7, Cyp8b1$^{-/-}$; n=8). * $p<0.05$, vs. WT mice (two-way
175 ANOVA).

176

177 Supplementary Figure 5. Effect of arvanil on liquid gastric emptying. Mice were fasted for 4-h,
178 then administered arvanil (2.5 mg/Kg, i.p.). One hour later, a fat-rich meal (Ensure:Corn Oil =
179 17:3) was given by oral gavage and 1-h later, acetaminophen (100 mg/kg) was administered by
180 oral gavage. (WT; n=12, Cyp8b1$^{-/-}$; n=13). * $p<0.05$ (one-way ANOVA).

181

182 Supplementary Figure 6. Effect of BIIE on defecation. Mice were fasted for 4.5-h, then given the
183 BIIE (2 mg/kg, i.p.). Thirty minutes later, a fat-rich meal (Ensure:Corn Oil = 17:3) was given by
184 oral gavage and 10-min later 5-THP (10 mg/Kg) was given intraperitoneally. Fecal pellets were
185 collected for 1-h. (n=5-6/each group). * $p<0.05$, ** $p<0.01$ (one-way ANOVA).

186

Higuchi S, *et al. Gut* 2020; 69:1620–1628. doi: 10.1136/gutjnl-2019-319693

187 Supplementary Figure 7. GLP-1 and PYY levels after intestinal infusion of olive oil. (A-B) Plasma
188 GLP-1 levels and area under the curve (AUC) after fat rich meal gavage. (C-D) Plasma PYY levels
189 and AUC after fat-rich meal gavage. Mice were fasted for 5-h, then mice were given the fat-rich
190 meal (Ensure:Corn Oil = 17:3) by oral gavage (n=7 for each group). $^{\$}p<0.05$ between Cyp8b1$^{-/-}$
191 and DKO (one-way ANOVA). (E) Systemic plasma GLP-1 and (F) Systemic plasma PYY levels
192 after olive oil infusion via chronic indwelling duodenal catheters. (n=8 for each group).

193

194 Supplementary Figure 8. Plasma hormone levels. (A) Plasma GIP levels and (B) area under the
195 curve (AUC). Mice were fasted for 5-h, then mice were given the fat-rich meal (Ensure:Corn Oil =
196 17:3) by oral gavage (n=7 for each group). (C) Plasma ghrelin and (D) GDF15 levels during the
197 solid-phase gastric emptying test. Mice were fasted overnight, then allowed access to chow diet
198 for 1-h, then food-deprived again for 2-h. Plasma samples were collected after 2-h food
199 deprivation and used for ghrelin and GDF15 assay. (WT; n=16, Cyp8b1$^{-/-}$; n=10, GPR119$^{-/-}$; n=19,
200 DKO; n=9).

201

202

Higuchi S, *et al. Gut* 2020; 69:1620–1628. doi: 10.1136/gutjnl-2019-319693