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Fiber consumption stimulates the activity of microbial bile salt hydrolases

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ABSTRACT

Previously we reported a microbiota-dependent caloric restriction (CR)-triggered increase in the levels of taurine and taurine-conjugated bile acids (BA) in the gut. Now, we show that restrictive diets, including intermittent fasting and fasting-mimicking diet, had a similar impact to CR. The type of cage bedding that CR mice were housed with affected the levels of BAs and taurine in the ileum. Removal of cage bedding neutralized CR phenotype in terms of taurine levels, BAs deconjugation, and fecal microbiota composition. Microbiota transplant from CR mice housed with bedding increased BAs deconjugation. Inhibition of bile salt hydrolase (BSH) prevented the increase in free taurine concentration while increasing taurine-conjugated BA levels. *Ad libitum* consumption of diets high in fiber increased the levels of taurine conjugates but did not elevate the levels of BAs. Dietary restriction is required to stimulate BAs secretion, while ingestion of fiber stimulates the capacity of microbiota to deconjugate BAs.

1. Introduction

Over a century of scientific evidence proves that caloric restriction (CR) is associated with extended health span and life span. CR has been shown to act powerfully against oxidative stress, inflammation, hypertension, cardiovascular, and metabolic disease via a complex series of intricate events (Duszka, Gregor, Guillou, Konig, & Wahli, 2020; Duszka & Wahli, 2020). As the first organ affected by diets, the gut dynamically adapts to CR by adjusting metabolic and immune processes (Duszka et al., 2018). CR involves restricted intake of energy, and therefore, it is often associated with limitation of nutrient intake. Consequently, it results in a depletion of the levels of numerous metabolites in the gut (Duszka et al., 2018). However, as we reported, bile acids (BA) and taurine are exempted from this reduction. On the contrary, we found their levels to be elevated in CR mice's intestinal lumen and mucosa (Duszka et al., 2018; Gregor et al., 2021). This phenotype results from the stimulation of hepatic BAs production and secretion triggered by prolonged CR (Fu, Cui, & Klaassen, 2015; Fu & Klaassen, 2013; Gregor et al., 2021). Bile acids produced in the liver from cholesterol are conjugated to taurine or glycine prior to secretion. Following transport into the intestine, BAs can undergo deconjugation by bacterial bile salt hydrolases (BSHs), subsequently releasing unconjugated BAs and free taurine or glycine. The deconjugation of BAs is mediated by all major bacterial species in the resident gut microbiota (Archer, Chong, & Maddox, 1982; Gilliland & Speck, 1977; Jones, Begley, Hill, Gahan, & Marchesi, 2008; Ridlon, Kang, & Hylemon, 2006), and it may award a nutritional benefit by processing freed amino acid to carbon, nitrogen, sulfur, and energy (Tanaka, Hashiba, Kok, & Mierau, 2000; Van Eldere, Celis, De Pauw, Lesaffre, & Eyssen, 1996). Moreover, BSHs facilitate the incorporation of cholesterol or BAs into bacterial membranes (Dambekodi & Gilliland, 1998; M. Taranto et al., 1997; Taranto, Fernandez Murga, Lorca, & de Valdez, 2003), which impacts the physical properties of the membranes (Boggs, 1987), changing their sensitivity to host defense molecules (Peschel et al., 2001; Wilson et al., 1999). Furthermore, deconjugation is also consequential to the host as it reduces the BAs' efficiency in emulsifying dietary lipids, the formation of micelles, lipid digestion, absorption of fatty acids, and monoglycerides (De Smet, Van Hoorde, De Saeyer, Vande Woestyne, & Verstraete, 1994; Ridlon, Kang, Hylemon, & Bajaj, 2014). Importantly, deconjugation is considered an essential step facilitating further modification to secondary BAs

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(Batta et al., 1990; Stellwag & Hylemon, 1979; White, Lipsky, Fricke, & Hylemon, 1980); however, this concept has been questioned recently (Mythen, Devendran, Mendez-Garcia, Cann, & Ridlon, 2018). Depending on their conjugation status and secondary modifications, a wide range of BAs with radically varying properties are present in the gut, and the processes mediated by them are adjusted accordingly. Depending on the type, BAs can have a positive, negative, or neutral impact on colonic fluid secretion, gut motility and affect rectal sensory thresholds (Min, Rezaie, & Pimentel, 2022). They also affect secondary structure formation in RNA, induce DNA damage, oxidative stress, and regulate various processes vital for the host via its multiple BAs' sub-type specific receptors (Duszka, 2022).

Importantly, we recently discovered (Gregor et al., 2021) that taurine freed from BAs in the gut undergoes conjugation among other molecules, with glutathione (GSH) forming a taurine-GSH conjugate. Intestinal uptake of taurine is limited by the saturability of the taurine transporters (TauT) in the intestine (Anderson, Howard, Walters, Ganapathy, & Thwaites, 2009). However, we found that the formation of the taurine-GSH conjugate increases intestinal absorption of taurine and strengthens its recycling through the enterohepatic circulation in CR animals (Gregor et al., 2021). This enhanced reuptake mechanism during CR suggests taurine's crucial physiological role during energy scarcity. Considering its beneficial impact on multiple organs, it is not surprising that taurine has been suggested as a supplement mimicking the beneficial outcomes of CR (Wang et al., 2020). Importantly, we also showed that CR strongly impacts the intestine and fecal microbiota composition (Duszka et al., 2018), and the occurrence of taurine and its conjugates in the intestinal mucosa of CR mice depends on the microbiota composition (Gregor et al., 2021). Finally, we have also pointed out that CR-related hunger leads to increased cage bedding consumption and; therefore, high fiber ingestion in mouse laboratory models. Consequently, the type of cage bedding used during the experiment strongly affects the outcomes of dietary interventions, including the composition of the microbiota and the intestinal metabolome (Gregor

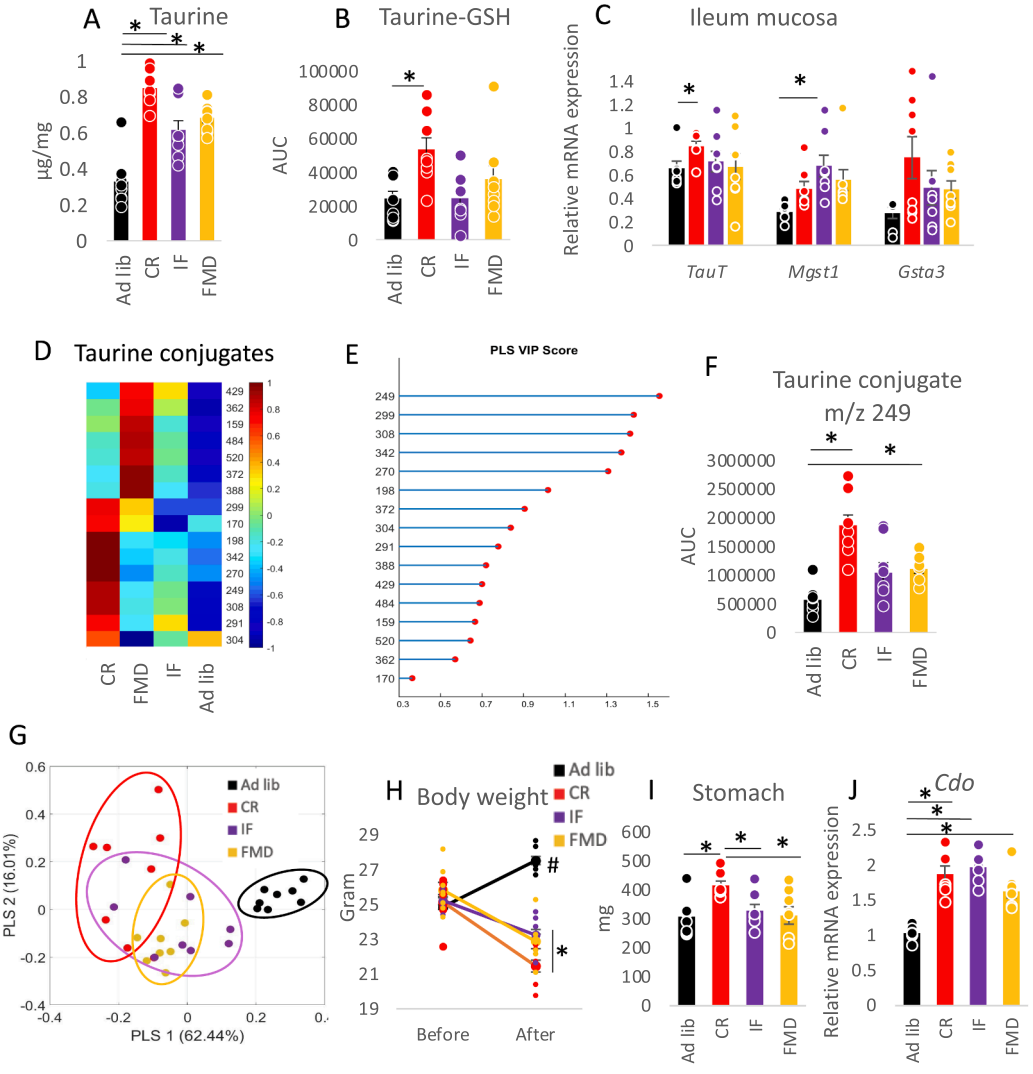

Fig. 1. Restrictive diets increase the levels of free taurine and its conjugates in the intestinal mucosa. The animals were submitted to 14 days of caloric restriction (CR), 28 days of intermittent fasting (IF), four cycles of fasting-mimicking diet (FMD), or *ad libitum* feeding (Ad lib). The levels of free taurine (A) and taurine-GSH conjugate (B), as well as mRNA expression of *TauT*, *Mgst1*, and *Gsta3* (C), were measured in the ileum mucosa of the Ad lib, CR, IF, and FMD animals. The abundance of various taurine conjugates (D), including the most abundant *m/z* 249 (F), were measured in the mucosa of ileum. The most statistically important variables among the conjugates were scored and arranged according to their importance (E). Regression of taurine conjugates levels based on covariance is depicted by a partial least square (PLS) chart (G). The weight of the whole mouse body (H) and stomach with its content (I) was measured during the dissection. mRNA expression of *Cdo* was quantified in the liver (J). *Cdo*: cysteine dioxygenase. Statistical significance was assessed using an ANOVA. * indicates statistical significance; n=8. Error bars stand for ±SEM.

et al., 2020). Kastl A. et al. (Kastl et al., 2022) very recently published that soluble fiber consumption has a stimulatory effect on BSH activity. Accordingly, our current study shows that cage bedding-derived fiber, as well as a high-fiber diet, increase BAs deconjugation and release of free taurine by enteric bacteria. Therefore, hunger-triggered cage bedding consumption by CR mice affects BAs deconjugation by acting on the gut microbiota.

2. Materials and methods

2.1. Animal care and experimental procedures

Male C57Bl/6 mice were purchased from Janvier Labs (Le Genest-Saint-Isle, France) and kept under a 12 h light/12 h dark cycle in standard specific-pathogen-free (SPF) conditions. The mice were given V153x R/M-H auto diet from SSNIFF-Spezialdiäten GmbH (Soest, Germany) and housed with wooden bedding (Lignocel select; J. Rettenmaier & Söhne GmbH + Co KG; Vienna, Austria) unless otherwise specified. A graphical representation of the experimental groups is presented in Supplementary Figures 1-3, in the order of data presentation in the results section. The groups did not differ significantly in body weight at the beginning of the experimental procedures.

Experiment 1: Mice were randomly split into groups of eight and submitted to CR, intermittent fasting (IF), or fasting-mimicking diet (FMD). Mice from the CR groups were submitted to 14 days of CR with a reduction to 80 % of daily food intake. Mice from the IF group were fasted every other day for 24 h (100% restriction) with a 24 h period of chow refeeding *ad libitum* repeated over a total of 28 days. The FMD group was submitted to three cycles of 4 days of fasting (50% kcal of *ad libitum* intake on day one and 10% kcal on days 2–4) with seven days of refeeding with standard chow as described before (Brandhorst et al., 2015). The FMD diet applied during the fasting period consists of vitamin and mineral-rich broth, vegetable mix, and oils blended in a low-caloric paste and dosed daily. The amount given to each mouse was calculated based on the energy requirements of the animals and the caloric density of the diet. For this experiment, mice from each experimental group were housed in single cages with wooden bedding. Due to the differences in the experimental procedures for CR, IF, and FMD, matching the length of the exposure to each diet would result in very different levels of restriction. Therefore, the duration of the experiment was adjusted, aiming at the most comparable conditions.

Experiment 2: To expose mice to different cage beddings, animals were divided into control *ad libitum* and CR groups. Each of these groups was separated into three subgroups (n=8) by the bedding type (Supplementary Figure 1 B): wooden (Lignocel select), corncob (RehoFix MK 3500), or cellulose (Arborcel Performance Small; all beddings from J. Rettenmaier & Söhne GmbH + Co KG).

Experiment 3: In the next experiment, mice were fed *ad libitum* or submitted CR. Half of the Ad lib and CR mice were housed in standard cage conditions, which in our case include wooden cage bedding. The rest of the *ad libitum* and CR animals were housed in cages without cage bedding (NB) (Supplementary Figure 2A). The bottom of the cage not containing cages bedding, was wiped daily to remove urine and reduce potential coprophagy.

Experiment 4: In experiments requiring fecal transplant (FT) (Supplementary Figure 2B), first, the animals were prepared for FT by dosing

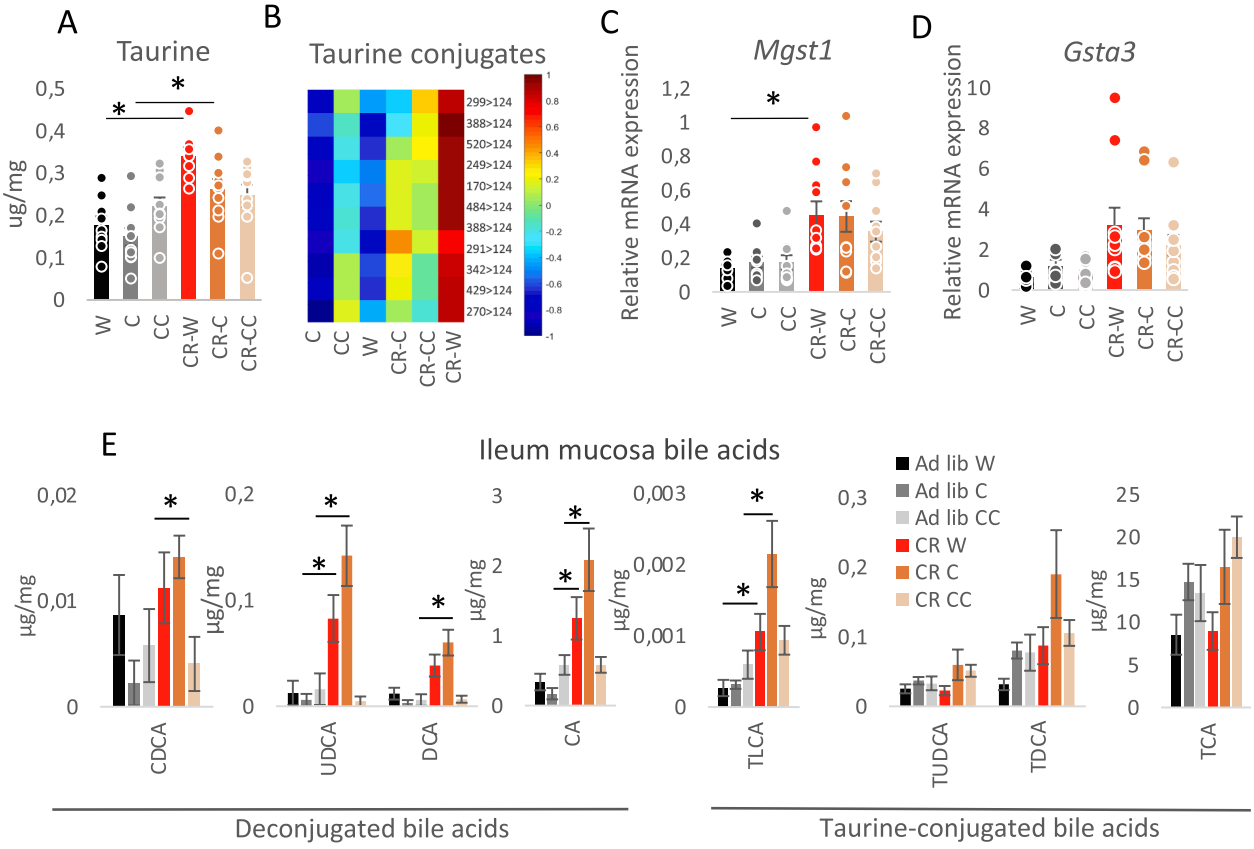

Fig. 2. Consumption of cage bedding impacts levels of taurine, taurine conjugates, and bile acids (BA). Mice were housed with wooden (W), cellulose (C), or corncob (CC) bedding and divided into *ad libitum* or CR groups. The levels of free taurine (A), taurine conjugates (B), and mRNA expression of *Mgst1* (C) and *Gsta3* (D), as well as BAs (E) were measured in the ileum mucosa of the animals. CA: cholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; DHCA: dehydrocholic acid; TCA: taurocholic acid; TDCA: taurodeoxycholic acid; TLCA: taurolithocholic acid; TUDCA: tauroursodeoxycholic acid; UDCA ursodeoxycholic acid; *Gsta3*: GSH S-transferase α 3; *Mgst1*: microsomal GSH S-transferase 1. The experimental groups were compared using ANOVA and * indicates statistical significance. Bars represent the mean of eight to nine biological replicates ±SEM.

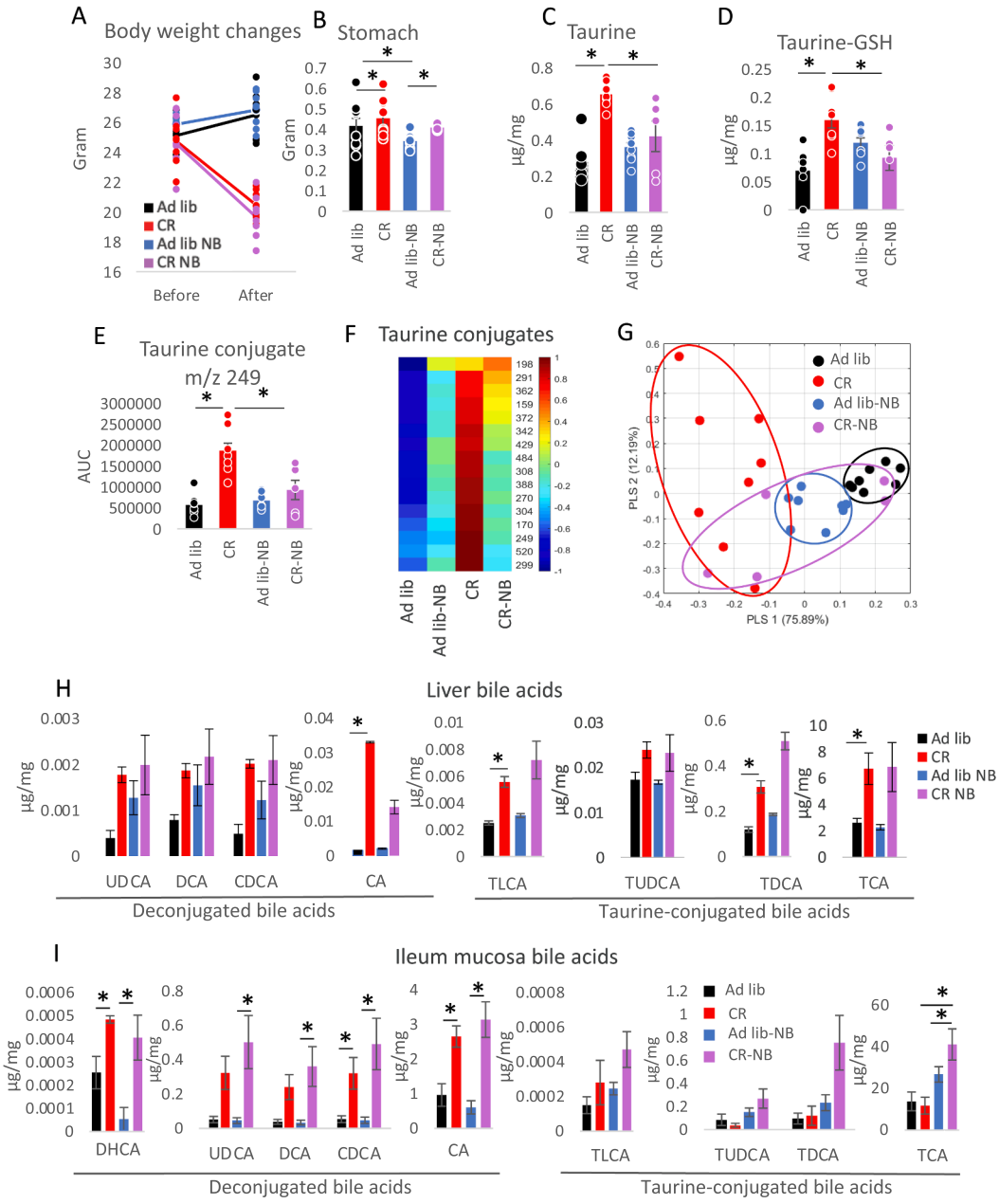

Fig. 3. Cage bedding triggers BAs deconjugation in CR mice intestine. Mice were submitted *ad libitum* feeding or CR and divided into groups housed with or without cage bedding (NB). Mice's body (A) and stomach (B) weight were measured. The levels of taurine (C), taurine-GSH (D), taurine conjugate *m/z* 249 (E), and taurine conjugates (F) levels were measured in the intestinal mucosa. Taurine conjugates are depicted in PLS chart (G). BAs levels were assessed in the ileum mucosa (H) and liver (I) of *ad libitum*-fed and CR mice housed with or without (NB) cage bedding. ANOVA was applied to assess statistical differences between the groups. * indicates statistical significance. Error bars represent ±SEM.

antibiotics in order to deplete gut flora. Mice from the FT groups (n=8) were gavaged twice with 200 µl of an antibiotic cocktail (vancomycin 0.5 g/l, neomycin 1 g/l, ampicillin 1 g/l, metronidazole 1 g/l; all from Sigma-Aldrich, Vienna, Austria). The antibiotics were given 5 and 3 days before the microbiota transplant. Afterward, the mice from the FT groups were gavaged twice at a 2-day interval with freshly extracted fecal microbiota from CR or *ad libitum* mice housed with wooden bedding (n=4). To obtain inoculants for FT, fresh fecal pellets were mixed with sterile PBS. The mixture was vortexed and centrifuged for 3 min at 1000 ×g, and the isolated supernatant was immediately gavaged into FT mice. FT mice were killed seven days after the first gavage.

Experiment 5: In order to inhibit BSH activity, *ad libitum* and CR mice (n=8 per group) were gavaged caffeic acid phenethyl ester (CAPE) (Zhong et al., 2022) 30 mg/kg daily for 14 days (Supplementary Figure 3A). The control *ad libitum* and CR mice received vehicle gavage.

Experiment 6: For the high-fiber diet experiment (Supplementary Figure 3D), mice were fed *ad libitum* control chow, high cellulose (20%), or high-soluble fiber diets (10% oligofructose and 10% pectin) for six weeks (n=8 per group). The diets were custom-made by SSNIFF-Spezialdiäten GmbH. The composition of the diets is available in Supplementary Table 1.

All animal experimentation protocols were approved by the Bundesministerium für Wissenschaft, Forschung und Wirtschaft, Referat für Tierversuche und Gentechnik (BMBWF-66.006/0008-V/3b/2018, BMWFW-66.006/2020, and 2022–0.257.032). All experiments were carried out according to animal experimentation Animal Welfare Act

guidelines.

2.2. Detection and identification of taurine, taurine conjugates, and GSH

The protocol for taurine, taurine conjugates, and GSH analysis was performed as published previously (Gregor et al., 2021). Briefly, 7–10 mg of scraped mucosa of the intestine was homogenized with a syringe. Additionally, the samples were disrupted by freezing the samples on dry ice with 70% ethanol followed by thawing in a 37 °C waterbath repeated in five cycles. Following that, based on the samples weight, nine times the volume of ice cold 100% ethanol was added, the suspension was vortexed, centrifuged for 10 min at 18,000 g at 4 °C, and the supernatants were analyzed using an LCMS-8040 (Shimadzu Corporation, Kyoto, Japan) with an Atlantis T3 3 μm column (2.1x150 mm, Waters, Milford, MA, USA). The column temperature was 40 °C. Water with 0.1% formic acid and acetonitril with 0.1% formic acid were used as solvents A and B, respectively.

2.3. Assessment of BAs deconjugation by fecal bacteria

Fresh feces were collected and 10 mg was minced in 240 μl Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich) containing taurine conjugated-BA mixture including taurocholic acid (TCA), taurolithocholic acid (TLCA), tauroursodeoxycholic acid (TUDCA), and taurodeoxycholic acid (TDCA) (each 1 μg per reaction; all Sigma-Aldrich). The reaction was incubated in a 37 °C water bath for 30 min. Afterward, the samples were spun down and the supernatant was collected, evaporated, and frozen at -20 °C until measurement of BAs levels.

2.4. Bile acid analysis

BAs were measured as reported previously (Gregor et al., 2021). Briefly, liver samples were homogenized in Precellys tubes with 1.4 mm ceramic beads, and nine times the volume of ice cold 100% methanol was added. After homogenization in the Precellys 24 Tissue Homogenizer (Bertin Instruments, Montigny-le-Bretonneux, France) samples were incubated for 10 min shaking on ice, vortexed, and centrifuged for 10 min at 12,000 g at 4 °C. To remove the remaining debris, the supernatants were transferred to Eppendorf tubes and centrifuged again for 10 min at 12,000 g at 4 °C. Supernatants were transferred into HPLC vials and stored at 4 °C until measurement. Intestinal samples were processed as described above in the method for GSH and taurine analysis. Plasma samples (50 μl) were extracted with 150 μl 100% methanol, vortexed for 30 s, and shaken on ice for 10 min with a laboratory rocker. 100 μl of the supernatant was evaporated, re-dissolved in 50 μl methanol, and transferred into an HPLC vial. Samples transferred to HPLC vials were handled alike. Analysis was conducted in positive modus using an LCMS-8040 Liquid Chromatograph Mass Spectrometer (Shimadzu Corporation, Kyoto, Japan) with an Atlantis T3 3 μm column (2.1 × 150 mm, Waters, Milford, MA, USA). The column temperature was 30 °C. Solvent A consisted of water and solvent B consisted of acetonitrile/methanol (3/1, v/v), both contained 0.1% formic acid and 20 mmol/l ammonium acetate. The solvent gradient was 30% B for 5 min, then it was increased to 100% B at 25 min and was kept constant for 20 min. For re-equilibration, the composition was set back to the initial ratio of 30% B for 10 min.

2.5. Ex vivo intestinal sacs assay

The freshly dissected ileum was flushed with PBS, and ends were loosely tied with a thread leaving a four cm-long sac. The intestine was filled with 200 μl taurine (25 mg/ml) and GSH (61.5 mg/ml) solution. The sacs were closed tightly and incubated in a 37 °C water bath in 10 ml of prewarmed Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich, St. Louis, MO, USA). Medium samples were collected over 2 h for measurement of taurine transport.

2.6. Gene expression analysis

RNA from the liver and intestine was isolated using the RNeasy mini kit (Qiagen, Hilden, Germany). SuperScript® II Reverse Transcriptase (Invitrogen™, Life Technologies, Carlsbad, CA, USA) was used for reverse transcription. Quantitative real-time PCR (qRT-PCR) reactions were performed using the QuantStudio™ 6 Flex Real-Time PCR System with the SYBR Green PCR Master Mix (both from Applied Biosystems, Life Technologies, Carlsbad, CA, USA). *Eef1a1* was used as a housekeeping gene. The sequences of the primers used are shown in Supplementary Table 2.

2.7. Fecal microbiota characterization

Fecal pellets were collected a day before dissection from mice from all four experimental groups of *experiment 3* (see section 4.1.): *ad libitum* (n=8), CR (n=8), *ad libitum* NB (n=8) and CR NB (n=8) (Supplementary Figure 2A), snap frozen and stored at -80 °C until further processing. 16S rRNA gene sequencing of the fecal microbiota was performed at the Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna (JMF) under the project ID JMF-2206–08. Briefly, DNA from fecal material was extracted with the QIAamp Fast DNA Stool Mini Kit (Qiagen, Germany) following manufacturer's instructions. Amplicons were generated using primers targeting the V4 region of bacterial and archaeal 16S rRNA genes (Apprill et al., 2015; Parada, Needham, & Fuhrman, 2016), barcoded in a unique dual setup, and sequenced on the Illumina MiSeq system as further described by Pjevac et al. (Pjevac et al., 2021). After sequencing, amplicon sequence variants (ASVs) were inferred (Callahan, Sankaran, Fukuyama, McMurdie, & Holmes, 2016) and classified following the analysis workflow detailed by Pjevac et al. (Pjevac et al., 2021).

Downstream analyses were performed using R v4.2.1 and Bioconductor v3.16 packages SummarizedExperiment v1.28, SingleCellExperiment v1.20, TreeSummarizedExperiment v2.6 (Huang et al., 2020), mia v1.6 (https://github.com/microbiome/mia), vegan v2.6–4 (https://CRAN.R-project.org/package=vegan), phyloseq v1.42 (McMurdie & Holmes, 2013), microbiome v1.20 (https://microbiome.github.io), microViz v0.10.5 (Barnett, Arts, & Penders, 2021), DESeq2 v1.38.3 (Love, Huber, & Anders, 2014), and ALDEx2 v1.30 (Fernandes et al., 2014). Alpha diversity was calculated on rarified data (5428 read pairs per sample) using vegan and mia. Beta diversity was calculated by performing a PCoA on an Aitchison distance matrix using microViz. The difference in per-group centroids was tested with a PERMANOVA on an Aitchison distance using vegan and microViz. Pairwise differential ASV abundance testing was performed using DESeq2 with alpha=0.05 and otherwise default parameters after adding a pseudocount of 1 to the data. Correlation between ASV centered log ratio transformed counts and environmental variables was calculated for the top most 20 abundant ASVs using ALDEx2′s correlation test using Pearson correlation with FDR multiple testing correction.

2.8. Data availability

The microbiota sequencing datasets for *experiment 2* in the European Nucleotide Archive [https://www.ebi.ac.uk/ena/browser/view/PRJEB37837]. This dataset has been generated and made available together with our previous publication (Gregor et al., 2020). However, the data presented in this manuscript have not been published before.

Amplicon sequencing datasets generated in this study for *experiment 3* are deposited in the NCBI SRA (https://www.ncbi.nlm.nih.gov/sra/) under the BioProject accession numbers PRJNA928685.

2.9. Statistics

Heatmaps comparing the levels of taurine conjugates between experimental groups were created using Z-Scored data, showing the relative deviation from the groups' mean value, and visualized using the MATLAB extension COVAIN. The heatmaps were designed to arrange groups according to their similarity in terms of the pattern of the occurrence of the measured metabolites.

For analysis of statistical differences between more than three groups, one-way ANOVA with Bonferroni correction for multiple testing was applied using SPSS Statistics 26 (IBM Corp., Armonk, NY, USA). Data sets with two or three groups were compared using a two-sided Student's *t*-test and a p-value lower than 0.05 was considered statistically significant.

3. Results

3.1. Restrictive diets, in particular CR, increase the levels of free taurine and its conjugates in the intestinal mucosa

We reported previously that CR increases the level of taurine and its conjugates, including taurine-GSH, in the intestinal mucosa (Gregor et al., 2021). In order to verify if other restrictive diet conditions could also trigger this phenotype, mice were submitted to various restrictive diets: CR, IF, and FMD (Suppl Fig. 1A). All of the diets triggered an increase in taurine (Fig. 1A), and CR raised taurine-GSH conjugate levels (Fig. 1B) in the ileum mucosa. CR showed the most substantial impact on the level of taurine, taurine-GSH, as well as *TauT* mRNA expression (Fig. 1A-C). In accordance, the expression of GSH S-transferases (GST) (*Mgst1* and *Gsta3*) tended to be elevated; however, statistically significant changes were observed only for IF (Fig. 1C). Also, the levels of multiple taurine conjugates in the mucosa increased following the restrictive diets; however, the impact was not even in terms of types of conjugates and the magnitude of increase (Fig. 1D). The occurrence of an unidentified taurine conjugate with *m/z* 249, which is the most abundant of all detected taurine conjugates, distinguished the diets the most (Fig. 1E). This conjugate closely followed the pattern of free taurine concentration changes, with increased levels for CR and IF (Fig. 1F). Overall, CR resulted in the greatest changes in taurine conjugates composition, resulting in the most distinct profile of all restrictive diets compared to *ad libitum* feeding (Fig. 1G). Simultaneously, the CR mice had the lowest body weight at the end of the experiment (Fig. 1H), suggesting the strictest overall energy restriction. CR mice also had the heaviest stomachs (Fig. 1I) despite the last food portion being delivered to them ca. 14 h before the dissection. Corresponding with increased levels of taurine in the intestine, the expression of genes coding *Cdo*, one of the taurine-producing factors, was increased in the liver of mice from all restrictive diet groups (Fig. 1J).

3.2. Cage bedding influences the levels of taurine, its conjugates, and BAs in the intestinal mucosa of CR mice

As we have shown previously, mice submitted to restrictive diets consume cage bedding, which influences the outcomes of CR and overnight fasting (Gregor et al., 2020). We housed *ad libitum*-fed and CR mice with one of three different beddings: wooden (W), cellulose (C), and corncob (CC) (Suppl Fig. 1B). Cage bedding affected CR-triggered increase in taurine concentration in the intestinal epithelium (Fig. 2A). W bedding stimulated the increase in taurine concentration in CR mice the strongest. Accordingly, the levels of taurine conjugates were much higher in CR-W compared to other CR and *ad libitum* groups (Fig. 2B). The occurrence of conjugate with *m/z* 249 again showed a similar pattern as taurine (Suppl Fig. 1C). This conjugate was once more the most differentiating factor for all the experimental groups (Suppl Fig. 1D). CR-W mice showed the strongest trend among CR groups to stimulate *TauT* expression (Suppl Fig. 1E). Taurine-GSH conjugate levels partially followed the taurine occurrence pattern; however, the results were not statistically significant (Suppl Fig. 1F). Nevertheless, the expression pattern of GSTs (*Mgst1*, *Gsta3*) clearly pictured the impact of the bedding mediated via GSH; however, the differences were statistically significant only for CR *Mgst1* (Fig. 2C-D).

Furthermore, bedding also influenced the composition of BAs. W and C beddings showed the strongest stimulating impact increasing the levels of BAs in the intestinal epithelium (Fig. 2E). CC bedding triggered a much milder increase in BAs in the intestine, which contrasts particularly strongly with the results for W and C in the case of ursodeoxycholic acid (UDCA), deoxycholic acid (DCA), and cholic acid (CA) (Fig. 2E). Importantly, the increase in BAs levels in the ileal mucosa of CR mice was observed for deconjugated BAs and much less for taurine-conjugated BAs (Fig. 2E). However, the levels of both deconjugated as well as conjugated BAs were statistically significantly increased in the liver of CR mice (Suppl Fig. 1G), indicating a high rate of BAs deconjugation upon secretion into the small intestine.

3.3. The presence of cage bedding is responsible for increased levels of taurine in the intestinal mucosa and BAs deconjugation during CR

In order to verify the contribution of cage bedding to the observed phenotype, we submitted mice to *ad libitum* or CR feeding while housing in standard conditions with wooden bedding or in cages without cage bedding (NB) (Suppl Fig. 2A). Both CR and CR NB groups lost comparable weight during 14 days of the dietary regime (Fig. 3A). CR mice had heavier stomachs than *ad libitum* mice (Fig. 2B), suggesting accumulation of hard-to-digest mass, usually cage bedding. Surprisingly, the difference was also present for mice housed without bedding, implying that CR NB mice likely also consumed feces and fur. Removal of bedding neutralized the differences between *ad libitum* and CR mice in terms of the concentrations of taurine (Fig. 3C), taurine-GSH (Fig. 3D), *m/z* 249 conjugate (Fig. 3E), as well as other taurine conjugates (Fig. 3F) in the intestine mucosa. In fact, the composition of taurine conjugates differentiated the CR-NB group taurine conjugates profile from CR and shifted in the direction of *ad libitum* (Fig. 3G).

In the liver, CR stimulated levels of BAs regardless of the presence of bedding (Fig. 3H). Importantly, the levels of taurine-conjugated BAs in the liver (Fig. 3H) were elevated, which is in contrast to the measurements in the intestine mucosa (Fig. 3I). In the ileal mucosa, the level of unconjugated BAs was comparable between the ileal epithelium of CR mice housed with or without cage bedding (Fig. 3I). However, the levels of taurine-conjugated BAs were much higher in CR NB compared to CR and Ad lib groups. These results align well with the lack of increase in free taurine and its conjugates (Fig. 3D-F) levels in CR NB mucosa, further indicating that the taurine-conjugated BAs are produced in the liver and deconjugated in the conditions where cage bedding is present. When bedding consumption is circumvented, the deconjugation rate is reduced.

3.4. The presence of cage bedding is responsible for the majority of fecal microbiota composition changes during CR

Since gut microbiota is responsible for BAs deconjugation, we assessed changes in fecal microbial composition in the *ad libitum*, CR, *ad libitum* NB, and CR NB groups via 16S rRNA gene amplicon sequencing. We showed that CR impacts the fecal microbiota composition, resulting in an increased species richness (Fig. 4A) and significantly distinct microbial community composition in CR mice (Fig. 4B), confirming our previous report (Duszka et al., 2018). Differential abundance analysis indicated that, in particular, ASVs affiliated with the genera *Mucispirillum*, *Parabacteroides*, and *Prevotellacea* were significantly more relatively abundant under CR, while ASVs affiliated with the order *Gastronanerophilales*, the family *Rikenellaceae,* the ASVs affiliated to various genera amongst the *Firmicutes* were relatively less abundant under CR (Fig. 4C). Importantly, the removal of cage bedding reduced

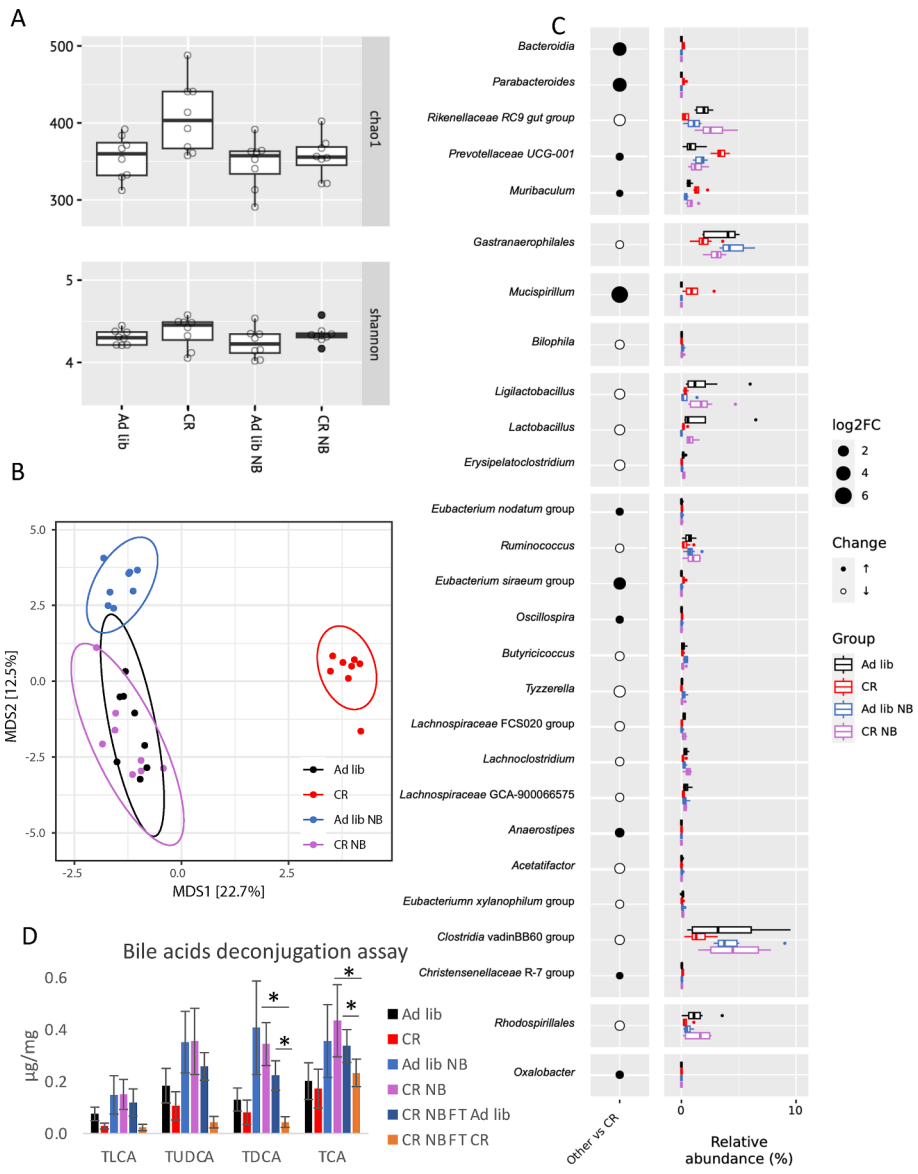

Fig. 4. Microbial community analysis to assess the effect of bedding consumption on intestinal microbiota under CR. Fecal pellets were collected from ad lib, CR, ad lib NB, and CR NB animals. The samples from eight replicates per experimental group were analyzed for microbial composition using Illumina MiSeq sequencing. Species richness (chao 1) and alpha diversity (Shannon) (A), and microbial community beta diversity (B) of the four treatment groups were analyzed. The relative abundance of taxonomic groups that were statistically significantly more or less relatively abundant in the CR group when compared to all other treatment groups is displayed in panel C. For the following experiment, besides ad lib, CR, ad lib NB, and CR NB groups, additional CR groups were housed without cage bedding and received fecal transplants from *ad libitum* (CR NB FT Ad lib) or CR (CR NB FT CR) mice housed with bedding. Fresh fecal samples were collected, disrupted, and incubated with taurine-conjugated BAs. The levels of taurine-conjugated BAs remaining after 30 min incubation were measured to estimate the deconjugation rate (D). Microbiota sequencing results were analyzed using R v4.2.1 and Bioconductor v3.16 packages Alpha diversity was calculated on rarified data using vegan and mia. Beta diversity was calculated by performing a PCoA on an Aitchison distance matrix using microViz. The difference in per-group centroids was tested with a PERMANOVA on an Aitchison distance using vegan and microViz. ANOVA was applied to assess statistical differences between the groups in panel D; * represents statistical significance n=8 in panels A-C, n=4 (ad lib and CR fecal transplant donors) and n=8 (Ad lib-NB, CR-NB, CR NB FT Ad lib, and CR NB FT CR groups) in panel D.

the impact of CR, resulting in fecal microbiota composition of CR NB mice that much closer resembles the *ad libitum* group (Fig. 4B).

The abundance of *Mucispirillum* and *Parabacteroides* was also increased in the cecum of CR mice housed with W, C, and CC beddings (Suppl Fig. 2 B-C). Importantly, the abundance pattern of *Parabacteroides* in CR groups with different beddings fits well with free taurine and taurine conjugates levels (Fig. 2A-B) as well as GSTs expression in CR groups (Fig. 2C-D). Taken together, these results underline our previous observations on the strong impact of cage bedding on CR outcomes (Gregor et al., 2020).

Next, the role of the intestinal microbiota in response to fiber and the modulation of taurine levels was assessed. Firstly, *ad libitum* and CR mice were housed with or without cage bedding. Additionally, two groups of CR mice housed without bedding received FT from *ad libitum* fed with bedding (CR NB FT Ad lib) or CR with bedding (CR NB FT CR) (Suppl Fig. 2D). Fecal samples from all six groups were collected to assess the capacity of microbiota to deconjugate BAs. The pellets were minced in a solution containing a mix of taurine-conjugated BAs, including TLCA, TUDCA, TDCA, and TCA. Afterward, the reaction was incubated at 37C for 30 min, and taurine-conjugated and deconjugated BAs were measured. Solutions containing samples from CR mice tended to have reduced levels of conjugated BAs compared to *ad libitum*; however, the observed differences were not statistically significant (Fig. 4D). Incubation of fecal samples from *ad libitum* NB and CR NB mice showed no difference in taurine-BAs levels in the assessed reaction solutions, and the BAs levels remained high compared to other tested groups (Fig. 4D). Most importantly, FT from CR mice triggered a strong deconjugating capacity of fecal samples compared to FT from the *ad libitum* group (Fig. 4D).

3.5. Bacterial BSH is responsible for BAs deconjugation and release of taurine during CR

To challenge the contribution of microbial BSH to the observed phenotype, *ad libitum* and CR mice were gavaged with BSH inhibitor (BSHi) CAPE daily for 14 days (Suppl Fig. 3A). BSHi treatment did not impact levels of most deconjugated BAs besides CA (Fig. 5A). However, it increased the levels of taurine-conjugated BAs in the ileum mucosa of *ad libitum* and CR animals (Fig. 5A) without impacting the typical *ad libitum* and CR pattern of BAs levels (Fig. 5B) and gene expression (Fig. 5C) in the liver. And so, the expression of genes connected with taurine synthesis (*Cdo*), taurine conjugation to BAs (*Bal*), BA production (*Cyp7a1*), and transport (*Ntcp*), were increased in CR compared to *ad libitum* (Fig. 5C), while mRNA levels of a BA synthesis inhibitor (*Shp*)

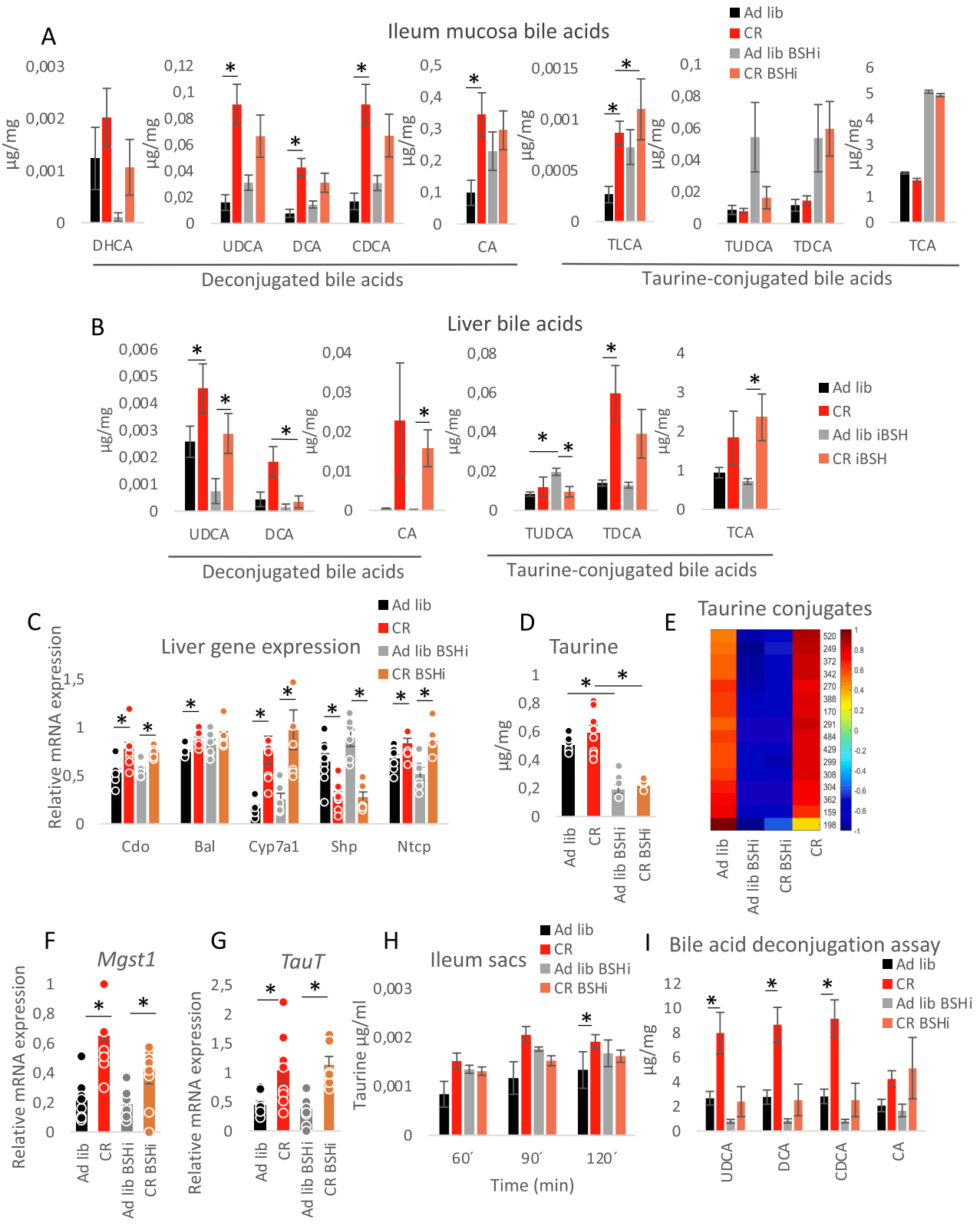

Fig. 5. Bacterial BSHs deconjugate BAs upon cage bedding indigestion. The concentration of BAs was measured in the ileum mucosa (A) and liver (B) of mice submitted *ad libitum* feeding or CR and gavaged vehicle solution or caffeic acid phenethyl ester (CAPE) daily to inhibit bile salt hydrolase (BSH). The expression of *Cdo*, *Bal*, *Cyp7a1*, *Shp*, and *Ntcp* genes was measured in the mouse liver using qRT-PCR (C). The levels of taurine (D) and its conjugates (E) were assessed in the intestinal mucosa. Expression of *Mgst1* and *TauT* mRNA was measured in the mucosa of the ileum (F and G). *Ex vivo* intestine sacs assay was applied to measure taurine uptake (H). Freshly collected fecal samples were incubated with taurine-conjugated BAs, and the appearance of deconjugated BAs was measured after 30 min incubation (I). *Bal*: bile acid CoA ligase; *Cyp7a1*: cholesterol 7 a-hydroxylase; *Ntcp*: Na+/taurocholate cotransporting polypeptide; *Shp*: small heterodimer partner; *Taut*: taurine transporter. ANOVA was applied to assess statistical differences between the groups. * represents statistical significance; n=8 in panels A-H, n=4 in panel I. Error bars stand for ±SEM.

were reduced in the liver of CR mice (Fig. 5C). In accordance with the reduced deconjugation of BAs (Fig. 5A), the levels of free taurine (Fig. 5D) and taurine conjugates (Fig. 5E) decreased in the mucosa of the ileum. Among them, the taurine conjugate *m/z* 249 (Suppl Fig. 3B) and taurine-GSH (Suppl Fig. 3C) were affected. Despite the lack of changes in CR vs. *ad libitum* expression pattern of *Mgst1* (Fig. 5F) and *TauT* (Fig. 5G) upon BSHi treatment, the CR-specific increase in uptake of taurine from the intestine was not observed in CR BSHi animals when assessed in *ex vivo* sacs assay (Fig. 5H), suggesting that an increase in taurine concentration is needed to stimulate taurine uptake. Next, mice feces were incubated with a mix of taurine-conjugated BAs (TUDCA, TDCA, TCDCA, and TCA) followed by a measurement of corresponding deconjugated BAs (UDCA, DCA, CDCA, and CA). The results indicated a much stronger increase in deconjugated BAs in samples from CR mice compared to *ad libitum*, whereas the deconjugating capacity of samples from CR BSHi animals was reduced (Fig. 5I).

3.6. Fiber stimulates deconjugation but not secretion of BAs

To verify if it is the fiber in cage bedding that influences BAs deconjugation, mice were fed standard chow, high-cellulose (C; 20% cellulose), or high-soluble fiber (S; 10% oligofructose and 10% pectin) diets (Suppl Fig. 3E). High-fiber feeding triggered an increase in free taurine (Fig. 6A) as well as several taurine conjugates (Fig. 6B), including taurine-GSH (Fig. 6C) levels in the intestine epithelium. Soluble fiber had a greater impact on conjugates level compared to

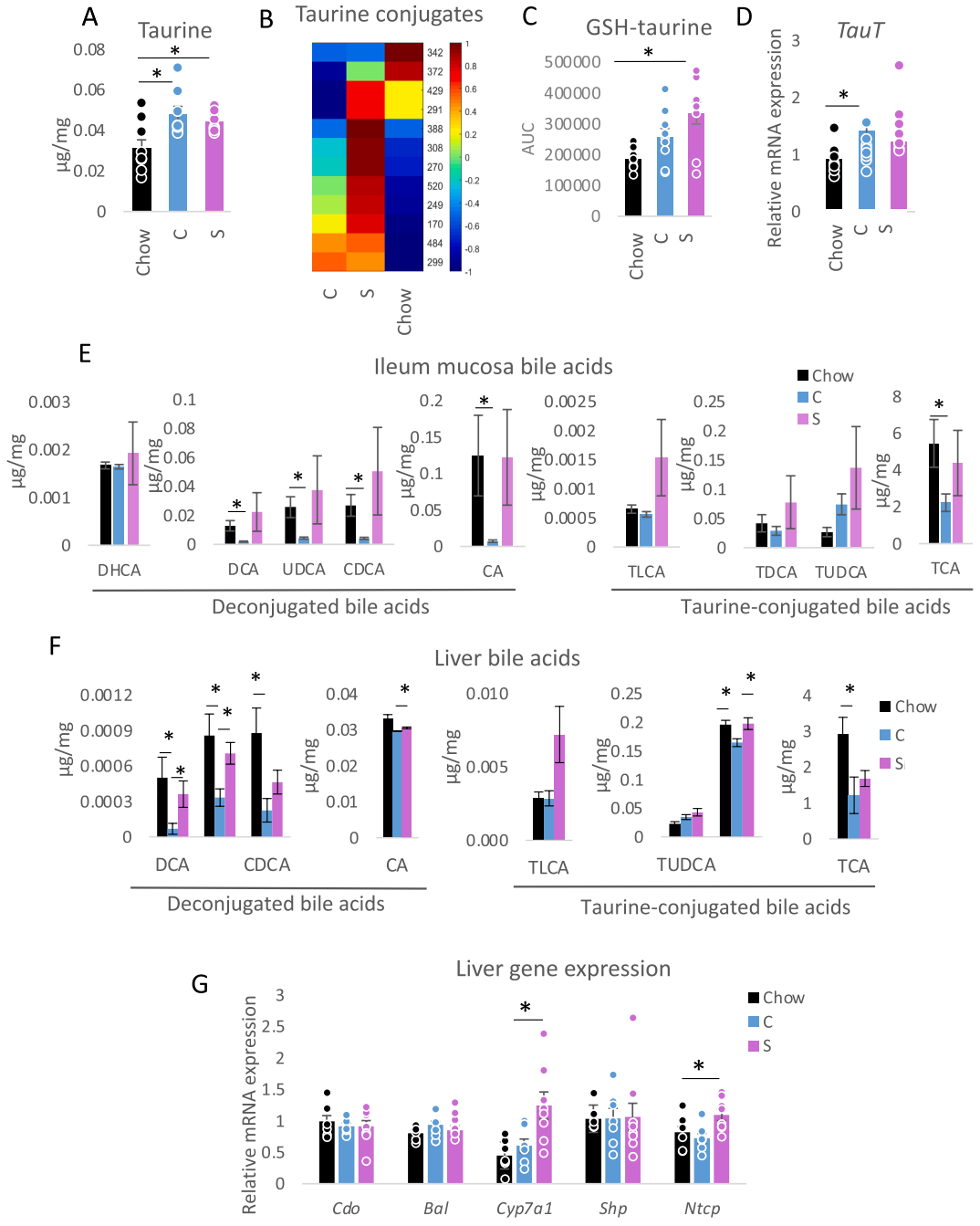

Fig. 6. Fiber consumption stimulates BAs deconjugation. The levels of taurine (A), its conjugates (B), GSH-taurine (C), mRNA of *TauT* (D), and BAs (E) were measured in the ileum mucosa of mice fed chow, high-cellulose (C), or high-soluble (S) fiber diets. BA concentration (F) and gene expression (G) were also assessed in the liver (F). Two-tailed Student's *t*-test was applied to assess statistical differences between the groups; *p<0.05; n=8. Error bars represent ±SEM.

cellulose. Also, only soluble fiber supplementation resulted in an increased *Mgst1* expression (Suppl Fig. 3F). While the high-cellulose diet stronger increased intestinal *TauT* mRNA expression (Fig. 6D). Furthermore, the high-cellulose diet resulted in decreased levels of most BAs in the ileal epithelium (Fig. 6E). Following the intestinal pattern, hepatic levels of several BAs decreased when challenged with a diet high in cellulose (Fig. 6F). Importantly, fiber consumption did not increase the levels of any BAs in the liver (Fig. 6F). The diets also did not affect the expression of genes connected with taurine production (*Cdo*) and taurine conjugation to BAs (*Bal*) in the liver (Fig. 6G). The mRNA expression of the positive regulator of BAs synthesis *Cyp7a1* was elevated in the C group, while the levels of mRNA of the negative regulator *Shp* were not changed (Fig. 6G). Finally, the mRNA of BA absorptive *Ntcp* was higher in the liver of the S compared to the C group (Fig. 6G).

4. Conclusions

In summary, we show that various types of energy restrictions trigger an increase in taurine levels and its conjugates in the small intestine of mice, relative to weight loss and hunger. Further, the presence and type of cage bedding affect the levels of taurine and BAs in the ileal epithelium. Next, the gut microbiota is affected by cage bedding and stimulates CR- and fiber consumption-associated deconjugation of taurine-conjugated BAs. Our results suggest that bacteria-derived BSH deconjugates BAs and releases free taurine. Finally, we prove that solely fiber consumption without CR can stimulate the occurrence of taurine conjugates in the ileal mucosa.

As we showed previously (Gregor et al., 2020), during CR, mice elevate their bedding consumption to up to 8% of their body weight daily, thus considerably increasing fiber intake. Also, IF and FMD, same as CR, increase BAs levels in the intestine (Gregor et al., 2022), and taurine-conjugated BAs secreted upon CR are deconjugated in the intestine (Gregor et al., 2021). Therefore, the presented results build upon our previous data but also further support it and show that any type of dietary restriction that triggers hunger in rodents, likely increases cage bedding and, thus, fiber intake. Consequently, BAs secreted into the GI tract are deconjugated by fiber-stimulated, BSH-active bacteria. However, here assessed diets differed in the level of restriction and the resulting hunger, which was reflected in body weight loss and stomach weight and likely influenced the amount of bedding consumed. This was further manifested as distinct levels of taurine and its conjugates in the intestinal mucosa. This conclusion is supported by the fact that the heaviest stomachs have been previously measured in CR-W bedding mice compared to other beddings, which corresponded to the highest bedding intake (Gregor et al., 2020). Surprisingly, we also observed an increase in stomach weight for mice housed without cage bedding. This likely indicates coprophagy and increased fur intake, which based on taurine and BAs levels, do not affect the main investigated phenotype.

The link between the amount of bedding consumed and the levels of taurine points toward microbiota as a mediator of the investigated phenotype. This further support our earlier report (Gregor et al., 2021), which indicated that antibiotics treatment neutralized CR-triggered increase in taurine levels while microbiota transplant from CR mice partly mimicked it. Similarly, in this study, microbiota transplant influenced fecal deconjugation of BAs. Intriguingly, deconjugation was much more efficient in microbiota recipients than donors. Likely, antibiotics treatment preceding the transplant removed the majority of commensal bacteria, creating conditions for effective colonization by CR-specific microbes with high BSHs activity.

Importantly, the assay that we applied to assess bacterial BSH activity does not deliver direct evidence for BAs deconjugation. As a proxy for BSH activity, we applied the changes in the levels of taurine-conjugated BAs before and after incubation with mice feces. Regrettably, we did not account for other types of microbial modifications of BAs, including re-conjugation to other molecules, generation of secondary transformation, dehydrogenation, etc. Despite the imperfectness of this assay, our other measurements indicating differences between animal groups in various experimental conditions in terms of BAs conjugation status as well as taurine levels, support the conclusions drawn from this assay.

Since multiple strains express BSH (Archer et al., 1982; Gilliland & Speck, 1977; Jones et al., 2008; Ridlon et al., 2006), it was surprising to detect only several ASVs positively responding to CR with bedding. Moreover, *Parabacteroides* is the only significantly affected AVS whose abundance follows taurine levels upon housing with different beddings. Nevertheless, more in-depth studies will be required to establish causality. Particularly applicable would be an experiment involving the supplementation of a range of diets containing various types of fibers and correlating outcomes in terms of bacteria abundance, BSH expression, and activity. Ultimately, inoculation with specific bacterial strains and verification of BAs composition will be needed.

Importantly, one of the limitations of our study concerns the choice of samples used for microbiota sequencing. For technical and feasibility reasons, we have chosen feces instead of ileum content. Clearly, ileum content would deliver more accurate information concerning bacteria directly involved in BAs deconjugation in the small intestine, and our aim was not to use fecal microbiota as a proxy for the whole GI tract. However, we were still able to prove the main idea that the presence of cage bedding has a much stronger impact on bacteria composition compared to CR. Nevertheless, a follow-up analysis of the intestinal microbiota will be required to identify bacteria holding fiber-activated BSH.

The difference in the impact of W, C, and CC cage beddings on the investigated CR phenotype shows that the fiber type may influence BAs' intestinal level and/or taurine deconjugation. Therefore, we performed an experiment feeding mice two contrasting types of fiber. Both types did not affect the levels of BAs in the liver. However, cellulose, contrary to soluble fiber, strongly decreased the levels of BAs in the intestine and liver. We conclude that it is due to a well-recognized feature of fiber relying on binding and helping remove BAs with feces, an attribute accountable for the cholesterol level-reducing properties of fiber (Soliman, 2019). Importantly, contrary to CR, fiber consumption did not increase the level of BAs.

The presented results indicate that feeding high-fiber diets triggers an increase in taurine and taurine conjugates levels, reflecting a fiber-elicited increase in BSH activity. We also show that enhanced BA production and secretion into the intestine requires a restrictive diet, while *ad libitum* fiber consumption does not stimulate it. Therefore, the relatively mild increase in taurine levels likely results from *ad libitum* feeding and consequent lack of restriction-prompted increase in taurine-conjugated BAs, which would provide a substrate for BSHs. The mechanism behind the stimulatory impact of CR on the production and secretion of BAs is currently under investigation.

A recent report showed increased BSH activity upon supplementation with soluble fiber but not following cellulose intake (Kastl et al., 2022). Furthermore, the authors proved a correlation between microbial glycoside hydrolases and BSH activity in response to dietary fiber. However, the background behind the coregulation of the two enzymes is not known. In our study, we similarly see a stronger impact of soluble fiber on the investigated phenotype but also an increase in taurine, taurine conjugates, and *TauT* expression upon cellulose supplementation. Additionally, our results from the fiber-supplementation study are supported by another experiment showing that consumption of wooden and cellulose beddings, which are high in insoluble fiber, increased deconjugated BAs, free taurine, and taurine conjugates levels.

BSH is capable of deconjugating both glycine and taurine from BAs (Begley, Gahan, & Hill, 2005). However, in our previous study, we measured an increase specifically in the levels of free taurine but not glycine in the intestine of CR mice. In this context, it is important to notice that in mice, only 5% of bile acids are conjugated to glycine and 95% to taurine. Therefore, in humans, where approximately 70% of BAs

are conjugated to glycine, the outcome of fiber consumption or increased BSH activity may differ from the one measured in mice. Kastl et al. (Kastl et al., 2022) investigated the impact of fiber supplementation in human subjects and observed changes in the levels of taurine as well as glycine-conjugated BAs, suggesting the impact on both types of BAs. However, the authors did not report measuring the levels of free taurine or glycine to provide a more direct indication that the observed changes were due to deconjugation and not resulting from other types of BAs' modifications.

Multiple consequences, as well as therapeutic applications of the modulation of BAs levels as well as conjugation status, are in sight. Firstly, deconjugation reduces the efficiency of BAs in emulsifying, digesting, and absorption of dietary lipids (De Smet et al., 1994). Consequently, microbial BSH activity has been linked to growth reduction in chickens (Feighner & Dashkevicz, 1987, 1988). Hence the adjustment of BSH activity in humans may aid body weight control. On the other hand, this regulation could potentially have important applications in the therapy of malabsorption, e.g., in the case of short bowel syndrome.

Further, conjugation with taurine or glycine changes the polarity and water solubility of BAs, thus affecting its capacity for passive or transporter-supported reabsorption (Cohen & Carey, 1990; Hofmann & Roda, 1984). Unconjugated BAs bind with a lower affinity to the transporters resulting in enhanced fecal loss of BAs. Consequently, increased demand for cholesterol for *de novo* BAs synthesis lowers serum cholesterol levels. Therefore, BSH activation may be one of the mechanisms contributing to the fiber-mediated reduction in cholesterol levels, as confirmed by the fact that administration of BSH-active bacteria reduces serum cholesterol levels in pigs, minipigs, and mice (Chikai, Nakao, & Uchida, 1987; De Smet, De Boever, & Verstraete, 1998; De Smet et al., 1994; du Toit et al., 1998; Pereira, McCartney, & Gibson, 2003; Tannock, 1995). Adjustment of the levels of BAs may have application in the therapy of multiple diseases, including various subtypes of inflammatory bowel disease, some characterized by reduced while others by excessive BAs levels.

Furthermore, deconjugation increases the antibacterial potency of BAs (Mullish et al., 2019; Sannasiddappa, Lund, & Clarke, 2017). Therefore, fiber supplementation or BSH-active probiotics may have direct applications supporting the therapy of microbial gut infections.

Importantly, deconjugation is considered a prerequisite for some of the enzymatic reactions converting primary to secondary BAs and therefore affect the levels of ligands for various BAs receptors (Batta et al., 1990; Stellwag & Hylemon, 1979; White et al., 1980). CDCA serves as the most potent ligand of the farnesoid X receptor (FXR), followed by CA, DCA, and LCA. While taurine-conjugated MCAs are competitive FXR antagonists (Makishima et al., 1999; Parks et al., 1999; H. Wang, Chen, Hollister, Sowers, & Forman, 1999). The main function of FXR is the prevention of BAs synthesis within a negative feedback loop activated upon increased BAs levels. Applying tempol to selectively reduce the genus Lactobacillus and BSH activity leads to the accumulation of FXR antagonists. Consequently, tempol prevents FXR-driven CYP7a1 inhibition, resulting in increased BA production (Li et al., 2013; Sayin et al., 2013). Importantly, besides BAs synthesis, FXR takes part in the inflammatory processes as it reduces the expression of cytokines, production of leukotrienes, as well as NKT cell and NFkB activation (Gai et al., 2018; Mencarelli et al., 2009; Vavassori, Mencarelli, Renga, Distrutti, & Fiorucci, 2009).

Contrary to FXR, taurine conjugation increases BAs' affinity for Takeda G protein-coupled receptor 5 (TGR5) (Duboc, Tache, & Hofmann, 2014). The main outcomes of TGR5 signaling activity are connected with improved liver function, reduced hepatic inflammation, steatosis, and fibrosis. It also contributes to hepatic glucose metabolism, insulin signaling, maintaining metabolic homeostasis, as well as cell differentiation and proliferation (Guo, Chen, & Wang, 2016; Pols, Noriega, Nomura, Auwerx, & Schoonjans, 2011). Thus, depending on conjugation status, BAs will affect different processes via their receptors. In general, taurine-conjugated BAs are less toxic than unconjugated BAs and exhibit some beneficial properties. Particularly, TUDCA has broad therapeutic applications and it is more efficient in treating liver cirrhosis than its deconjugated counterpart UDCA (Azer, Canfield, & Stacey, 1995; Pan et al., 2013). Importantly, besides BSH activity, diet composition is a vital factor affecting taurine conjugation status. The ratio of glycoconjugates to tauroconjugates may shift to 9:1 in humans inhabiting rural Africa or reduce to 0.1:1 upon taurine supplementation (Hardison, 1978; Sjovall, 1959). Also, a high-fat diet increases levels of taurine-conjugated BAs (Devkota et al., 2012). Therefore, planning for the therapeutic application of BSH-activating fiber or BSH-expressing probiotics, it is important also to consider overall nutrition.

As shown previously, CR intensified the production of taurine-conjugated BAs in the liver and their secretion into the GI tract. The GI tract microbiota then deconjugates BAs, increasing free taurine levels. Therefore, the hepatic expression of *Cdo* and taurine production influences the intestinal levels of taurine. This was visible in the case of restrictive diets, but fiber feeding did not impact *Cdo* expression.

Conjugation of taurine released from BAs to other molecules likely regulates taurine's biological activity. As we have shown (Gregor et al., 2021), CR, in conjunction with the occurrence of taurine-GSH, results in an increase in taurine uptake from the intestine. Therefore, despite increased *TauT* expression in CR ileum mucosa samples, treatment with a BSH inhibitor prevented increased intestinal uptake of taurine. Interestingly, solely an increase in taurine conjugates levels, triggered by a high-fiber diet, induced *TauT* mRNA expression. On the other hand, low taurine levels did not reduce the CR-triggered increase in *TauT* expression upon BSH inhibition. Therefore, CR and taurine abundance likely impact *TauT* expression via separate mechanisms. In the GI tract, taurine plays multiple roles. It has anti-inflammatory effects, shapes the microbiota, accelerates the production of SCFA, reduces LPS concentration, maintains the intestinal tight junction barrier, and mediates long-term metaorganism colonization resistance (Ainad-Tabet et al., 2019; Duszka, 2022; Fang et al., 2019; Shimizu, Zhao, Ishimoto, & Satsu, 2009; Wen et al., 2020; Zhao et al., 2008). Importantly, certain bacteria, including *Deltaproteobacteria*, has the capacity to convert BA-derived taurine to sulfide. Subsequently, sulfide serves as an inhibitor of the cellular respiration of numerous pathogens (Stacy et al., 2021). However, sulfide can also be toxic to the host as it increases cell proliferation in the gut, induces inflammatory pathways, triggers oxidative stress and DNA damage in the human colon (Attene-Ramos et al., 2010; Attene-Ramos, Wagner, Gaskins, & Plewa, 2007; Attene-Ramos, Wagner, Plewa, & Gaskins, 2006; Christl, Eisner, Dusel, Kasper, & Scheppach, 1996; Deplancke & Gaskins, 2003). Hence, the here-described regulation of taurine release but also the rapidity of its reuptake may have consequences for gut health. Notably, the function of the various conjugates created by taurine in the gut needs further investigation.

In summary, cage bedding consumption modifies intestinal levels of taurine and its conjugates, while BAs secretion depends on the dietary regime. The connection between fiber and BSH in bacterial metabolism remains to be studied. Also, the therapeutic application of fiber supplementation in modulating BAs composition opens a promising field of research. Importantly, we further prove that hunger-triggered cage bedding consumption impacts microbiota and host. Consequently, thus far published rodent studies involving dietary restriction reported the combined impact of diet and fiber consumption.

Ethics statement

All animal experimentation protocols were approved by the Bundesministerium für Wissenschaft, Forschung und Wirtschaft, Referat für Tierversuche und Gentechnik (BMBWF-66.006/0008-V/3b/2018, BMWFW-66.006/2020, and 2022–0.257.032). All experiments were carried out according to animal experimentation Animal Welfare Act guidelines.

Authors contribution statement

AG performed animal experiments, analyzed metabolites, and analyzed the data; SAH analyzed samples and the data, MM analyzed

metabolites and the data; SB analyzed metabolites and the data; JS and PP analysed the microbiome data, MP assisted with the development of the methodology for metabolites detection; KD designed and performed the experiments, and wrote the manuscript. All authors corrected and approved the final manuscript.

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CRediT authorship contribution statement

András Gregor: . **Sandra Auernigg-Haselmaier:** Data curation. **Manuel Malleier:** Data curation. **Stefan Bruckberger:** Data curation. **Joana Séneca:** . **Petra Pjevac:** Data curation, Formal analysis. **Marc Pignitter:** Methodology. **Kalina Duszka:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2023.105707.

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