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Original article

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

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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 μmol/l) increase vs. individuals without (delta BA ≤1 μmol/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.

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,

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Abbreviations

ABCC2	ATP binding cassette subfamily C member 2
ALT	alanine aminotransferase
AP	alkaline phosphatase
AST	aspartate aminotransferase
BA	bile acids
BMI	body mass index
BUN	blood urea nitrogen
CA	Cholic acid
CAP	controlled attenuation parameter
CDCA	cheno-deoxycholic acid
DCA	deoxycholic acid
EEC	enteroendocrine cells
FF	fast-food
FGF19/21	fibroblast growth factor 19/21
FXR	farnesoid-x-receptor
GCA	glyco-cholic acid
GCDCA	glycol-cheno-deoxycholic acid
GDCA	glyco-deoxycholic acid
GIP	gastric inhibitory polypeptide
GLP-1	glucagon-like peptide 1
GPBAR1	G protein-coupled bile acid receptor
GUDCA	glycol-urso-deoxycholic acid
γGT	gamma-glutamyl transferase
HDL	high density lipoprotein
LBP-1	lipopolysaccharide binding protein
LDL	low density lipoprotein
LSM	liver stiffness measurement
M30	apoptosis marker M30
M65	cell death marker M65
NAFLD	non-alcoholic fatty liver disease
TCA	tauro-cholic acid
TCDCA	tauro-cheno-deoxycholic acid
TDCA	tauro-deoxycholic acid
TLCA	tauro-lithocholic acid
TNFα	tumor necrosis factor alpha
TUDCA	tauro-urso-deoxycholic acid
UDCA	urso-deoxycholic acid

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. Methods

2.1. Sample collection

All studies were performed at the University Hospital Essen. All subjects provided informed written consent and the Ethics Committee (Institutional Review Board) of the University Hospital Essen approved the study prior to beginning (reference number: 15-6730-BO). The study protocol conformed to the ethical guidelines of the declaration of Helsinki. For this study, we recruited 25 healthy volunteers without significant alcohol consumption during 14 days prior to the study and collected serum samples in fasted state in the morning and stored aliquots at −80 °C until use to measure cell death markers (M65, M30), bile acids profile, FGF19 and inflammatory cytokines while general clinical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), gamma-glutamyl transferase (γGT), total BA, bilirubin, cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), fasting glucose, blood urea nitrogen (BUN), and creatinine were evaluated via the central laboratory of the University Hospital Essen. Additionally, transient elastography as well as controlled attenuation parameter (CAP) to assess hepatic steatosis were performed. Later that day, the subjects received a high caloric FF meal of their choice and were asked to continue eating for a two-hour period or until fully saturated. The next day, we repeated all the studies mentioned above. Additionally, we collected stool samples for microbiota analysis prior to the food binge.

2.2. Patient and public involvement statement

Study design, feasibility and outcome measurement, as well as recruitment were conducted according to ethical issues and without direct involvement of subjects or the public. It was not possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research as we planned this study as a prospective observational study with volunteers and no specific patient group.

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 QIamp-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 (2 × 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, Pielou's diversity, Shannon and Simpson indices. 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 $\leq$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 TecoMedical (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.

Table 1
Baseline characteristics, calorie consumption during fast-food meal and baseline fasting laboratory results of the cohort. n.s. stands for not significant.

	Males ($n = 13$)	Females ($n = 12$)	p-value
Age (years)	24.23 ± 0.95	24.17 ± 0.93	n.s.
Height (cm)	181.70 ± 1.70	167.90 ± 1.90	<0.0001
Weight (kg)	82.07 ± 2.18	60.78 ± 2.49	<0.0001
BMI (kg/m^2)	24.90 ± 0.72	21.50 ± 0.72	<0.005
Calories/2 h period (kcal)	2831.00 ± 164.20	1683.00 ± 90.31	<0.0001
Calories from Carbohydrates (%)	46.99 ± 1.20	47.67 ± 1.72	n.s.
Calories from Fat (%)	40.85 ± 0.97	39.38 ± 1.07	n.s.
Calories from Protein (%)	12.16 ± 0.57	12.95 ± 0.60	n.s.
Fasting glucose (mg/dl)	86.54 ± 1.75	78.73 ± 1.20	<0.005
BUN (mg/dl)	15.77 ± 1.22	12.17 ± 0.59	<0.05
Creatinine (mg/dl)	1.10 ± 0.50	0.88 ± 0.02	<0.005

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).

Table 2
Basic data in serum before (Day 0) and after (Day 1) the fast-food meal of the cohort ($n = 25$). n.s. stands for not significant.

	Day 0	Day 1	p-value
Weight (kg)	71.85 ± 2.70	72.48 ± 2.78	<0.001
Fasting glucose (mg/dl)	82.96 ± 1.34	86.04 ± 1.60	n.s.
CAP (dB/m)	218.60 ± 11.16	231.30 ± 10.03	n.s.
LSM (kPa)	5.22 ± 0.42	5.50 ± 0.42	n.s.
AST (U/L)	24.84 ± 2.49	20.64 ± 1.73	<0.0001
ALT (U/L)	25.67 ± 3.17	24.38 ± 2.74	n.s.
Alkaline phosphatase (U/L)	56.52 ± 3.41	57.38 ± 3.79	n.s.
γGT (U/L)	18.48 ± 1.94	17.44 ± 1.89	<0.05
Bilirubin (mg/dl)	0.68 ± 0.07	0.43 ± 0.04	<0.0001
Direct bilirubin (mg/dl)	0.23 ± 0.02	0.15 ± 0.02	<0.001
M65 (U/l)	135.80 ± 13.24	123.80 ± 9.15	n.s.
M30 (U/l)	117.50 ± 7.00	122.70 ± 10.90	n.s.
Cholesterol (mg/dl)	173.60 ± 4.99	164.70 ± 4.55	<0.0001
LDL-cholesterol (mg/dl)	84.75 ± 3.93	80.59 ± 3.77	n.s.
HDL-cholesterol (mg/dl)	173.60 ± 4.99	164.70 ± 4.55	<0.0001
Triglycerides (mg/dl)	106.20 ± 9.38	101.80 ± 12.23	n.s.
GLP1 (pg/ml)	449.0 ± 49.02	581.70 ± 52.65	<0.01
FGF21 (pg/ml)	110.70 ± 18.28	79.12 ± 10.78	<0.05
GIP (pg/ml)	122.50 ± 12.26	114.00 ± 7.85	n.s.
Adiponectin (ng/m)	6.38 ± 0.63	6.74 ± 0.77	n.s.
Leptin (ng/ml)	22.63 ± 3.69	20.71 ± 3.24	n.s.
FGF19 (pg/ml)	132.30 ± 14.69	123.80 ± 16.08	n.s.
Richness	182.20 ± 8.39	204.30 ± 11.29	n.s.
Pielou's Evenness Index	0.75 ± 0.01	0.74 ± 0.01	n.s.
Shannon Index	3.90 ± 0.09	3.94 ± 0.09	n.s.
Simpson Index	0.95 ± 0.01	0.95 ± 0.01	n.s.

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 $\leq$ 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 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.

	Day 0	Day 1	*p*-value
Total bile acids (μmol/l)	3.91 ± 0.25	8.26 ± 1.80	<0.001
Conjugated bile acids (μmol/l)	41.86 ± 5.54	64.12 ± 9.90	<0.05
Unconjugated BA (μmol/l)	16.29 ± 2.95	60.92 ± 17.18	<0.0001
Primary bile acids			
CA (μmol/l)	4.85 ± 1.77	19.12 ± 5.90	<0.05
CDCA (μmol/l)	4.25 ± 1.12	20.25 ± 7.33	<0.0001
GCA (μmol/l)	4.66 ± 0.73	6.79 ± 1.15	n.s.
TCA (μmol/l)	0.89 ± 0.17	1.37 ± 0.46	n.s.
GCDCA (μmol/l)	18.47 ± 2.60	27.68 ± 4.04	<0.05
TCDCA (μmol/l)	3.41 ± 0.58	4.78 ± 1.04	n.s.
Sum of primary bile acids (μmol/l)	36.40 ± 6.09	80.00 ± 16.00	<0.001
Primary unconjugated bile acids (μmol/l)	9.10 ± 2.80	39.00 ± 13.00	<0.01
Primary conjugated bile acids (μmol/l)	27.30 ± 3.85	40.60 ± 6.04	<0.05
Secondary bile acids			
DCA (μmol/l)	7.19 ± 0.97	21.55 ± 4.83	<0.0001
GLCA (μmol/l)	0.20 ± 0.03	0.25 ± 0.06	n.s.
GDCA (μmol/l)	8.17 ± 1.27	14.25 ± 2.48	<0.05
TDCA (μmol/l)	1.27 ± 0.19	14.18 ± 2.48	<0.05
TLCA (μmol/l)	0.07 ± 0.01	0.03 ± 0.01	<0.001
Sum of secondary bile acids (μmol/l)	17.20 ± 1.97	38.60 ± 6.71	<0.0001
Secondary unconjugated bile acids (μmol/l)	7.19 ± 0.97	21.50 ± 4.82	<0.0001
Secondary conjugated bile acids (μmol/l)	9.71 ± 1.42	17.01 ± 3.22	<0.05
Tertiary bile acids			
UDCA (μmol/l)	1.30 ± 0.26	2,72 ± 0.82	n.s.
GUDCA (μmol/l)	3.37 ± 0.81	3.55 ± 0.73	n.s.
TUDCA (μmol/l)	0.19 ± 0.04	0.23 ± 0.05	n.s.
Tertiary bile acids (μmol/l)	4.85 ± 1.07	6.49 ± 1.39	n.s.

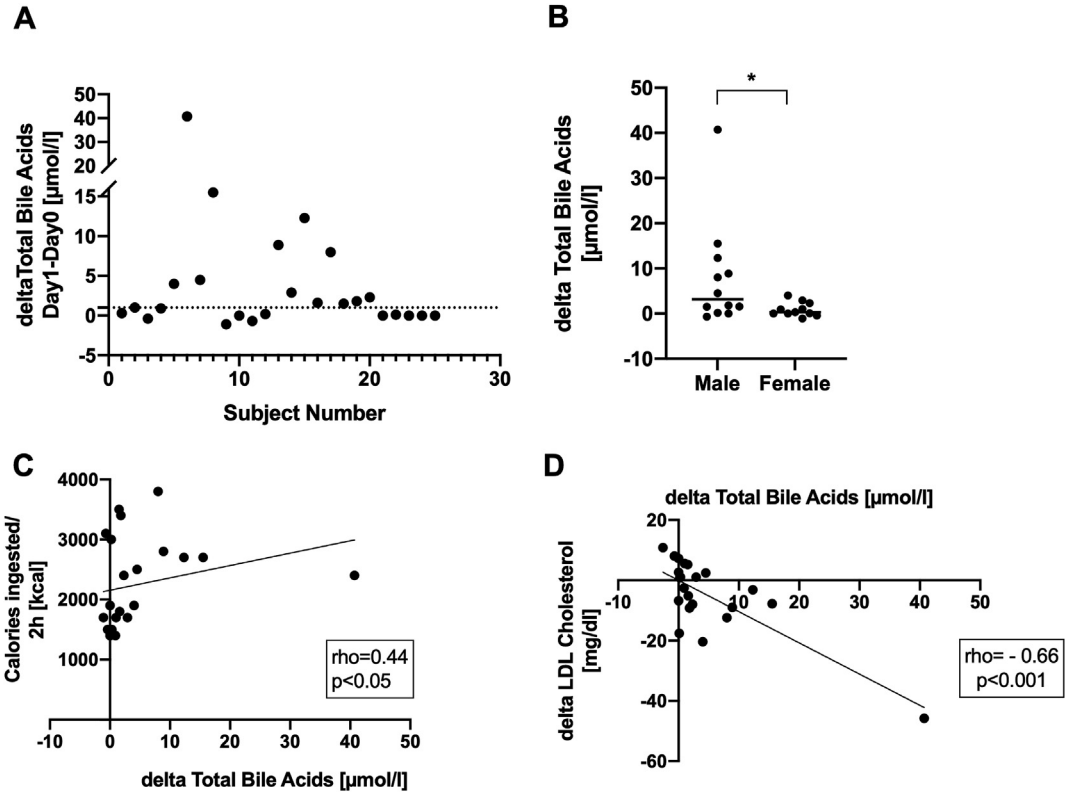

Fig. 1. Increase of bile acids (delta BA) on day 1 after the fast-food meal in the individuals of the study cohort are shown in response to a cutoff of ≤1 μmol/l or >1 μmol/l of total bile acid change (A). Differences of bile acid changes between day 0 and day 1 comparing male and female subjects (B). 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) *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

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 4
Characteristics and alterations of diagnostic parameters in subjects with low (delta Total Bile Acids ≤1) vs. high (delta Total Bile Acids >1) increase in fasting bile acid levels following the fast-food meal. n.s. stands for not significant.

	delta Total BA ≤ 1 ($n = 13$)	delta Total BA > 1 ($n = 12$)	p-value
Male/Female	4/9	9/3	<0.05
Age (years)	24.31 ± 0.95	24.08 ± 0.92	n.s.
BMI (kg/m^2)	23.50 ± 0.90	23.10 ± 0.70	n.s.
Calories/2 h period (kcal)	1954.00 ± 190.00	2633.00 ± 194.00	<0.05
Calories from Carbohydrates (%)	46.23 ± 1.26	48.35 ± 1.62	n.s.
Calories from Fat (%)	40.94 ± 1.32	39.28 ± 1.35	n.s.
Calories from Proteins (%)	12.69 ± 0.65	12.37 ± 0.52	n.s.
delta LSM (kPa)	1.18 ± 0.65	−0.69 ± 0.40	<0.05
delta CAP (dB)	−4.17 ± 12.85	28.33 ± 19.83	n.s.
delta AST (U/L)	−4.83 ± 2.17	−3.50 ± 0.77	n.s.
delta ALT (U/L)	1.09 ± 1.31	1.22 ± 1.41	n.s.
delta AlkPhos (U/L)	−0.64 ± 1.63	−2.00 ± 0.71	n.s.
delta γGT (U/L)	−1.17 ± 0.52	−1.08 ± 0.56	n.s.
delta Bilirubin (mg/dl)	−0.16 ± 0.04	−0.38 ± 0.09	n.s.
delta Cholesterol (mg/dl)	−7.50 ± 2.35	−11.08 ± 2.50	n.s.
delta LDL-Cholesterol (mg/dl)	0.60 ± 2.53	12.28 ± 18.45	n.s.
delta HDL-Cholesterol (mg/dl)	−3.27 ± 1.64	−3.75 ± 1.58	n.s.
delta Triglycerides (mg/dl)	−15.27 ± 7.01	10.17 ± 20.20	n.s.
delta Fasting Glucose (mg/dl)	3.17 ± 2.24	2.33 ± 2.13	n.s.
delta BUN (mg/dl)	1.17 ± 0.68	2.33 ± 0.57	n.s.
delta M30 (U/l)	2.56 ± 6.78	9.93 ± 6.81	n.s.
delta M65 (U/l)	12.71 ± 15.08	−34.53 ± 16.59	n.s.
delta Adiponectin (ng/ml)	−0.42 ± 0.76	0.64 ± 1.44	n.s.
delta Leptin (ng/ml)	−1.13 ± 3.46	5.21 ± 4.43	n.s.
delta GLP1 (pg/ml)	219.90 ± 104.60	107.90 ± 45.36	n.s.
delta GIP (pg/ml)	−2.74 ± 12.72	0.93 ± 7.84	n.s.
delta FGF19 (pg/ml)	6.03 ± 17.31	−24.34 ± 33.0	n.s.
delta FGF21 (pg/ml)	−26.66 ± 13.37	−38.72 ± 35.1	n.s.
delta LBP (ng/ml)	11.80 ± 18.21	−21.19 ± 16.25	n.s.
delta TNFα (pg/ml)	1.53 ± 1.31	−2.65 ± 1.67	<0.05
Abundance (%) *Dialister* spp	0.16 ± 0.15	1.59 ± 0.89	n.s.
Abundance (%) *Bacteroides massiliensis*	2.21 ± 0.74	0.17 ± 0.16	<0.05
Abundance (%) *Sutterella wadsworthensis*	0.66 ± 0.32	1.48 ± 0.37	<0.05
Richness	187.30 ± 15.30	177.50 ± 8.42	n.s.
Pielou's Evenness Index	0.76 ± 0.01	0.74 ± 0.03	n.s.
Shannon Index	3.97 ± 0.12	3.84 ± 0.16	n.s.
Simpson Index	0.96 ± 0.004	0.94 ± 0.02	n.s.

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 delta 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 glyco-chenodeoxycholic 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 total 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 solubilization and absorption of dietary fat and lipid-soluble vitamins, BA also modulate the expression and release of various 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

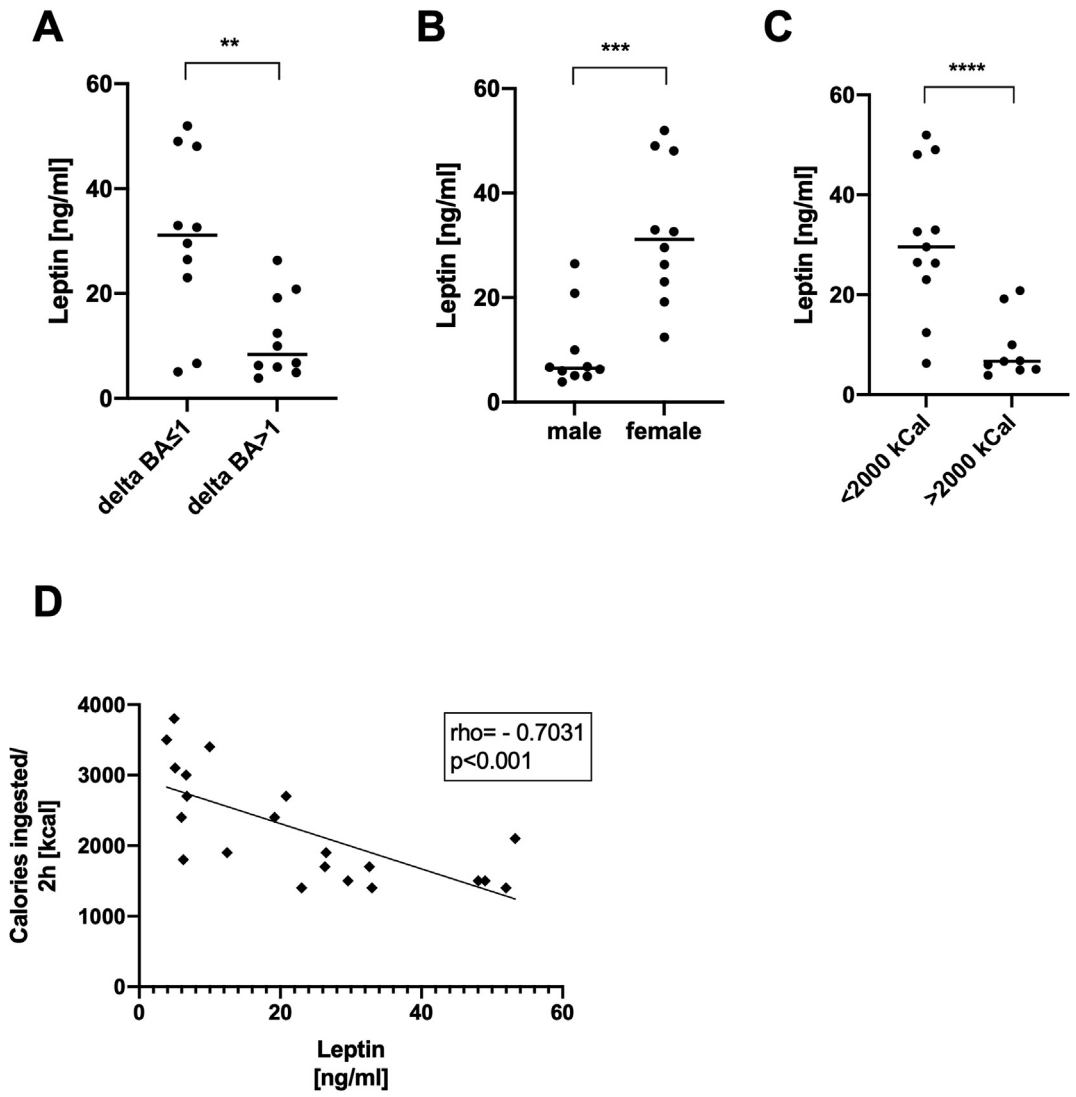

Fig. 2. Leptin serum levels comparing the groups with low or high increase of bile acids (A), male and female (B) and in those with a high or low intake of calories during the fast-food meal (C). Leptin serum levels correlate with calories intake (D). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

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 *phylae* 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

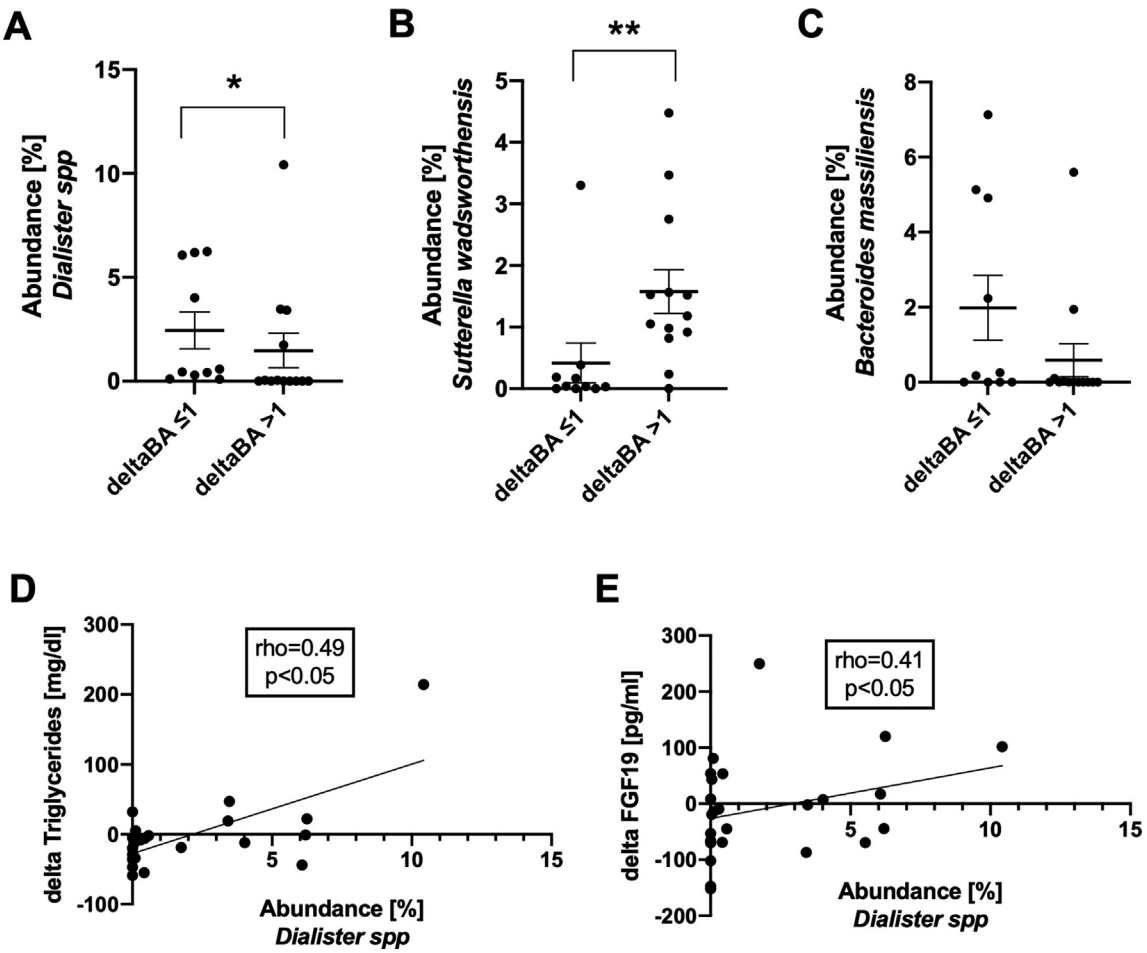

Fig. 3. Abundances of *Dialister* spp. (A), *Bacteroides massiliensis* (B) and *Sutterella wadsworthensis* (C) comparing individuals with delta BA ≤ 1 and delta BA > 1. Differences in the serum levels of triglycerides (D) and FGF19 (E) correlate with the abundance of *Dialister* spp. *$p < 0.05$, **$p < 0.01$.

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 increased abundance of *S. wadsworthensis* in individuals with a high BA response to the FF challenge, a profound correlation with serum GCDCA levels and an association with increased hepatic steatosis (CAP) and an inverse association with liver stiffness. While we observed associations here of the abundance of *Dialister* spp 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 studies, 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

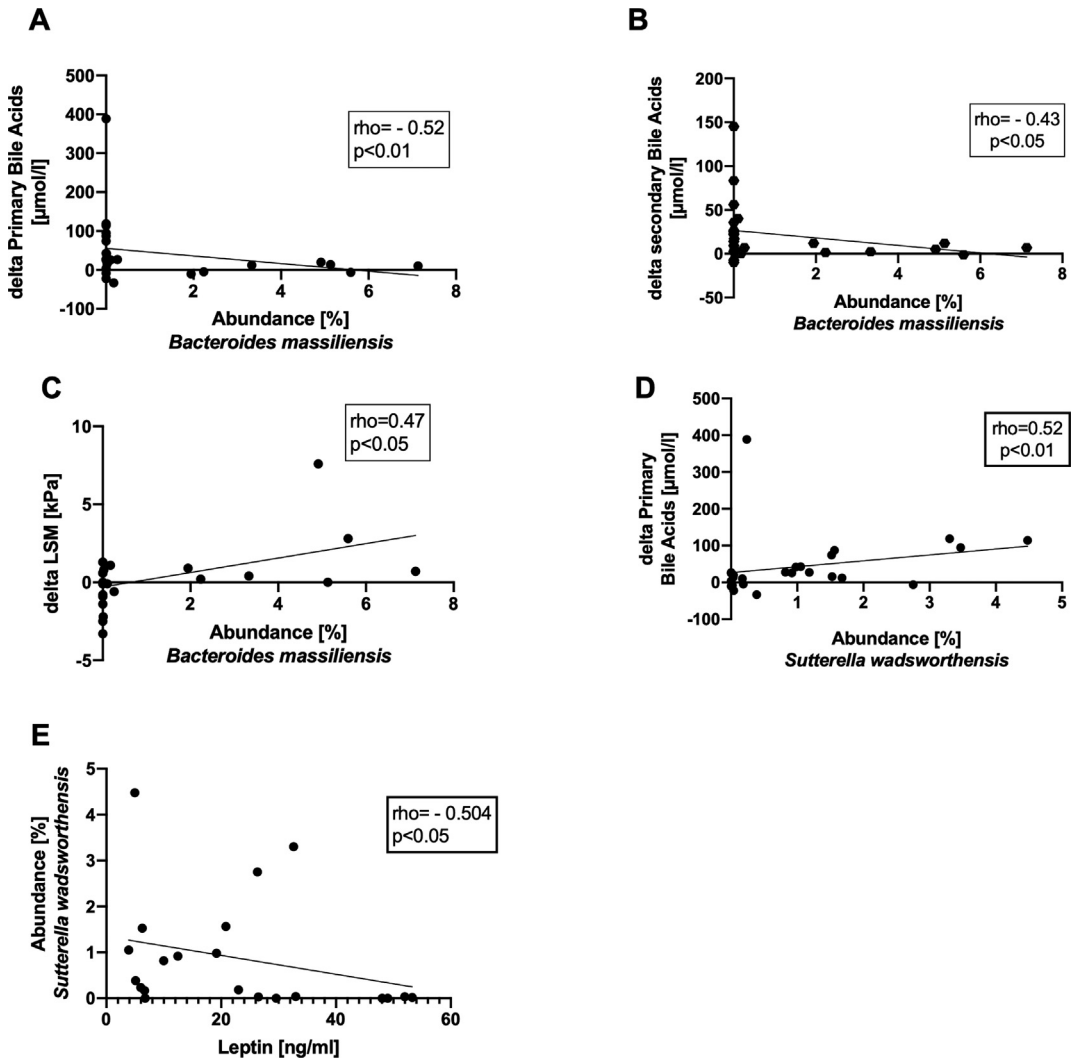

Fig. 4. The abundance of *Bacteroides massiliensis* in correlated to changes of the primary (A) and secondary (B) bile acids levels as well as with the transient elastography (LSM, C). The abundance of *Sutterella wadsworthensis* is correlated to changes of the primary BA (D) and leptin serum levels (E).

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

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), RVV (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), HM (critical revision of the manuscript for important intellectual content; technical support), MPÖ (critical revision of the manuscript), GG (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).

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Conflict of interest

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

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Appendix A. Supplementary data

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

References

[1] Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A. The interaction of hepatic lipid and glucose metabolism in liver diseases. J Hepatol 2012;56:952–64.
[2] Kliewer SA, Mangelsdorf DJ. Bile acids as hormones: the FXR-FGF15/19 pathway. Dig Dis 2015;33:327–31.
[3] Boursier J, Diehl AM. Nonalcoholic fatty liver disease and the gut microbiome. Clin Liver Dis 2016;20:263–75.
[4] Sydor S, Best J, Messerschmidt I, Manka P, Vilchez-Vargas R, Brodesser S, et al. Altered microbiota diversity and bile acid signaling in cirrhotic and non-cirrhotic NASH-HCC. Clin Transl Gastroenterol 2020;11:e00131.
[5] Bechmann LP, Kocabayoglu P, Sowa J-P, Sydor S, Best J, Schlattjan M, et al. Free fatty acids repress small heterodimer partner (SHP) activation and adiponectin counteracts bile acid-induced liver injury in superobese patients with nonalcoholic steatohepatitis. Hepatology 2013;57:1394–406.
[6] Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab 2009;10:167–77.
[7] Ramos Pittol JM, Milona A, Morris I, Willemsen ECL, van der Veen SW, Kalkhoven E, et al. FXR isoforms control different metabolic functions in liver cells via binding to specific DNA motifs. Gastroenterology 2020;159(5):1853–1865.e10.
[8] Gonzalez FJ, Jiang C, Patterson AD. An intestinal microbiota-farnesoid X receptor axis modulates metabolic disease. Gastroenterology 2016;151:845–59.
[9] Farr S, Stankovic B, Hoffman S, Masoudpoor H, Baker C, Taher J, et al. Bile acid treatment and FXR agonism lower postprandial lipemia in mice. Am J Physiol Gastrointest Liver Physiol 2020;318:G682–93.
[10] Kumari A, Pal Pathak D, Asthana S. Bile acids mediated potential functional interaction between FXR and FATP5 in the regulation of lipid metabolism. Int J Biol Sci 2020;16:2308–22.
[11] Ridlon JM, Kang DJ, Hylemon PB, Bajaj JS. Bile acids and the gut microbiome. Curr Opin Gastroenterol 2014;30:332–8.
[12] Rath S, Heidrich B, Pieper DH, Vital M. Uncovering the trimethylamine-producing bacteria of the human gut microbiota. Microbiome 2017;5:54.
[13] Hammer O, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. 2001. p. 9.
[14] Pham HT, Arnhard K, Asad YJ, Deng L, Felder TK, John-Williams LS, et al. Inter-laboratory robustness of next-generation bile acid study in mice and humans: international ring trial involving 12 laboratories. J Appl Lab Med 2016;1:129–42.
[15] Sowa J-P, Heider D, Bechmann LP, Gerken G, Hoffmann D, Canbay A. Novel algorithm for non-invasive assessment of fibrosis in NAFLD. PLoS One 2013;8:e62439.
[16] Abumrad NA, Davidson NO. Role of the gut in lipid homeostasis. Physiol Rev 2012;92:1061–85.
[17] Xie C, Jones KL, Rayner CK, Wu T. Enteroendocrine hormone secretion and metabolic control: importance of the region of the gut stimulation. Pharmaceutics 2020;12.
[18] Adrian TE, Gariballa S, Parekh KA, Thomas SA, Saadi H, Al Kaabi J, et al. Rectal taurocholate increases L cell and insulin secretion, and decreases blood glucose and food intake in obese type 2 diabetic volunteers. Diabetologia 2012;55:2343–7.
[19] Gao F, Zhang X, Zhou L, Zhou S, Zheng Y, Yu J, et al. Type 2 diabetes mitigation in the diabetic Goto-Kakizaki rat by elevated bile acids following a common-bile-duct surgery. Metabolism 2016;65:78–88.
[20] Spiller RC, Frost PF, Stewart JS, Bloom SR, Silk DB. Delayed postprandial plasma bile acid response in coeliac patients with slow mouth-caecum transit. Clin Sci (Lond) 1987;72:217–23.
[21] Pennington CR, Ross PE, Bouchier IA. Fasting and postprandial serum bile acid concentrations in normal persons using an improved GLC method. Digestion 1978;17:56–62.
[22] Katsuma S, Hirasawa A, Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochem Biophys Res Commun 2005;329:386–90.
[23] Shima KR, Ota T, Kato K-I, Takeshita Y, Misu H, Kaneko S, et al. Ursodeoxycholic acid potentiates dipeptidyl peptidase-4 inhibitor sitagliptin by enhancing glucagon-like peptide-1 secretion in patients with type 2 diabetes and chronic liver disease: a pilot randomized controlled and add-on study. BMJ Open Diabetes Res Care 2018;6:e000469.
[24] Zhu C, Sawrey-Kubicek L, Beals E, Rhodes CH, Houts HE, Sacchi R, et al. Human gut microbiome composition and tryptophan metabolites were changed differently by fast food and Mediterranean diet in 4 days: a pilot study. Nutr Res 2020;77:62–72.
[25] Martinez KB, Leone V, Chang EB. Western diets, gut dysbiosis, and metabolic diseases: are they linked? Gut Microbes 2017;8:130–42.
[26] Wan Y, Yuan J, Li J, Li H, Zhang J, Tang J, et al. Unconjugated and secondary bile acid profiles in response to higher-fat, lower-carbohydrate diet and associated with related gut microbiota: a 6-month randomized controlled-feeding trial. Clin Nutr 2020;39:395–404.
[27] Gérard P. Metabolism of cholesterol and bile acids by the gut microbiota. Pathogens 2013;3:14–24.
[28] Pudlo NA, Urs K, Kumar SS, German JB, Mills DA, Martens EC. Symbiotic human gut bacteria with variable metabolic priorities for host mucosal glycans. mBio 2015;6. e01282-01215.

[29] Mbakwa CA, Hermes GDA, Penders J, Savelkoul PHM, Thijs C, Dagnelie PC, et al. Gut microbiota and body weight in school-aged children: the KOALA birth cohort study. Obesity (Silver Spring) 2018;26:1767–76.
[30] Paramsothy S, Nielsen S, Kamm MA, Deshpande NP, Faith JJ, Clemente JC, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. Gastroenterology 2019;156:1440–1454.e2.
[31] Fève B, Bastard J-P. From the conceptual basis to the discovery of leptin. Biochimie 2012;94:2065–8.
[32] Pénicaud L, Meillon S, Brondel L. Leptin and the central control of feeding behavior. Biochimie 2012;94:2069–74.
[33] Lawler K, Huang-Doran I, Sonoyama T, Collet T-H, Keogh JM, Henning E, et al. Leptin-mediated changes in the human metabolome. J Clin Endocrinol Metab 2020:105.
[34] Giordano C, Barone I, Vircillo V, Panza S, Malivindi R, Gelsomino L, et al. Activated FXR inhibits leptin signaling and counteracts tumor-promoting activities of cancer-associated fibroblasts in breast malignancy. Sci Rep 2016;6:21782.
[35] Grases-Pintó B, Abril-Gil M, Castell M, Rodríguez-Lagunas MJ, Burleigh S, Fåk Hållenius F, et al. Influence of leptin and adiponectin supplementation on intraepithelial lymphocyte and microbiota composition in suckling rats. Front Immunol 2019;10:2369.
[36] Holscher HD, Guetterman HM, Swanson KS, An R, Matthan NR, Lichtenstein AH, et al. Walnut consumption alters the gastrointestinal microbiota, microbially derived secondary bile acids, and health markers in healthy adults: a randomized controlled trial. J Nutr 2018;148:861–7.
[37] Trauner M, Claudel T, Fickert P, Moustafa T, Wagner M. Bile acids as regulators of hepatic lipid and glucose metabolism. Dig Dis 2010;28:220–4.
[38] Matsubara T, Li F, Gonzalez FJ. FXR signaling in the enterohepatic system. Mol Cell Endocrinol 2013;368:17–29.
[39] Guo J-L, Kuang W-M, Zhong Y-F, Zhou Y-L, Chen Y-J, Lin S-M. Effects of supplemental dietary bile acids on growth, liver function and immunity of juvenile largemouth bass (Micropterus salmoides) fed high-starch diet. Fish Shellfish Immunol 2020;97:602–7.
[40] Dai D, Pan Y, Zeng C, Liu S, Yan Y, Wu X, et al. Activated FXR promotes xenobiotic metabolism of T-2 toxin and attenuates oxidative stress in broiler chicken liver. Chem Biol Interact 2020;316:108912.
[41] Kälsch J, Bechmann LP, Heider D, Best J, Manka P, Kälsch H, et al. Normal liver enzymes are correlated with severity of metabolic syndrome in a large population based cohort. Sci Rep 2015;5:13058.