Gender and gut microbiota composition determine hepatic bile acid, metabolic and inflammatory response to a single fast-food meal in healthy adults


Department of Internal Medicine, University Hospital Knappschaftskrankenhaus, Ruhr-University Bochum, In der Schornau 23-25, 44892 Bochum, Germany
Department of Gastroenterology and Hepatology, University Hospital Essen, Hufelandstrasse 55, 45147 Essen, Germany
Department of Gastroenterology, Hepatology, and Infectious Diseases, Otto-von-Guericke-University Hospital Magdeburg, Leipziger Strasse 44, 39120 Magdeburg, Germany
CECAD Research Center, CECAD Lipidomics Facility, University of Cologne Joseph-Stelzmann-Str. 26, 50931 Cologne, Germany
Department of Immunology, Ophthalmology and ORL, Complutense University School of Medicine, Avenida de Séneca 2, 28040 Madrid, Spain
12 de Octubre Health Research Institute (imas 12), Madrid, Spain
Department of Gastroenterology and Hepatology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 EZ Groningen, the Netherlands
Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9713 EZ Groningen, the Netherlands

Article info
Article history:
Received 9 December 2020
Accepted 2 April 2021

Keywords:
Bile acids
Binge eating
Fast food
Microbiota
Liver
Steatosis

Abstract
Background & aims: Regular consumption of fast-food (FF) as a form of typical Western style diet is associated with obesity and the metabolic syndrome, including its hepatic manifestation nonalcoholic fatty liver disease. Currently, it remains unclear how intermittent excess FF consumption may influence liver metabolism. The study aimed to characterize the effects of a single FF binge on hepatic steatosis, inflammation, bile acid (BA), glucose and lipid metabolism.

Methods: Twenty-five healthy individuals received a FF meal and were asked to continue eating either for a two-hour period or until fully saturated. Serum levels of transaminases, fasting BA, lipid profile, glucose and cytokine levels as well as transient elastography and controlled attenuation parameter (CAP; to assess hepatic steatosis) were analyzed before (day 0) and the day after FF binge (day 1). Feces was collected prior and after the FF challenge for microbiota analysis.

Results: The FF meal induced a modest increase in CAP, which was accompanied by a robust increase of fasting serum BA levels. Surprisingly, levels of cholesterol and bilirubin were significantly lower after the FF meal. Differentiating individuals with a relevant delta BA (>1 mmol/l) increase vs. individuals without (delta BA ≤1 mmol/l), identified several gut microbiota, as well as gender to be associated with the BA increase and the observed alterations in liver function, metabolism and inflammation.

Conclusion: A single binge FF meal leads to a robust increase in serum BA levels and alterations in parameters of liver injury and metabolism, indicating a novel metabolic aspect of the gut–liver axis.

© 2021 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

1. Introduction

High caloric Western type diet is associated with obesity, the metabolic syndrome and its hepatic manifestation nonalcoholic fatty liver disease (NAFLD). Fast-foods (FF) are quick alternatives to home cooked meals, high in saturated fat, sugar, salt and calories,
which is frequently consumed in excess and represents the preferred food type of several eating disorders including bulimia and binge eating. So far, it remains unclear how intermittent excess food intake and especially FF consumption may influence liver metabolism.

Bile acids (BA) are crucial mediators of lipid- and glucose-metabolism and their actions extend far beyond their critical role in fat absorption via emulsification and formation of micelles in the intestinal lumen [1]. Primary BA are synthesized in hepatocytes from cholesterol in a multistep process and then secreted into the bile. The expression of key proteins in this process is regulated by the BA-activated nuclear receptor farnesoid X receptor (FXR). FXR and its gut-derived downstream target fibroblast growth factor 19 (FGF19) regulate BA de novo synthesis, secretion, and reabsorption within the enterohepatic circulation [2]. In the intestine, BA are modified by bacterial enzymes, reabsorbed from the terminal ileum, and returned to the liver via the enterohepatic circulation. Among other mechanisms, diet induced microbiome alterations and associated BA compositions may promote the progression of NAFLD and its complications [3–5]. FXR activation as well affects glucose-metabolism via induction of the release of glucagon-like peptide 1 (GLP1) from enteroendocrine cells, hepatic glucose-homeostasis and insulin sensitivity [6–8]. Additionally, FXR activation affects postprandial lipid levels and enteric lipid resorption and fatty acid transport [9,10].

On the one hand, BA are potent activators of FXR, but following their release into the intestine, bacterial hydroxylation and deconjugation produces secondary BA that carry differential FXR activating potential [11]. On the other hand, the composition of the BA pool itself reflects and potentially affects gut microbiota diversity and richness. However, little is known how dietary interventions, especially short-term effects of FF and binge episodes might alter the FXR signaling pathway, gut–liver interactions and associated metabolic pathways. Therefore, the aim of this study was to characterize the effects of a single FF binge on hepatic steatosis, inflammation, BA, glucose and lipid metabolism as well as potentially predisposing microbiota-related factors.
2.3. Fecal samples and microbiome analysis

Fecal samples were collected in sterile tubes and stored at –80 °C until to DNA isolation. Individuals did not receive antibiotic treatment during the past 4 weeks. DNA was isolated using the Qamp-DNA isolation kit following manufacturer’s instructions (Qiagen, Hilden, Germany) including a mechanical lysis step using dry bead tubes (MoBio Laboratories Inc., Carlsbad, CA, USA) and the Fast Prep™-24 instrument (MP Biomedicas, Solon, OH, USA) at 6.0 m/s for 45 s (two times). Amplicon libraries were generated as previously described [12] and sequenced on a MiSeq (2x 300 bp, Illumina, Hayward, CA, USA). All FastaQ files were analyzed using dada2 package in R (www.r-project.org) and as result a unique table containing all samples with the sequence reads and abundances was generated. All samples were resampled to equal the smallest library size of 19,380 reads per sample using the phyloseq package and returning 2999 phylotypes. Sequence reads were assigned to a taxonomic affiliation based on the naïve Bayesian classification with a pseudo-bootstrap threshold of 80%. Relative abundances, in percentage of Phylotypes, Genus, Family, Order, Class and Phylum were used for downstream analyses. The vegan package (http://CRAN.R-project.org/package-vegan) was used to generate rarefaction curves and for calculating the richness, Piec, and PDest-abundant. Multivariate analyses were performed with Past3 [13] and univariate analyses were performed with GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA). The data comprising the six taxonomy ranks were used to construct sample-similarity matrices using the Bray–Curtis algorithm, where samples were clustered with 1000 bootstrap. Significant differences between a priori predefined groups of specimens were evaluated using Permutational multivariate analysis of variance (PERMANOVA with 9,999 permutations) and groups were considered significantly different if the p value was ≤0.05. Differences between a priori defined groups were calculated using Mann–Whitney test in PRISM 8. All p values were corrected by applying the Benjamini–Hochberg false-discovery rate correction (desired FDR 0.5%) and it was considered significant if the corrected p value was <0.05. Only phylotypes with a mean of abundant >1% were considered.

2.4. Serum bile acid profiling

Quantification of primary and secondary BA in serum was performed by Liquid Chromatography coupled to Electrospray Ionization Tandem Mass Spectrometry (LC–ESI-MS/MS) using the Biocrates® Bile Acids Kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) which covers 16 individual BA. Data analysis was completed using the Biocrates MetIDQ software [14].

2.5. ELISA

Serum levels of the overall cell death marker M65, and apoptosis marker M30 as well as leptin were measured using commercially available kits from TecnoMedical (Sissach, Switzerland). Serum levels of fibroblast growth factor 19 and 21 (FGF19/21) or tumor necrosis factor alpha (TNFα) were quantified using the Quantikine ELISA-kit from R&D Systems (Minneapolis, MN, USA), levels of glucagon like peptide 1 (GLP1) by using the GLP1 ELISA kit from Abcam (Cambridge, United Kingdom). Lipopolysaccharide binding protein (LBP) was quantified with the human LBP ELISA Kit from Hycult Biotech (Uden, the Netherlands). Serum amounts of the gastric inhibitory polypeptide (GIP) were assessed using the human GIP ELISA kit from Novus Biologicals (Centennial, CO, USA). All ELISA Kits were performed concerning manufacturers constructions.

2.6. Statistical analysis

Statistical significance was determined using a Wilcoxon matched-pairs signed rank test, normality of the data was tested with the Shapiro–Wilk test. Correlation analysis was performed using a simple linear regression analysis. Multivariate logistic regression was performed to validate independent effects of select variables; all analyses were performed with GraphPad Prism 8 and/or SPSS 25 (SPSS Inc., Chicago, IL, USA). If not stated otherwise all data are presented as mean ± SEM. Significance was assumed at p < 0.05. For correlation analysis, Spearman’s Rank Correlation Coefficient was used. Univariate logistic regression analysis was used to identify the predictive value of a parameter. This was followed by binary logistic regression analysis. Variables with a p value < 0.05 in univariate analysis were included in a stepwise backward elimination multivariate regression analysis.

3. Results

3.1. Gender differences in baseline characteristics and tolerable fast-food consumption over a two-hour period

As expected, baseline characteristics differed between male and female subjects in line with gender-adjusted normal limits for individual parameters assessed in a fasting state before the dietary intervention. Following the assessment of baseline characteristics as mentioned above, the study subjects were invited to place orders for FF provided by two major international franchises as well as a local pizza kitchen. In the evening of day 0, the subjects gathered in groups up to five to consume the ordered meals within a two-hour period until complete satiety. Additionally, the subjects were offered non-diet sodas ad libitum. The amount of ingested food and soda was documented, and calories calculated from the caloric tables provided by the vendors. The average amounts of the various food types consumed comparing male and female subjects are shown in Supplementary Fig. 1A. While we observed a trend towards a higher consumption of burgers, beverages and deserts in male subjects, statistical analysis remained non-significant. Looking at the overall caloric intake, since female subjects were significantly smaller, weighed less and thus had a lower BMI, they ingested significantly less calories over the given two-hour period. However, the macronutritional composition of ingested foods was comparable between male and female subjects (Table 1). Baseline fecal bacterial communities did not differ between males and females and between before and after the fast-food binge based on PERMANOVA analysis.

3.2. Acute effects of a one-time fast-food intervention on liver stiffness, steatosis and liver injury

At day one after the dietary intervention, the subjects returned to our laboratory following an overnight fast of at least 12 h. At day one, we observed a modest increase in body weight (Table 2). Liver stiffness as assessed by transient elastography and hepatic steatosis as assessed by CAP were not changed after the FF meal (Table 2). In order to assess potential hepatocyte injury, we quantified hepatic apoptosis (M30) and overall cell death markers (M65) in the subjects serum as previously reported [15]. Here, we found no signs of acute hepatocyte cell death (Table 2). Surprisingly, serum LDH levels were even significantly lower the day after the FF intervention (Table 2). While LDH is not only secreted from injured liver cells, but this might also even indicate a protective effect of the FF binge on hepatocytes.
3.3. Suppression of serum bilirubin and transaminase levels following the intervention

Additionally, to the above-mentioned reduction in serum LDH levels, we observed a significant reduction of fasting serum AST and γGT levels the day after the FF meal (Table 2). Moreover, this reduction was accompanied by a robust suppression of total and direct serum bilirubin levels but with no effect on Alkaline Phosphatase levels (Table 2).

3.4. Metabolic effects of a single fast-food meal

In line with our previous findings described above, serum lipids appeared lower the day after the intervention. More specifically, serum cholesterol was significantly lower with a significant decrease in HDL-cholesterol and a modest decrease in LDL-cholesterol (Table 2). Similarly, we observed a modest, yet non-significant decrease in serum triglyceride and fasting glucose levels were slightly increased, which was accompanied by a significant increase in serum GLP1 levels and a decrease in FGF21 (Table 2). No effects were observed on serum levels of GIP, adiponectin and leptin in this cohort before and after the FF meal (Table 2).

3.5. Induction of fasting serum BA levels the day after a binge fast-food meal

Overall, we observed a significant increase of total serum BA levels at day 1 (Table 3). When differentiating into primary and secondary BA (mean abundance relative to fixed library standards), we observed an increase in primary and secondary BA, both from the conjugated and unconjugated ones and a diverse pattern of distribution (Table 3) while serum levels of FGF19 were not changed (Table 2). Cholic acid (CA) was the predominant primary BA induced at day 1, while the other primary BA remained unchanged (Table 3). Within the secondary BA, deoxycholic acid (DCA); glyco-deoxycholic acid (GDCA) and tauro-deoxycholic acid (TDCA) were significantly induced, while the serum concentration of tauro-lithocholic acid (TLCA) even decreased (Table 3).

Furthermore, there was also a slight increase in tertiary BA, but this difference was not significant, and the concentration of tertiary BA was also very small (Table 3).

Interestingly, when looking at the distribution of total BA increase (delta total BA), we observed that in about half the study population, there was only a modest increase or even a decrease, compared to the other half with an increase of total BA, including a dramatic increase of up to 40.7 μmol/l in one subject (Fig. 1A).

For further analyses, we therefore divided the cohort into two groups (delta BA < 1 μmol/l; n = 13 vs. delta BA > 1 μmol/l n = 12). Individuals with a high increase in serum BA the day after the intervention were more predominantly male, ingested significantly more calories (while the male subjects did not eat significantly higher average amounts of food; see Supplementary Fig. 1B) during the intervention, had a decrease in liver stiffness compared to day 0 as well as a decrease in serum TNFα levels (Table 4; Fig. 1B). Accordingly, delta total BA was associated with caloric intake and serum LDL levels (Fig. 1C–D). In contrast, the individuals with lower delta BA had significantly higher serum levels of leptin (Fig. 2A) and were mainly female (Fig. 2B). As expected, individuals with higher leptin levels had a lower intake of calories and the intake of calories was inversely associated with serum levels of leptin (Fig. 2C–D).

For a more detailed analysis of the BA profile its potential effects, we analyzed correlations utilizing the Spearman’s rank correlation coefficient. Hereby, we further analyzed the differences of total unconjugated and conjugated BA before and after the intervention (delta total unconjugated BA and delta total conjugated BA). However, we did not find significant differences comparing groups with a higher or a lower increase of total unconjugated or conjugated BA (Supplementary Tables 1 and 2).

3.6. Gut microbiota composition is associated with the BA response to a single fast-food meal

In order to evaluate a potential effect of the microbiome on the changes observed here, we collected stool at day 0 and day 1 and performed 16S rRNA gene sequencing. The short duration between the intervention and the second stool sample, we did not observe any effect on the microbiota composition between day 0 and day 1 as analyzed via PERMANOVA. No significant differences in microbiota composition were observed between individuals with a high vs. low increase in BA regarding diversity (including Pielou’s diversity, Shannon and Simpson index) and richness (Table 4), as well as on higher-ranking taxonomy levels. We performed a comparison at Phylum and Genus levels and presented both when comparing Day 0 vs. Day 1, BA increase, caloric intake, and gender but there

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2</td>
</tr>
<tr>
<td>Table 2</td>
</tr>
</tbody>
</table>
were no differences in these comparisons (Supplementary Fig. 2). However, when comparing on Phylotype level, we identified a number of individual phylotypes, that statistically differed in abundance in the stool of subjects with low vs. high BA response. Following alignment using the ribosomal database project (RDP) database (http://rdp.cme.msu.edu/), these are supposably the

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bile acids (μmol/l)</td>
<td>3.91 ± 0.25</td>
<td>8.26 ± 1.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Conjugated bile acids (μmol/l)</td>
<td>41.86 ± 5.54</td>
<td>64.12 ± 9.90</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Unconjugated BA (μmol/l)</td>
<td>16.29 ± 2.95</td>
<td>60.92 ± 17.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Primary bile acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA (μmol/l)</td>
<td>4.85 ± 1.77</td>
<td>19.12 ± 5.90</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CDCA (μmol/l)</td>
<td>4.25 ± 1.12</td>
<td>20.25 ± 7.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GCA (μmol/l)</td>
<td>4.66 ± 0.73</td>
<td>6.79 ± 1.15</td>
<td>n.s.</td>
</tr>
<tr>
<td>TCA (μmol/l)</td>
<td>0.89 ± 0.17</td>
<td>1.37 ± 0.46</td>
<td>n.s.</td>
</tr>
<tr>
<td>GCDCA (μmol/l)</td>
<td>18.47 ± 2.60</td>
<td>27.68 ± 4.04</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TCDCA (μmol/l)</td>
<td>3.41 ± 0.58</td>
<td>4.78 ± 1.04</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sum of primary bile acids (μmol/l)</td>
<td>36.40 ± 6.09</td>
<td>80.00 ± 16.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Primary unconjugated bile acids (μmol/l)</td>
<td>9.10 ± 2.80</td>
<td>39.00 ± 13.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Primary conjugated bile acids (μmol/l)</td>
<td>27.30 ± 3.85</td>
<td>40.60 ± 6.04</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Secondary bile acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCA (μmol/l)</td>
<td>7.19 ± 0.97</td>
<td>21.55 ± 4.83</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GLCA (μmol/l)</td>
<td>0.20 ± 0.03</td>
<td>0.25 ± 0.06</td>
<td>n.s.</td>
</tr>
<tr>
<td>GDCA (μmol/l)</td>
<td>8.17 ± 1.27</td>
<td>14.25 ± 2.48</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TDCA (μmol/l)</td>
<td>1.27 ± 0.19</td>
<td>14.18 ± 2.48</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TILCA (μmol/l)</td>
<td>0.07 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sum of secondary bile acids (μmol/l)</td>
<td>17.20 ± 1.97</td>
<td>38.60 ± 6.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Secondary unconjugated bile acids (μmol/l)</td>
<td>7.19 ± 0.97</td>
<td>21.50 ± 4.82</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Secondary conjugated bile acids (μmol/l)</td>
<td>9.71 ± 1.42</td>
<td>17.01 ± 3.22</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Tertiary bile acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UDCA (μmol/l)</td>
<td>1.30 ± 0.26</td>
<td>2.72 ± 0.82</td>
<td>n.s.</td>
</tr>
<tr>
<td>GUDCA (μmol/l)</td>
<td>3.37 ± 0.81</td>
<td>3.55 ± 0.73</td>
<td>n.s.</td>
</tr>
<tr>
<td>TUDCA (μmol/l)</td>
<td>0.19 ± 0.04</td>
<td>0.23 ± 0.05</td>
<td>n.s.</td>
</tr>
<tr>
<td>Tertiary bile acids (μmol/l)</td>
<td>4.85 ± 1.07</td>
<td>6.49 ± 1.39</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Table 3
Levels of total serum bile acids and individual bile acids profile in fasted individuals before (day 0) and following (day 1) a binge fast-food meal. n.s. stands for not significant.

Fig. 1. Increase of bile acids (delta BA) on day 1 after the fast-food meal in the individuals of the study cohort are shown in response to a cutoff of ≤1 μmol/l or >1 μmol/l of total bile acid change (A). Differences of bile acid changes between day 0 and day 1 comparing male and female subjects (B). Calorie intake was associated with the changes in total BA (C) levels and LDL-cholesterol levels are negatively correlated with the changes in bile acid levels (D) *p < 0.05, ***p < 0.001, ****p < 0.0001.
phylotypes Dialister spp, Bacteroides massiliensis and Sutterella wadsworthensis (Fig. 3A–C).

While the abundance of Dialister spp appeared to be independent of gender and caloric intake (Supplementary Fig. 3A–B), we found a significant association with the response in serum triglyceride levels and FGF19 (Fig. 3D–E). Abundance of B. massiliensis and S. wadsworthensis was significantly lower in individuals with a high BA increase (Table 4). Furthermore, there was a significant negative association of B. massiliensis abundance with decreasing levels of several primary and secondary BA (Fig. 4A–B; Supplementary Table 3) as well as a positive correlation with liver stiffness LSM (Fig. 4C). This appeared to be independent of gender and caloric intake (Supplementary Fig. 3C–D). In contrast, the abundance of S. wadsworthensis was significantly higher in BA >1 individuals (Table 4). Abundance of S. wadsworthensis was independent of gender and caloric intake (Supplementary Fig. 3E–F) but significantly associated with primary BA as well as leptin levels (Fig. 4E–F), in particular with the primary BA glycochenodeoxycholic acid (GCDCA) (Supplementary Table 3). However, multivariate regression only revealed B. massiliensis and gender to be independently associated with BA increase (Supplementary Table 4).

4. Discussion

In this study, we demonstrate that a single binge FF meal leads to a robust increase of fasting serum BA, accompanied by alterations in several parameters of liver injury and hepatic lipid and glucose metabolism. Furthermore, we identified male gender as well as the abundance of specific gut microbiota, to be associated with the above-mentioned FF binge induced BA increase, underlining the importance of the gut–liver axis in metabolic response to dietary challenges.

Physiologically, upon entering the duodenum, food induces the release of bile from the gallbladder. BA then aid in emulsification of dietary fat and thereby promoting fat absorption in the small intestine. Down the digestive tract, BA are subsequently actively reabsorbed in the terminal ileum by transport proteins and then directed towards the liver via the portal vein as part of the enterohepatic circulation [16]. BA do not only play an essential role in the regulation of nutrient factors (glucose, fat), GLP1 release from EECs is triggered as well as lipid- and glucose-metabolism. Along with other mediators regulating glucose and lipid homeostasis, Postprandial stimulation of entero-endocrine cells (EECs) induces the release of several mediators involved in digestive function, regulation of appetite as well as lipid- and glucose-metabolism. Along with other mediators, GLP1 is released from EECs in the distal gut. Among nutrient factors (glucose, fat), GLP1 release from EECs is triggered by BA, most likely via stimulation of GPBAR1 (G protein-coupled bile acid receptor 1 also known as TGR5) [17]. Therefore, several therapeutic approaches, including FXR agonists, BA substitution and even bariatric surgery have been evaluated regarding their antidiabetic properties, based on this BA–GLP1 axis [18,19]. While this rapid postprandial interaction of BA and glucose metabolism has been widely recognized, current data indicates this process to be a rather acute effect, that vanishes within a few hours following a meal in healthy individuals [6,20,21]. Since GLP1 has a rather
short physiological half-life, it was found that in our cohort the GLP1 levels were still significantly increased 12 h after the FF meal. Since GLP1 levels can be increased by BA via TGR5 [22] it has also been shown that a GLP1 increase following UDCA-treatment and increased BA levels can potentially enhance the effects of dipeptidyl peptidase-4 (DPP4)- inhibitors in patients with type 2 diabetes [23]. However, in a subset of subjects, we found a sustained increase in serum BA levels and GLP1 even after a 12 h fasting period following the FF meal but without alterations in GIP levels, which suggests that GLP1 secretion is triggered by the increase of secondary BA. This observation was associated with male gender and the abundance of selected microbiota in the stool.

Surprisingly little human data on the effects of FF in the context of BA metabolism is available. Recently, Zhu et al. published data on a short-term trial of a four-day dietary intervention in which 10 healthy subjects were randomly assigned to start either with a FF or a Mediterranean diet in a crossover design with a four-day washout phase in between [24]. Unlike in our “quasi”-pre-test post-test trial, comparing post-interventional results to baseline following a single meal, the authors did not observe alterations in BA homeostasis. There were no clear differences concerning diversity of the intestinal microbiome, but even after a four-day dietary intervention, FF led to significant alterations in microbiota composition. Especially interesting was that following the FF diet some phyla of bile-tolerant bacteria increased (e.g. Bilophila, Collinsella, Alistipes) even though no clear influence on the BA pool was detected [24]. Few publications observed diet-induced modifications of gut microbiota composition within the first three days of diet [25]. Therefore, we did not expect an effect on abundance following our one-time FF intervention between baseline and day 1. However, abundances of these above-mentioned two phylotypes (B. massiliensis and S. wadsworthensis) were associated with BA increase and metabolic alterations observed in our study.

While in the above-mentioned publication, the authors did not observe alterations in BA levels, Wan et al. found changes in serum BA levels similar to our observation in a randomized-controlled trial, feeding diets with different fat-content (diet containing 40% fat as compared to a lower fat content of 20%) compared to healthy individuals over six months [26]. These alterations in mainly secondary BA were furthermore associated with differences in the abundance of several gut bacteria involved in BA homeostasis (Bacteroides, Clostridium, Bifidobacterium and Lactobacillus), similar

![Fig. 2. Leptin serum levels comparing the groups with low or high increase of bile acids (A), male and female (B) and in those with a high or low intake of calories during the fast-food meal (C). Leptin serum levels correlate with calories intake (D). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.](image)
to our observation in patients with NAFLD and liver cancer [4]. Among others, *Bacteroides* species, like *B. massiliensis* frequently express BA hydrolases, enzymes involved in deconjugation of BA [27]. *B. massiliensis* in our cohort was inversely associated with increase in primary and secondary BA and correlated with an increase in liver stiffness at day 1. *B. massiliensis* has furthermore been linked to mucin-degrading properties, which directly and indirectly affect gut microbiota homeostasis and degradation of carbohydrates, which in turn reach the liver via the enterohepatic circulation and are added to the body as additional calories and can thus promote fatty liver and fibrogenesis [28].

Analyzing data from 295 school children, Mbakwa et al. found an inverse association of the abundance of *S. wadsworthensis* with body weight, as well as a modest inverse association of *Dialister* with an obese phenotype [29]. Interestingly, in patients with ulcerative colitis undergoing a prospective randomized-controlled trial on the effect of fecal microbiota transplant in order to induce clinical remission, a high abundance of *S. wadsworthensis* in stool was associated with poor outcome and lower secondary BA synthesis indicating a link between this phylum and BA metabolism [30]. In comparison with this observation, we found significantly elevated fasting leptin levels in individuals without a significant increase in serum BA (delta BA ≤ 1 μmol/l) following the FF meal. Furthermore, we observed an inverse association of *S. wadsworthensis* with serum leptin levels, which was itself inversely correlated to caloric intake. While the latter observation is non-surprising, as the role of leptin in hunger and satiety has been widely studied, little is known about potential interactions between leptin and BA as well as a potential effect of *S. wadsworthensis* [31,32]. In leptin-deficient individuals, leptin treatment leads to alterations in the metabolome including an induction of primary and secondary BA and FXR activation has been reported to inhibit leptin signaling in cell culture and xenograft models [33,34]. Apart from an observed effect of leptin supplementation on the abundance of *Sutterella* species in rodents, no data is available on potential effects of leptin on this phylum, especially no human data [35].

A randomized-controlled trial investigating the effects of a walnut-rich diet on metabolism and microbiota revealed a diet-induced increase in the abundance of *Dialister* with FGF19, TNFα and triglyceride levels, little data is available on potential metabolic functions of this phylum. Additionally, we identified significantly elevated fasting leptin levels in individuals without a significant increase in serum BA (delta BA ≤ 1 μmol/l) following the FF meal. Furthermore, we observed an inverse association of *S. wadsworthensis* with serum leptin levels, which was itself inversely correlated to caloric intake. While the latter observation is non-surprising, as the role of leptin in hunger and satiety has been widely studied, little is known about potential interactions between leptin and BA as well as a potential effect of *S. wadsworthensis* [31,32]. In leptin-deficient individuals, leptin treatment leads to alterations in the metabolome including an induction of primary and secondary BA and FXR activation has been reported to inhibit leptin signaling in cell culture and xenograft models [33,34]. Apart from an observed effect of leptin supplementation on the abundance of *Sutterella* species in rodents, no data is available on potential effects of leptin on this phylum, especially no human data [35].

A randomized-controlled trial investigating the effects of a walnut-rich diet on metabolism and microbiota revealed a diet-induced increase in the abundance of *Dialister*. This diet was as well associated with a decrease in blood lipids and secondary BA [36]. In our study, given the short duration between intervention and analysis at day 1, we do not assume a diet-specific effect on gut microbiota and BA signaling, but rather a predisposing phenotype involving the three above-mentioned phylotypes, as well as gender

---

**Fig. 3.** Abundances of *Dialister* spp. (A), *Bacteroides massiliensis* (B) and *Sutterella wadsworthensis* (C) comparing individuals with delta BA ≤ 1 and delta BA > 1. Differences in the serum levels of triglycerides (D) and FGF19 (E) correlate with the abundance of *Dialister* spp. *p < 0.05, **p < 0.01.
in determining the BA response to a FF meal as suggested by others [11]. BA metabolism and transport is multifunctionally regulated, which involves a lot of different transport proteins regulating cellular import as well as export implementing detoxification of endogenous and exogenous substances with differing substrate specificities. Most of these transport proteins are regulated by FXR, which controls the communication between de novo BA synthesis in the hepatocytes, bile flow, reabsorption of BA via enterocytes in the gut as well as biliary lipid secretion. A canalicular phospholipid translocator, which is mainly involved in secretion of biliary phospholipid and especially conjugated bilirubin is ABCC2 (also known as MRP2) [37,38].

Interestingly, transaminase levels did not show any increase in response to the massive caloric intake, while AST and γGT levels were even decreased. It must probably be acknowledged here that the transaminases of the cohort were not initially increased, but were in the lower normal range, and measurable damage was not achieved by this intervention. Limited data is available on the effects of dietary interventions on transaminase levels. However, in fish, a high-starch diet resulted in a decrease in ALT and AST with comparable effects on blood lipids as seen in our study. Here, bile acid supplementation abolished these effects [39]. Other in vivo studies revealed that CA supplementation abolishes hepatotoxic effects and may even reduce oxidative stress following administration of a hepatotoxic compound or its metabolites [40]. However, observational studies in humans revealed that transaminases are most likely not a suitable parameter for NAFLD, since liver damage cannot be identified by interpreting transaminases even in severely overweight subjects [41].

There are certain limitations in our study. The present study was conducted with a rather small cohort and the influence of gender could be addressed more precisely by alternative study designs and increased group sizes accordingly. In addition, we performed a very short intervention (only one binge eating episode) with a relatively short follow-up. It would have been interesting to look at later time points, especially with regard to the microbiota analysis. Unfortunately, an exact mechanism including the interaction of BA and lipid metabolism in more detail also remains unexplained. Mechanisms that influence the transport and regulation of BA are obvious but cannot be investigated in detail in the current study.

In conclusion, after a single FF meal we observed a robust increase of fasting serum BA and GLP1, but surprisingly decreased levels of transaminases, cholesterol and bilirubin. Based on the present findings, we presume a potential microbiota-mediated

**Fig. 4.** The abundance of *Bacteroides massiliensis* is correlated to changes of the primary (A) and secondary (B) bile acids levels as well as with the transient elastography (LSM, C). The abundance of *Sutterella wadsworthensis* is correlated to changes of the primary BA (D) and leptin serum levels (E).
acute effect on FXR signaling, which may interact with GLP1 and leptin as potential novel links of the gut—liver-axis to hepatic lipid- and glucose metabolism.

Author contribution

AF (analysis and interpretation of data, drafting of the manuscript), SS (acquisition, analysis and interpretation of data; study design; technical support; statistical analysis; drafting of the manuscript), CW (acquisition, analysis and interpretation of data; statistical analysis, drafting of the manuscript), PM (analysis and interpretation of data; statistical analysis), SA (data acquisition), RY (acquisition and analysis of microbiome data; statistical analysis, drafting of the manuscript), AL (critical revision of the manuscript for important intellectual content), AJ (critical revision of the manuscript), SB (acquisition and analysis of data for bile acids profile; technical support), CL (measurement of bile acid profiles; technical support), YAN (critical revision of the manuscript), KNF (critical revision of the manuscript for important intellectual content; technical support), HIM (critical revision of the manuscript for important intellectual content; technical support), MPO (critical revision of the manuscript), GC (obtained funding; critical revision of the manuscript for important intellectual content), FJC (critical revision of the manuscript for important intellectual content), AC (obtained funding; critical revision of the manuscript for important intellectual content), LPB (study concept and design; acquisition, analysis and interpretation of data; statistical analysis; study supervision; drafting of the manuscript; obtained funding).

We especially thank the team of Data Integration Center of University Medicine Magdeburg which is supported by MIRACUM that is funded by the German Federal Ministry of Education and Research (BMBF) within the “Medical Informatics Funding Scheme” (PKZ 01ZZ1801H).

Funding

This work was supported by the German Research Foundation (DFG; project number BE-3967/3-1; LPB), Dr. Werner Jackstaedt Foundation (LPB), the European association for the study of the liver (EASL; Short-term fellowship; SS), Deutsche Leberstiftung (Vernetzungsstipendium; SS), Wilhelm-Laupitz Foundation (AC).

Conflict of interest

All authors declare that they do not have a conflict of interests.

Acknowledgments

We like to thank Martin Schlattjan, Ursula Stolz, Ilka Kramer and Simone Philipson for excellent technical assistance. Furthermore, we like to thank Robert Geffers and Michael Jarek from the Helmholtz Centre for Infection Research in Braunschweig (Germany) who performed illumina sequencing for the microbiome analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinu.2021.04.008.

References


