Original Article

MOLECULAR METABOLISM

Small intestinal taurochenodeoxycholic acid-FXR axis alters local nutrient-sensing glucoregulatory pathways in rats

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ABSTRACT

Objective: The mechanism of nutrient sensing in the upper small intestine (USI) and ileum that regulates glucose homeostasis remains elusive. Short-term high-fat (HF) feeding increases taurochenodeoxycholic acid (TCDCA; an agonist of farnesoid X receptor (FXR)) in the USI and ileum of rats, and the increase of TCDCA is prevented by transplantation of microbiota obtained from the USI of healthy donors into the USI of HF rats. However, whether changes of TCDCA-FXR axis in the USI and ileum alter nutrient sensing remains unknown.

Methods: Intravenous glucose tolerance test was performed in rats that received USI or ileal infusion of nutrients (i.e., oleic acids or glucose) via catheters placed toward the lumen of USI and/or ileum, while mechanistic gain- and loss-of-function studies targeting the TCDCA-FXR axis or bile salt hydrolase activity in USI and ileum were performed.

Results: USI or ileum infusion of nutrients increased glucose tolerance in healthy but not HF rats. Transplantation of healthy microbiome obtained from USI into the USI of HF rats restored nutrient sensing and inhibited FXR via a reduction of TCDCA in the USI and ileum. Further, inhibition of USI and ileal FXR enhanced nutrient sensing in HF rats, while inhibiting USI (but not ileal) bile salt hydrolase of HF rats transplanted with healthy microbiome activated FXR and disrupted nutrient sensing in the USI and ileum.

Conclusions: We reveal a TCDCA-FXR axis in both the USI and ileum that is necessary for the upper small intestinal microbiome to govern local nutrient-sensing glucoregulatory pathways in rats.

Keywords Small intestine; Nutrient sensing; Glucose tolerance; Bile acids; FXR

1. INTRODUCTION

The deconjugation of conjugated bile acids to liberate free bile acids in the intestinal lumen is facilitated by the gut microbial bile salt hydrolase [1–3]. High fat (HF)-induced changes of the gut microbiome alter intestinal microbial bile salt hydrolase activity and consequently bile acid levels [4]. In people with type 2 diabetes, gut microbial bile salt hydrolase activity is altered [5], and serum levels of taurine-conjugated bile acids, such as taurochenodeoxycholic acid (TCDCA) and tauroursodeoxycholic acid are elevated and positively correlated with elevated fasting glucose levels and insulin resistance [6].

We have recently documented that HF feeding in rats elevates TCDCA in the plasma, upper small intestine, ileum, and the dorsal vagal complex of the brain [7]. Transplantation of upper small intestinal healthy microbiome obtained from healthy donors into the USI of HF rats not only negates the ability of HF to increase TCDCA levels in the USI, but also in the ileum, plasma, and dorsal vagal complex [7]. The consequential lowering of TCDCA levels in the dorsal vagal complex of HF rats inhibits FXR and improves insulin sensitivity to lower hepatic glucose production [7]. The effect of TCDCA on FXR in the dorsal vagal complex is consistent with the fact that TCDCA is an agonist of FXR [8]. The transplantation of upper small intestinal healthy microbiome in HF rats also enhances oleic acid sensing in the USI to increase glucose tolerance independently of changes in plasma insulin levels [9], and the impact of oleic acid sensing in the USI is disrupted through the infusion of FXR agonist GW4064 into the USI [9]. In parallel, hepatic glucose production is inhibited by oleic acid sensing in the ileum [10] and glucose sensing in the USI [11] during the pancreatic-euglycemic clamps when plasma insulin is maintained at basal levels. However, it remains unclear whether glucose sensing in the USI and nutrient sensing in the ileum regulate glucose tolerance. In addition, it remains unknown whether changes of TCDCA in the USI and/or ileum of HF rats incurred by healthy microbiome transplantation may affect small intestinal nutrient sensing glucoregulatory pathways via FXR.

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Abbreviations: USI, Upper small intestine; HF, high-fat; TCDCA, taurochenodeoxycholic acid; FXR, farnesoid X receptor; *L. gasseri*, *Lactobacillus gasseri*

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In this study, we first aimed to confirm the effect of luminal oleic acid infusion into the USI on glucose tolerance as described in regular chow-fed, HF, and HF with upper small intestinal healthy microbiome transplanted rats [9]. We also investigated whether glucose sensing in the USI impacts glucose tolerance in association with changes in FXR signaling. We next infused TCDCA into the USI to overcome the expected drop in TCDCA levels incurred by upper small intestinal healthy microbiome transplant [7] and evaluated the response of FXR signaling and oleic acid or glucose sensing in the USI (Figure 1A). Second, we have repeated the same set of experiments but with oleic acid, glucose and/or TCDCA infused instead into the ileum of rats implanted with both USI (for microbiome transplant) and ileum luminal catheters and evaluated whether glucose tolerance and ileal FXR signaling changes (Figure 2A). Third, we have infused FXR antagonist glycine-β-muricholic acid either into the USI or ileum of HF rats and investigated the responses of nutrient sensing glucoregulation and FXR signaling in the USI or ileum (Figure 3A). Finally, we have evaluated whether inhibiting microbial bile salt hydrolase activity in the USI of healthy microbiome transplanted HF rats could alter USI and/or ileum nutrient sensing glucoregulation (Figure 4A). Altogether, these complementary studies have evaluated whether a TCDCA-FXR axis in USI and ileum is sufficient and necessary for upper small intestinal healthy microbiome transplant to alter USI and ileum nutrient sensing glucoregulation *in vivo*.

2. MATERIALS AND METHODS

2.1. Animal preparation

Male Sprague-Dawley (SD) rats (8—10 weeks of age) from Charles River Laboratories (Montreal, QC, Canada) were subjected to a standard 12-hour light—dark cycle and had ad libitum access to drinking water and a regular chow diet (Teklad Diet 7012, Harlan Laboratories, Madison, USA - 17% fat, 25% protein, and 58% carbohydrates; 3.1 kcal/g total metabolizable energy). Male SD rats were used because they develop hyperphagia immediately upon a HF diet. Rats were randomly assigned for a HF diet enriched with 10% lard oil (TestDiet 571R, Purina Mills, Richmond, USA containing 34% fat, 22% protein, and 44% carbohydrate; 3.9 kcal/g total metabolizable energy) for 3 d. Rats that did not develop hyperphagia were excluded. All animal protocols were approved by the UHN Animal Care and Use Committee in accordance with the Canadian Council on Animal Care guidelines.

2.2. Surgical procedures

Rats were anesthetized with an intraperitoneal injection of ketamine (Vetalar, Bioniche, Belleville, ON) and xylazine (Rompun, Bayer, Toronto, ON). Surgeries were performed 4—5 days prior to the experiments as described [9,10]. Rats either received a USI catheter that was placed 6 cm distal to the pyloric sphincter to target the lower

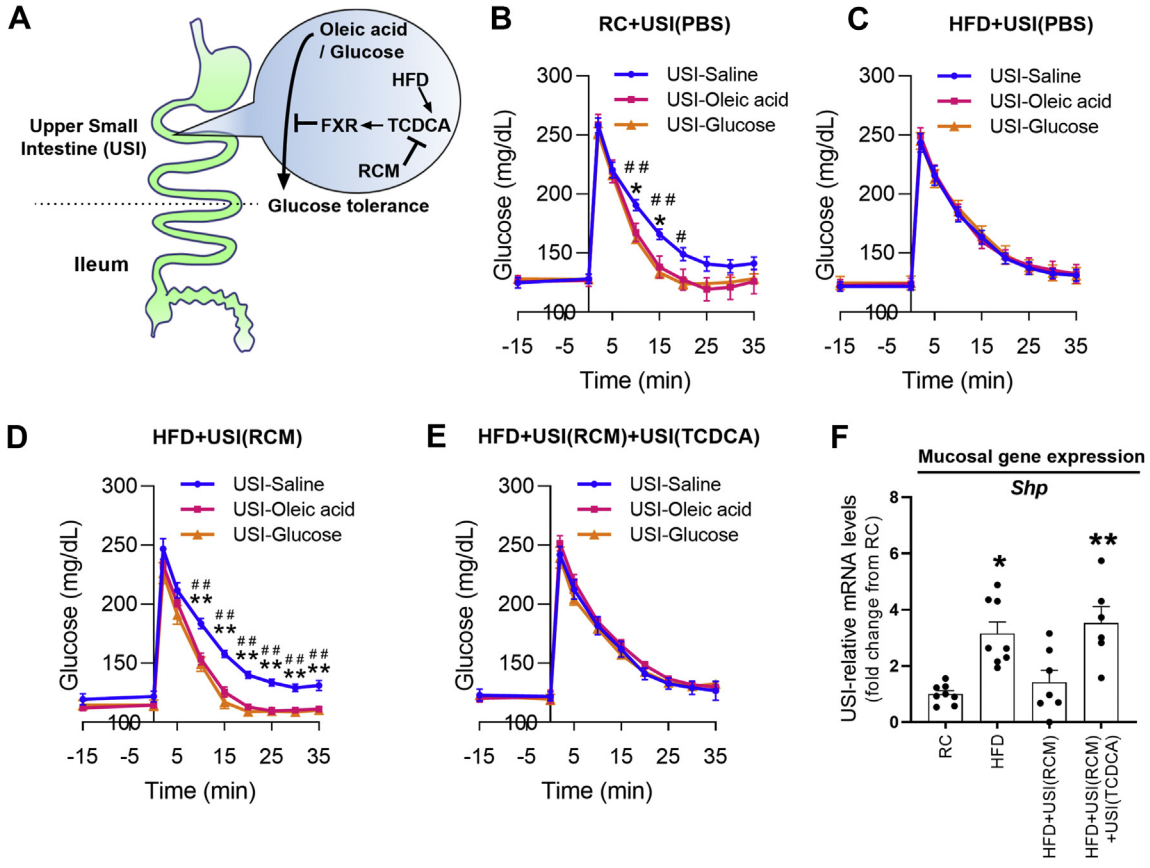

Figure 1: Healthy microbiome USI-transplant in HF rats restores USI oleic acid or glucose sensing glucoregulation and inhibits FXR via TCDCA changes. (A) Schematic representation of the working hypothesis. (B—E) Plasma glucose levels during IVGTT in response to USI saline, oleic acid, or glucose 50-min infusion in rats that received RC + USI (PBS) (saline n = 8, oleic acid n = 7, glucose n = 8) (B), HFD + USI (PBS) (saline n = 8, oleic acid n = 7, glucose n = 7) (C), HFD + USI (RCM) (saline n = 6, oleic acid n = 7, glucose n = 5) (D), or HFD + USI (RCM)+USI (TCDCA) (saline n = 5, oleic acid n = 6, glucose n = 5) (E). *p < 0.05 or **p < 0.01, saline versus oleic acid; and #p < 0.05 or ##p < 0.01, saline versus glucose. (F) Relative *Shp* mRNA expression in the USI mucosal layer of rats that received RC (n = 8), HFD (n = 8), HFD + USI (RCM) (n = 7), HFD + USI (RCM)+USI (TCDCA) (n = 6). *p < 0.05 or **p < 0.01, versus RC and HFD + USI (RCM). RC = regular-chow, RCM = regular chow-fed healthy microbiome.

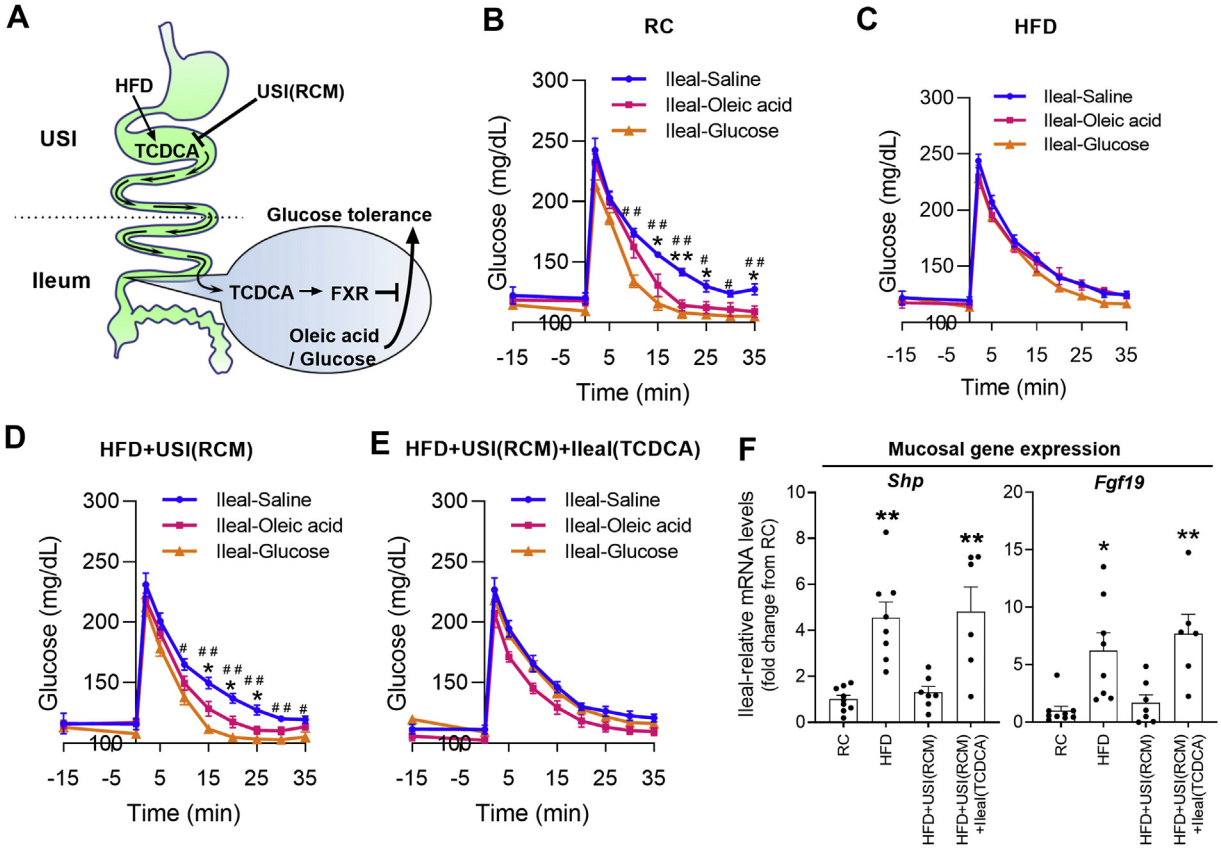

Figure 2: Healthy microbiome USI-transplant in HF rats restores ileal oleic acid or glucose sensing glucoregulation and inhibits FXR via TCDCA changes. (A) Schematic representation of the working hypothesis. (B–E) Plasma glucose levels during IVGTT in response to ileal 50-min infusion of saline, oleic acid, or glucose in rats that received RC (saline n = 6, oleic acid n = 7, glucose n = 5) (B), HFD (n = 5 per group) (C), HFD + USI (RCM) (saline n = 5, oleic acid n = 7, glucose n = 5) (D), or HFD + USI (RCM)+ileal (TCDCA) (saline n = 5, oleic acid n = 7, glucose n = 5) (E). *p < 0.05 or **p < 0.01, saline versus oleic acid; and #p < 0.05 or ##p < 0.01, saline versus glucose. (F) Relative *Shp* and *Fgf19* mRNA expression in the ileal mucosal layer of rats that received RC (n = 9), HFD (n = 8), HFD + USI (RCM) (n = 7), or HFD + USI (RCM)+ileal (TCDCA) (n = 6). *p < 0.05 or **p < 0.01, versus RC and HFD + USI-RCM. RC = regular-chow, RCM = regular chow-fed healthy microbiome.

duodenum and upper jejunum or an ileal catheter that was placed 2 cm proximal to the cecum or both (USI and ileal) for luminal infusion. Catheters were also inserted into the left carotid artery and right jugular vein for sampling and infusion. Rats were randomly assigned into groups and were excluded if the rats did not recover over 90% of pre-surgical weight.

2.3. Microbiota transplant

Upper small intestinal transplantation protocol was performed as described [9] (Supplementary Figure 1b and 2b). In brief, donor rats have received USI and vascular surgeries in order to ensure similar recovery as the recipient rats. The recipient rats received vascular surgeries and either USI or USI & ileal cannulations. The day before the intravenous glucose tolerance test (IVGTT), donor and recipient rats were fasted for 6–7 h (9:00 AM–3:00/4:00 PM) prior to transplantation. Donor rats were given 500 μl of phosphate-buffered saline (PBS) over 30 s into the USI and anesthetized, followed by abdomen incision. The small intestine luminal contents were removed over a 15- to 20-cm section starting from 6 cm distal to the pyloric sphincter, diluted (1:4 in PBS), homogenized with a hand-held homogenizer for 30 s, and finally filtered twice using a large strainer to remove all food particles. Five hundred microliters of luminal content or PBS vehicle was then infused over 30 s into the USI of recipient rats followed by a 120-μl saline flush over 30 s to cover the death volume of the gut catheters. It is important to note that using the same transplantation protocol, we documented that HF rats with healthy microbiome transplant exhibit normalization of microbiome in the USI to condition as seen in healthy chow-fed rats [9].

2.4. Intestinal infusions

Oleic acid (Sigma, St. Louis, MO) was infused at 800 mM or 200 mM (0.01 ml min^{-1} for 50 min; total amount delivered was 500 μl) into the lumen of USI or ileum, respectively. These concentrations were selected based on our previous studies documenting that USI [9] and ileal [10] oleic acid infusions given at the current amount do not increase fatty acids levels in the blood circulation of rats but exert glucoregulatory responses due to oleic acid sensing in the small intestine. Glucose (2 mM; Sigma) was infused into the USI or ileum at 2 μl min^{-1} for 50 min. This concentration was selected based on previous studies documenting that USI glucose infusion given at the current amount not only does not increase plasma glucose levels but instead lowers hepatic glucose production due to small intestinal glucose sensing [11]. The level of TCDCA in the ileal luminal content of 3-d HF-fed rats is elevated from 40 nmol/g of tissue (chow-fed) to 140 nmol/g [7]. Because the ileal luminal content we obtained on average weighed 300 mg, TCDCA is elevated in the ileum by 30 nmol in 3-d HF-fed rats. Since previous in vitro studies [12] report that 100 μM of TCDCA increases FXR activation (i.e., *Shp* expression) as well, we have decided to administer 100 μM of TCDCA (T6260, Sigma; dissolved in saline; 500 μl over 30 s (equal to 50 nmol infused)

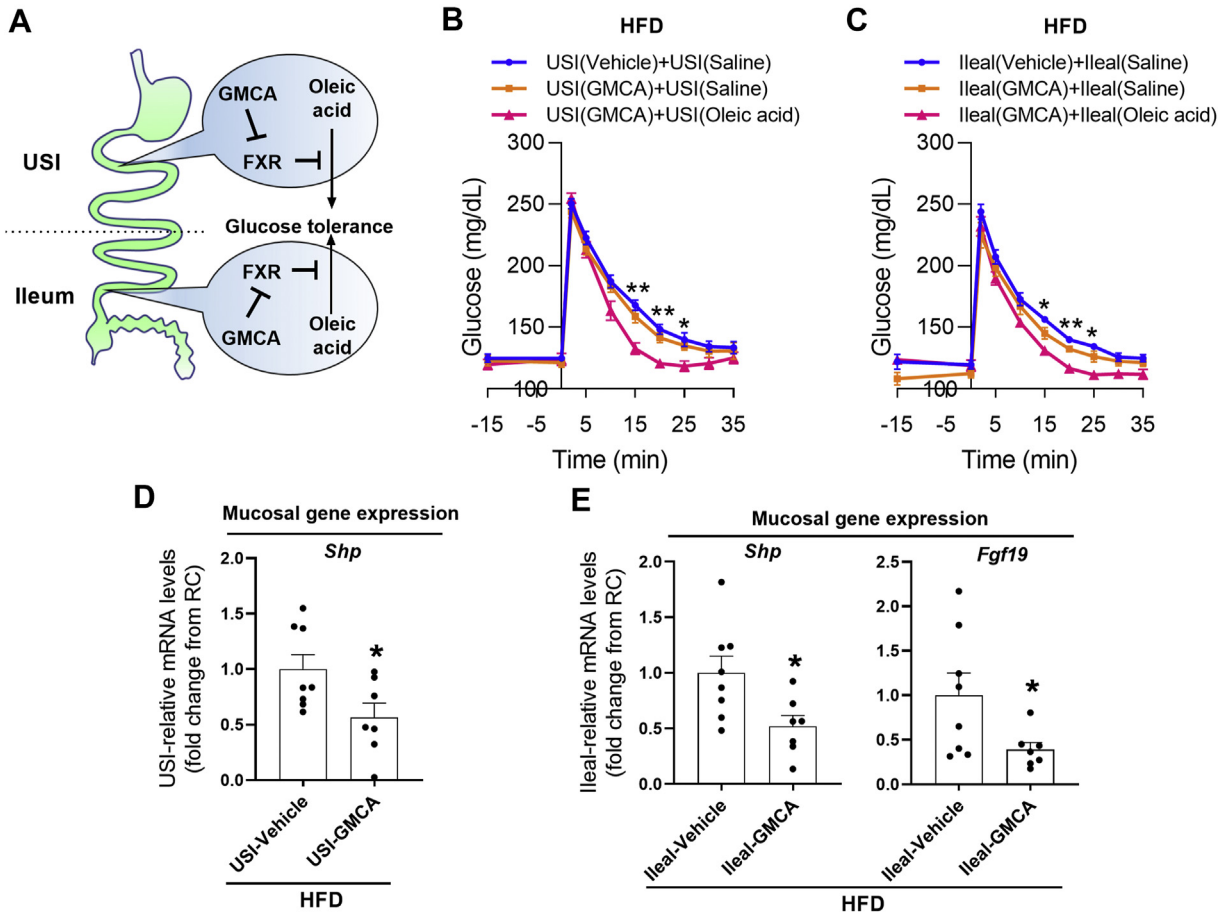

Figure 3: Inhibition of FXR in the USI and ileum of HF rats restores oleic acid sensing and inhibits FXR in the USI and ileum. (A) Schematic representation of the working hypothesis. (B) Plasma glucose levels during the IVGTT in HF rats that received USI-vehicle injections and USI-saline 50-min infusion (n = 7), USI-GMCA injections and USI saline (n = 7) or oleic acid (n = 6) 50 min infusion. *p < 0.05 or **p < 0.01, USI (GMCA)+USI-oleic acid vs. all other groups. (C) Plasma glucose levels during the IVGTT in HF rats that received ileal-vehicle injections and ileal-saline 50-min infusion (n = 5), ileal-GMCA injections, and ileal saline (n = 5) or oleic acid (n = 5) 50-min infusion. *p < 0.05 or **p < 0.01, ileal (GMCA)+ileal-oleic acid vs. all other groups. (D) Relative *Shp* mRNA expression in the USI mucosal layer of HF rats that received USI-vehicle (n = 8) or USI-GMCA (n = 7) injections. *p < 0.05, versus vehicle. (E) Relative *Shp* and *Fgf19* mRNA expression in the ileal mucosal layer of HF rats that received ileal-vehicle (n = 8/group) or ileal-GMCA (n = 7/group) injections. *p < 0.05, versus vehicle counterparts. GMCA = glycine-β-muricholic acid.

followed by a 120 μl over 10 s saline flash) into the ileum 30 min after the healthy microbiome transplantation into the USI and 90 min prior to the IVGTT (Supplementary Figure 2a). In parallel, HF feeding for 3 d increases TCDCA in the USI 10x higher than ileum in nmol/g [7]. We first injected 800 μM (equal to 400 nmol infused) of TCDCA into the USI (Supplementary Figure 1a) of HF rats with healthy microbiome transplant using the same infusion protocol as the ileum studies. However, after the second injection of TCDCA and the initiation of oleic acid infusion into the USI at −15 min, we have noticed that the rats (n = 2) experienced a severe intestinal cramp. As such, we infused TCDCA instead at 400 μM (200 nmol infused). Not only intestinal cramp is no longer observed, but TCDCA administered at this concentration negated USI nutrient sensing (Fig 1E) and activated USI FXR (i.e., *Shp* expression) in HF rats with healthy microbiome transplantation to a similar extent as HF rats (Fig 1F). FXR antagonist glycine-β-muricholic acid (dissolved in 1% carboxymethyl cellulose; a gift from Dr. Changtao Jiang, Peking University, China) was infused at 10 mg/kg into the USI or ileum (500 μl over 30 s followed by a 120 μl saline flash over 10 s) at 4:00 PM on the day before and 90 min prior to IVGTT (Supplementary Figure 3a). One percent carboxymethyl cellulose was used as vehicle. The dose of glycine-β-muricholic acid (10 mg/kg) was chosen based on previous work indicating that oral glycine-β-muricholic acid intake given at 10 mg/kg/day for 1d or 5d selectively inhibits small intestinal FXR signaling [13–15]. Riboflavin (555682, Sigma; dissolved in PBS - 20 mM stock) is a bile salt hydrolase inhibitor [16,17]. The rats received riboflavin (0.5 mM or 190 μg/ml) in the USI (diluted either in donor luminal content or PBS; 500 μl over 30 s) or ileum (diluted in PBS; 500 μl over 30 s) at the time of the transplantation and 120 min prior to IVGTT (Supplementary Figure 4a). Riboflavin was selected to infuse at 0.5 mM since riboflavin (0.5 mM) inhibits >96% of *Lactobacillus acidophilus* (*L. acidophilus)* bile salt hydrolase activity [16,17]. Importantly, the bile salt hydrolase gene (LGAS_0051; BshA) of *Lactobacillus gasseri* (*L. gasseri,* which is changed in the USI in our microbiome transplant conditions [9]) has a 98% identity of *L. acidophilus* bile salt hydrolase gene [18]. Further, riboflavin administered at higher concentration (i.e., >5 mM) may elucidate effects on inflammation and oxidative stress [19,20].

2.5. IVGTT

Rats were fasted (food removed at 9:00 AM) for 24 h prior to IVGTT, and IVGTT was performed as described [9] (Supplementary Figure 1a). Blood samples were collected in heparinized tubes and centrifuged at

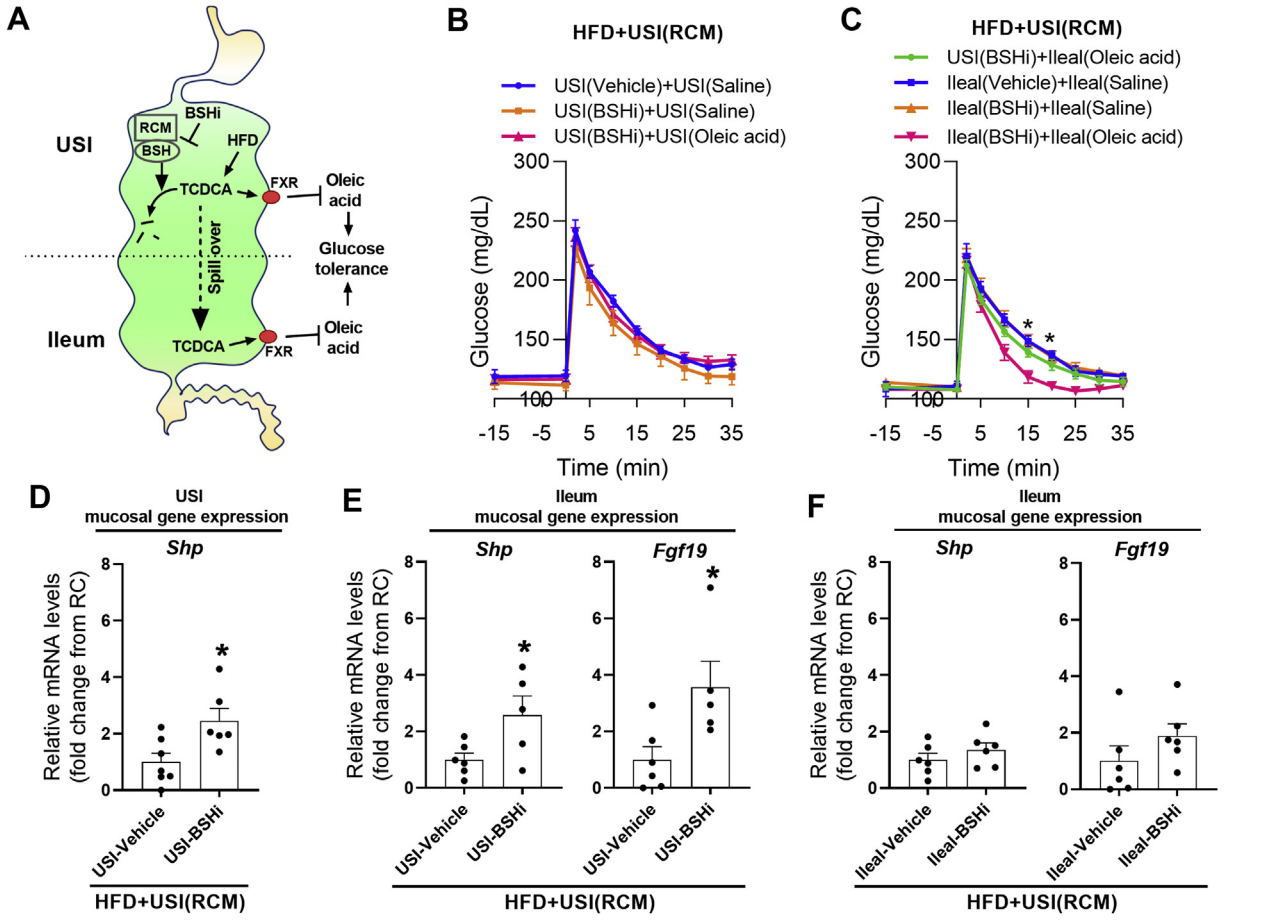

Figure 4: Inhibition of bile salt hydrolase in the USI of HF rats with healthy microbiome transplant negates oleic acid sensing and activates FXR in the USI and ileum. (A) Schematic representation of the working hypothesis. (B) Plasma glucose levels during the IVGTT in HFD + USI (RCM) transplant rats that received USI-vehicle injections and USI-saline 50-min infusion (n = 5), BSHi USI-injections, and USI saline (n = 5) or oleic acid (n = 7) 50 min infusion. (C) Plasma glucose levels during the IVGTT in HFD + USI (RCM) transplant rats that received USI-BSHi riboflavin injections and ileal oleic acid 50-min infusion (n = 5), ileal-vehicle (n = 5) or ileal-BSHi (n = 5) injections and ileal saline 50-min infusion, or ileal-BSHi injections and ileal 50-min oleic acid infusion (n = 5). *$p < 0.05$ or **$p < 0.01$, ileal (BSHi)+ileal-oleic acid versus all other groups. (D, E) Relative *Shp* mRNA expression in the USI mucosal layer (D) and *Shp* and *Fgf19* mRNA expression in the ileal mucosal layer (E) of HFD + USI (RCM) rats that received USI-vehicle (USI mucosa, n = 7; ileal mucosa n = 6/group) or USI-BSHi (USI mucosa, n = 6; ileal mucosa n = 5/group) injections. *$p < 0.05$, versus vehicle counterparts. (F) Relative *Shp* and *Fgf19* mRNA expression in the ileal mucosal layer of HFD + USI (RCM) rats that received ileal-vehicle or ileal-BSHi injections (n = 6/group). RCM = regular chow-fed healthy microbiome. BSHi = bile salt hydrolase inhibitor (riboflavin).

2,000×*g* for 30 s. Plasma glucose levels were determined by the glucose oxidase method using a GM9 glucose analyzer (Analox Instruments, Stourbridge, UK).

2.6. Virus infection

A subset of rats received an injection of lentivirus (1.0×10^6 infectious units) expressing shRNA FXR (shFXR; sc-108079-V, Santa Cruz Biotechnology, Inc.) or mismatch sequence shRNA (shMM; sc-108080, Santa Cruz Biotechnology, Inc.) into the USI prior to the insertion of the USI cannula [9,11,34,35]. Specifically, the USI was elevated and ligated with 4–0 sutures at 6 and 12 cm distal to the pyloric sphincter (to target the same region as the USI infusion protocol). This elevated 6 cm USI segment was flushed 3–4 times with 0.2 mL of saline via a 23-gauge needle inserted right below the 6 cm ligation. Subsequently, a 1:10 dilution of lentiviral particles in saline was injected (0.2 mL total) into the USI. After a 20 min incubation, sutures were removed, and the USI was flushed with saline. A catheter was then inserted into the site of the virus injection, and vascular cannulations were performed as described above. After 3 d of recovery, rats were fasted (food removed at 9:00 AM on the third day) for 24 h, and TCDCA or saline was administered into the USI as described above. On the morning of the 4th day, after 90 min of TCDCA or saline administration, rats received USI saline infusion for 50 min, and the mucosal layer of the USI was separated for qPCR analysis as described below.

2.7. qPCR analysis

An independent set of rats received gut and vascular surgeries and underwent the same experimental conditions and treatments (i.e., chow or HF fed, microbiota transplant, TCDCA, glycine-β-muricholic acid, and riboflavin administration) but did not undergo IVGTT. Instead, under basal conditions, rats received USI and/or ileum saline infusion for 50 min and were anesthetized. Approximately 75–100 mg of the mucosal layer of the upper small intestine (6–12 cm distal from the pyloric sphincter) and ileum (0–4 cm proximal from the cecum) were separated from the smooth muscle layer immediately following dissection. Mucosal scrapings were homogenized in lysis buffer (Ambion) using a PowerGen-125 homogenizer (Thermo Fisher Scientific, Toronto, ON), centrifuged at 12,500×*g* for 5 min, and RNA was

isolated using the Ambion PureLink RNA Mini Kit per kit guidelines (Thermo Fisher Scientific) and quantified using a Cytation 5 imaging reader (BioTek, Winooski, VT). Five micrograms of RNA was subjected to DNase digestion (Roche, Mannheim, Germany) at room temperature for 10 min, terminated by the addition of 25 mM of ethylenediaminetetraacetic acid (EDTA), and incubated at 70 °C for 15 min cDNA was generated from 1 μg of RNA using the SuperScript Vilo cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). qPCR was performed using 500 ng of cDNA, TaqMan Gene Expression master mix, and TaqMan primers (Thermo Fisher Scientific) for rat ribosomal protein *18s* (Rps 18; Assay ID: Rn01428913_gH), rat *Shp* (Nr0b2; Assay ID: Rn00589173_m1), rat *Asbt* (Slc10a2; Assay ID: Rn00691576_m1), rat *Fxr* (Nr1h4; Assay ID: Rn00572658_m1), or rat *Fgf19* (*Fgf19*; Assay ID: Rn00590708_m1) using a QuantStudio 7 Flex qPCR machine (Applied Biosystems). Relative gene expression was calculated using the ΔΔCt method, where each sample was normalized to *18s*.

2.8. Statistical analysis

All statistical analysis was performed using GraphPad Prism (version 8.0.1, GraphPad, La Jolla, CA, USA) based on measurements taken from distinct samples. The unpaired Student's t-test was used in comparing two groups. One-way analysis of variance (ANOVA) with Tukey post hoc test was performed for 3+ groups. Differences were considered significant at $p < 0.05$. All numerical results are presented as mean ± s.e.m.

3. RESULTS

Consistent with previous studies [9], we have confirmed that oleic acid infusion into the USI increased glucose tolerance in chow but not 3-d HF-fed rats with hyperphagia but comparable body weight (Figure 1B,C; Supplementary Figure 1c—f) and that healthy upper small intestinal microbiome transplantation into the USI of HF rats restored USI oleic acid sensing independent of weight changes (Figure 1D; Supplementary Figure 1d and g). Of note, 3-d HF vs. chow fed rats did not display glucose intolerance *per se* during IVGTT (Figure 1B,C). In the same models, we discovered for the first time that glucose infusion into the USI increased glucose tolerance in chow but not HF-fed rats (Figure 1B,C; Supplementary Figure 1e and f). Importantly, upper small intestinal healthy microbiome transplant also rescued the ability of glucose sensing in the USI to increase glucose tolerance in HF rats (Figure 1D; Supplementary Figure 1g). Thus, HF-induced changes of microbiome in the USI alter glucose and oleic acid sensing in the USI that regulate glucose tolerance.

Because upper small intestinal healthy microbiome transplant prevents HF feeding to increase TCDCA in the USI [7], we have postulated that the consequential lowering of TCDCA blunts FXR induction and enhances the ability of nutrient sensing in the USI to regulate glucose tolerance. As such, we have confirmed that both *Asbt* [conjugated bile acid (i.e., TCDCA or glycine-β-muricholic acid) transporter] and *Fxr* gene expression were detected in the mucosa of USI of chow fed rats, and the *Asbt* and *Fxr* expression levels were comparable to HF and HF with healthy microbiome transplant rats (Supplementary Figs. 1h and i). Next, we administered TCDCA into the USI of HF rats with healthy microbiome transplant to overcome the expected drop in TCDCA [7] (Supplementary Figure 1a and b). We found that these rats lost the glucoregulatory responses to oleic acid and glucose sensing in the USI independent of changes in weight (Figure 1E & Supplementary Figure 1d and j).

Among the various FXR-targeted genes, *Shp* and *Fgf19* (or *Fgf15* in mice) are commonly used to assess intestinal FXR activity [12,13,21—24]. The *Shp* expression was detected in the USI mucosa of rats (Figure 1F), while *Fgf19* expression in the USI mucosa was very low or undetectable. This latter observation is in agreement with the upper intestinal *Fgf15* gene expression pattern observed in mice [22]. In addition, we have found that HF increased *Shp* expression in the mucosa of the USI, and this *Shp* induction was prevented by healthy microbiome transplant (Figure 1F). These findings are consistent with the fact that HF feeding increases TCDCA levels in the USI and such elevation is prevented by healthy microbiome transplant [7]. More importantly, the administration of TCDCA into the USI prevented the ability of healthy microbiome transplant to lower *Shp* expression in HF rats (Figure 1F), suggesting that the infusion of TCDCA into the USI activates FXR to a similar extent as seen in HF rats (Figure 1F). To confirm that TCDCA increases *Shp* expression through FXR, we found that direct infusion of TCDCA into the USI increased *Shp* expression in rats injected with lentivirus expressing the mismatch sequence into the USI (Supplementary Figure 1k). On the other hand, TCDCA infusion into the USI failed to increase *Shp* expression in rats injected with lentivirus expressing the shRNA of FXR into the USI (Supplementary Figure 1k). Further, *Fxr* expression in the USI of rats injected with lentivirus shRNA FXR vs. mismatch was significantly reduced (Supplementary Figure 1l), confirming that TCDCA increases *Shp* expression in the USI via an FXR-dependent mechanism. Overall, the effects of healthy microbiome USI transplant in HF rats on the restoration of oleic acid and glucose sensing in glucoregulation and on FXR inhibition were negated by the infusion of TCDCA into the USI. These findings suggest that HF-induced changes in the microbiome of USI elevate TCDCA levels to impair oleic acid and glucose sensing in parallel to activate FXR in the USI.

More than 95% of small intestinal bile acids that are secreted into the USI are reabsorbed at the ileum [25,26]. We have discovered that upper small intestinal healthy microbiome transplant prevents HF feeding to increase ileal TCDCA as well [7]. Thus, we next investigated the glucoregulatory role of ileal oleic acid and glucose sensing and their respective interaction with the TCDCA in the ileum that is derived from the USI (Figure 2A; Supplementary Figure 2a and b). To our knowledge, we are the first to discover that ileal infusion of oleic acid or glucose increased glucose tolerance in chow fed but not in hyperphagic HF rats independent of weight changes (Figure 2B,C; Supplementary Figure 2c—f). Strikingly, the healthy microbiome obtained from the USI that was transplanted into the USI of HF rats was able to restore oleic acid and glucose sensing in the ileum and enhance glucose tolerance (Figure 2D; Supplementary Figure 2g). This restoration occurred in association with an inhibition of ileal *Shp* and *Fgf19* expression as compared to HF rats (Figure 2F), but independent of weight (Supplementary Figure 2d). Thus, we have postulated that the lowering of ileal TCDCA levels incurred by the upper small intestinal healthy microbiome transplant enhances ileal oleic acid and glucose sensing via FXR inhibition. To begin testing this hypothesis, we first administered TCDCA into the ileum of HF rats with healthy microbiome transplant to overcome the expected drop in TCDCA [7]. TCDCA infusion into the ileum of HF rats with healthy microbiome transplant was able to negate ileal oleic acid and glucose sensing to increase glucose tolerance (Figure 2E; Supplementary Figure 2h). This occurred in parallel to an induction of ileal *Shp* and *Fgf19* expression (Figure 2F) but independent of weight (Supplementary Figure 2d). Of note, *Asbt* and *Fxr* expression were also detected in the ileal mucosa (Supplementary Figure 2i and j) and were found to be higher than USI (*Asbt* Ct value: USI 31.1 vs. Ileum 25.3; *Fxr* Ct value: USI 26.4 vs. Ileum 24.8), as previously shown in humans for *Asbt* [32] and in mice for *Fxr* [33]. Further, the level of ileal Asbt and Fxr expression were comparable in chow, HF, and HF with healthy microbiome transplant rats (Supplementary Figure 2i and j). These findings illustrate that the effects of upper small intestinal healthy microbiome

transplant have on nutrient sensing and FXR in the ileum of HF rats are negated by TCDCA administration into the ileum. Taken together, TCDCA is highlighted as a common link and messenger in response to short-term HF feeding. Specifically, HF-induced microbiota changes in the USI elevate USI *and* ileal TCDCA levels [7] (Figure 1A & Figure 2A), leading to an impairment of oleic acid and glucose sensing in the USI and ileum in regulating glucose tolerance and an activation of FXR in the USI and ileum (Figures 1 & 2).

To directly evaluate the role of FXR in the USI and ileum on the glucoregulation of nutrient sensing, we administered chemical FXR inhibitor glycine-β-muricholic acid into the USI or ileum of HF rats (Figure 3A & Supplementary Figure 3a). Infusion of glycine-β-muricholic acid either into the USI or ileum *per se* at the current experimental short-term infusion dosage did not influence glucose tolerance or body weight (Figure 3B,C & Supplementary Figure 3b—e) but inhibited USI mucosal *Shp* expression (Figure 3D) or ileal mucosal *Shp* + *Fgf19* expression (Figure 3E), respectively. Importantly, glycine-β-muricholic acid infusion into the USI of HF rats restored oleic acid sensing in the USI to increase glucose tolerance (Figure 3B & Supplementary Figure 3d), while glycine-β-muricholic acid infusion into the ileum enhanced ileal oleic acid sensing to increase glucose tolerance as well (Figure 3C; Supplementary Figure 3e). Thus, inhibition of FXR in the USI and ileum of HF rats is sufficient to activate oleic acid sensing in the USI and ileum to regulate glucose tolerance. Collectively, these findings overall suggest that inhibition of FXR in the USI and ileum is necessary for upper small intestinal healthy microbiome transplant to restore nutrient sensing through a reduction of TCDCA in the USI and ileum of HF rats (Figures 1A,2A).

Finally, we investigated whether upper small intestinal healthy microbiome transplantation in HF rats induces microbial bile salt hydrolase-dependent changes in the USI and/or ileum to alter upper small intestinal and/or ileal TCDCA-FXR axis and nutrient sensing glucoregulation. The *Lactobacillus* genus is the dominant microbiota in the USI of rats and mice [9,27], and many *Lactobacillus* species deconjugate taurine-conjugated bile acids (i.e., TCDCA) via their respective bile salt hydrolase [28]. Given the current short term 3-d HF vs. chow-fed feeding rat protocol decreases *L. gasseri* in the USI and increases USI and ileal TCDCA levels, and that these HF-induced effects are prevented by upper small intestinal healthy microbiome transplantation [7,9], we reasoned that inhibiting bile salt hydrolase in the USI of HF rats with healthy microbiome transplant would prevent the inhibition of the TCDCA-FXR axis and negate nutrient sensing in the USI and ileum (Figure 4A).

Because riboflavin transporter is expressed in *L. gasseri* [29] and riboflavin is an inhibitor of *L. gasseri* bile salt hydrolase [30], we administered riboflavin into the USI of HF rats with healthy microbiome transplant (Supplementary Figure 4a). We first found that riboflavin vs. vehicle infusion into the USI resulted in no changes in weight (Supplementary Figure 4b and c) or glucose tolerance (Figure 4B; Supplementary Figure 4d) via the short-term injection protocol. However, riboflavin vs. vehicle increased both *Shp* expression (Figure 4D) in the USI and *Shp* & *Fgf19* expression in the ileum (Figure 4E) and negated the ability of oleic acid infusion into the USI and ileum to increase glucose tolerance (Figure 4B,C; Supplementary Figure 4d and e). In contrast, administering riboflavin into the ileum of HF rats with upper small intestinal healthy microbiome transplant failed to negate ileal oleic acid sensing (Figure 4C; Supplementary Figure 4e) and alter ileal *Shp* & *Fgf19* expression (Figure 4F). Body weight was comparable among groups (Supplementary Figure 4c). These data demonstrate that USI, *but not ileal*, administration of riboflavin into HF rats with upper small intestinal healthy microbiome transplant negates oleic acid sensing in the USI and ileum to increase glucose tolerance and activates FXR in the USI and ileum. Although it remains to be investigated, we propose that (i) upper small intestinal transplantation of healthy microbiome into HF rats elucidates upper small intestinal (but not ileal) microbial bile salt hydrolase-dependent changes and subsequently enhances upper small intestinal and ileal nutrient sensing glucoregulation through a reduction of the TCDCA-FXR axis (Figure 4A), and (ii) the changes of ileal FXR signaling and nutrient sensing glucoregulation (Figure 2D) in HF rats incurred by small intestinal healthy microbiome transplant are not dependent on changes in the ileal microbiome but rather spillover-changes of TCDCA from USI into the ileum (Figure 4A).

4. DISCUSSION

In this set of studies, we unexpectedly discovered that transplantation of USI-derived healthy microbiome into the USI of HF recipient rats enhances oleic acid- and glucose-sensing glucoregulation and inhibits FXR not only in the USI, but also in the ileum. In parallel, we have also discovered that healthy microbiome USI-transplant in HF rats lowers TCDCA levels not only in the USI, but also in the ileum [7]. The current results demonstrate that (i) preventing the lowering of USI and ileal TCDCA levels in HF rats with healthy microbiome USI-transplant activates FXR and impairs oleic acid and glucose sensing in the USI and ileum and (ii) inhibiting FXR directly in the USI and ileum of HF rats enhances oleic acid sensing glucoregulation in the USI and ileum. This large set of findings in rats unveil a TCDCA-FXR axis in both the USI and ileum that underlies the HF-induced impairment of small intestinal nutrient sensing and glucoregulation through changes in USI microbiome.

Consistently, inhibiting microbial bile salt hydrolase with riboflavin in the USI, but not ileal, lumen of HF rats with healthy microbiome transplant impairs USI and ileal nutrient-sensing glucoregulation. Although *L. gasseri* in the USI changes in inverse correlation with TCDCA levels [7] and FXR activity (Figure 1F) in the USI and ileum (consistent with the fact that blocking USI microbial bile salt hydrolase in HF rats with healthy microbiome transplant activates USI and ileal FXR), the definitive role of bile salt hydrolase in the USI of *L. gasseri* remains to be investigated. In addition, the role of cholecystokinin and glucagon-like peptide 1 in mediating the ability of small intestinal FXR to alter nutrient-sensing glucoregulatory pathways warrants future investigation, as cholecysokinin and glucagon-like peptide 1 have been implicated in the glucoregulatory effect of small intestinal oleic acid and glucose sensing [9,11].

In summary, we have revealed a USI and ileum TCDCA-FXR axis that is necessary for HF-induced changes of microbiota in the USI to alter small intestinal nutrient sensing and glucose tolerance in rats. Furthermore, for the first time to our knowledge, we show that direct short-term inhibition of FXR in the USI enhances nutrient sensing and glucose tolerance in HF rats, consistent with the glucose homeostatic regulations exert by FXR inhibition in the ileum of mice [12,13,21,31]. Together with the recent discoveries that HF-induced microbiota changes in the USI alter TCDCA levels in the plasma and the dorsal vagal complex and activate FXR axis in the dorsal vagal complex to induce insulin resistance [7], these collective findings highlight TCDCA as a potential link of HF-induced glucose dysregulation and insulin resistance in rodents *in vivo*.

AUTHOR CONTRIBUTION

T.M.Z.W. and Y-M.L. conducted and designed the experiments, performed the data analyses, and wrote the manuscript. Z.D. and S—Y.Z assisted with the experiments. T.K.T.L. supervised the project, designed the experiments, and edited the manuscript.

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CONFLICT OF INTEREST

None declared.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molmet.2020.101132.

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