The hepatic response to FGF19 is impaired in patients with non-alcoholic fatty liver disease and insulin resistance.

Running Title: The postprandial FGF19 response in NAFLD patients.

Tim C.M.A. Schreuder1,2*, Hendrik A. Marsman3*, Martin Lenicek4, Jochem R. van Werven5, Aart J. Nederveen5, Peter L.M. Jansen1,6, Frank G. Schaap6

*These authors contributed equally

Academic Medical Center, Departments of 1Gastroenterology and Hepatology, 3Surgery and 5Radiology, and 6AMC Liver Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

Department of Gastroenterology and Hepatology, Liver Unit, VU Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands

Institute of Clinical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles University in Prague, Czech Republic

Corresponding Author: Frank G. Schaap, PhD

AMC Liver Center
Meibergdreef 69-71
1105 BK Amsterdam
The Netherlands
P: +31 20 5668162
F: +31 20 5669190
f.g.schaap@amc.uva.nl
Abstract

Intestinal FGF19 has emerged as a novel endocrine regulator of hepatic bile salt and lipid metabolism. In patients with non-alcoholic fatty liver disease (NAFLD) hepatic lipid metabolism is deranged. A possible role of FGF19 in NAFLD has not been reported yet. In this study, we assessed intestinal FGF19 production and the hepatic response to FGF19 in NAFLD patients with and without insulin resistance (HOMA score ≥2.5 [n=12] and HOMA score <2.5 [n=8], respectively). To this end, NAFLD patients received a standardized oral fat challenge. Postprandial excursions of triglycerides, bile salts and FGF19 were monitored, and plasma levels of a marker for bile salt synthesis (7α-hydroxy-4-cholesten-3-one) were determined. Fasted FGF19 levels were comparable in a control group of healthy volunteers (n=15) and in NAFLD patients (0.26±0.28 ng/mL vs. 0.18±0.09 ng/mL, resp., P=0.94). Postprandial FGF19 levels in both controls and NAFLD patients peaked between 3-4 hrs and were three times higher than baseline levels. The areas under the postprandial FGF19 curve were similar in controls and in the HOMA score-based NAFLD subgroups. In NAFLD patients with HOMA score <2.5, the postprandial increase in plasma FGF19 was accompanied by a lowering of plasma levels of 7α-hydroxy-4-cholesten-3-one (-30%, P=0.015). This anticipated decline was not observed in insulin-resistant NAFLD patients (+10%, P=0.22). In conclusion, patients with NAFLD show an unimpaired intestinal FGF19 production. However, the hepatic response to FGF19 is impaired in NAFLD patients with insulin resistance (HOMA score ≥2.5). This impaired hepatic response to FGF19 may contribute to the dysregulation of lipid homeostasis in NAFLD.

Keywords: enterohepatic signaling, postprandial response, 7α-hydroxy-4-cholesten-3-one, CYP7A1, bile salt uptake
Introduction

Increased hepatic fat accumulation underlies the development of non-alcoholic fatty liver disease (NAFLD). The spectrum of liver abnormalities in NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) when accompanied by inflammation and fibrosis. One fifth of NASH patients ultimately develops cirrhosis (4). The etiopathology of NAFLD is far from understood. The progression of NAFLD towards NASH has traditionally been explained by a two-hit model in which initial accumulation of triglycerides (first hit) is followed by inflammatory and/or oxidative stress (second hit) resulting in steatohepatitis (8). More recently it has been suggested that hepatic deposition of saturated fat in particular, may directly damage the liver and mediate progression of plain steatosis to NASH (12). The increasing prevalence of this obesity-related disorder and its health consequences, necessitate further insight into the causes and consequences of hepatic lipid accumulation.

FGF19 belongs to a subfamily of FGFs that have an endocrine function and has emerged as a novel regulator of hepatic lipid homeostasis (5,17). Initial studies in mice transgenic for FGF19 indicated a role for FGF19 in whole-body energy and lipid homeostasis (11,32). Specifically, FGF19 transgenic mice had decreased adiposity and were resistant to high fat diet-induced weight gain. Furthermore, transgenic FGF19 expression or infusion of recombinant FGF19 protein reduced hepatic lipid accumulation and improved insulin-sensitivity in ob/ob mice (11). The observed in vivo effects of FGF19 have been attributed in part to reduced hepatic expression of acetyl-CoA carboxylase 2 (Acc2), a negative regulator of mitochondrial fatty acid oxidation. Repression of Acc2 in FGF19 transgenic mice may thus deplete hepatic lipid stores by promoting mitochondrial fatty acid degradation. In addition, a recent study revealed that FGF19 suppresses insulin-induced fatty acid synthesis in isolated hepatocytes (6). Thus, FGF19 appears to influence both hepatic fatty acid oxidation and hepatic lipid synthesis.
It is currently unknown if FGF19 plays a role in the etiopathogenesis of NAFLD. Reduced plasma FGF19 levels have been reported for patients with type 2 diabetes mellitus, but the cause of this decrease is unknown (30). FGF19 is produced by the small intestine in a bile salt-stimulated manner (16). Binding of FGF19 to its cognate receptor on hepatocytes activates intracellular signaling pathways resulting in repression of bile salt synthesis and, presumably, lipid synthetic pathways (6,14,16,17). FGF19 can thus be viewed as a feed-forward signal that informs the liver on dietary fat intake and return of bile salts. Reduced plasma FGF19 in type 2 diabetic subjects may be due to impaired intestinal FGF19 production, and may contribute to ongoing triglyceride synthesis and exacerbation of hepatic steatosis in subjects with NAFLD. In the present study, we examined the postprandial FGF19 response in subjects with NAFLD with or without insulin resistance.
Experimental Procedures

Patients and Study Design

From a prospective database on NAFLD patients, twenty patients between the age of 18 and 75 years were recruited for participation in this study. Initial diagnosis of NAFLD was based on elevated alanine aminotransferase levels (ALT ≥ 45 IU/L), increased echogenicity of the liver on ultrasound examination, and exclusion of other chronic liver diseases defined as normal iron and copper studies and absence of hepatitis B surface antigen, hepatitis C antibodies and autoimmune antibodies (antinuclear antibodies, antibodies to smooth muscle antigens and mitochondria). Patients with excessive alcohol intake, defined as more than 2 units/day in male and more than 1 unit/day in female, were excluded. Following study inclusion, all NAFLD patients underwent assessment of hepatic fat content by magnetic resonance spectroscopy ($^1$H-MRS).

Patients were divided into two groups according to severity of insulin resistance (homeostasis model of assessment - HOMA) (2). Following overnight fasting, all study subjects received a standardized oral fat challenge consisting of 30 gram cream (35 gram fat per 100 mL) per square meter of body surface area (26). Blood samples were taken from an indwelling cannula placed in a cubital vein at baseline and at hourly intervals for up to 7 hrs after oral fat intake. During the sampling period, patients were withheld from additional food but had free access to water. Patients gave their informed consent to the protocol of the study which was approved by the medical ethical committee of the Academic Medical Center in Amsterdam.

A previously described cohort consisting of 15 healthy volunteers with an irrelevant medical history (26), was used as a control group. These subjects underwent an identical oral lipid challenge with blood sampling at baseline and after 2, 3, 4 and 6 hrs.
**Determination of hepatic fat content**

The fat content in the liver of NAFLD patients was determined by $^1$H-MRS. All measurements were performed on a 3.0 Tesla Philips Intera scanner (Philips Healthcare, Best, The Netherlands). A voxel of 20 x 20 x 20 mm was positioned in the right hepatic lobe, avoiding vascular, biliary and extrahepatic structures. Spectra were acquired using first order iterative shimming, a PRESS sequence with TE/TR=35/2000 ms and 64 signal acquisitions. The water (4.7 ppm) and fat (1.3 ppm) resonance peaks were integrated using jMRUI software (25) and relative fat content was expressed as a ratio of the fat peak area over the cumulative water and fat peak areas. Calculated peak areas of water and fat were corrected for T2 relaxations ($T_{2\text{water}}=34\text{ms}, T_{2\text{fat}}=68\text{ms}$, ref. 9) and the percentage hepatic fat content was calculated according to Szczepaniak *et al.* (31).

**Blood chemistry**

Following collection of blood in EDTA tubes, plasma was prepared and analyzed for levels of glucose, insulin, C-reactive protein, and liver enzymes at baseline (t=0 hrs). Triglycerides (TG), total cholesterol, FGF19 and total bile salts (Diazyme, Poway CA) were assayed at baseline and at the respective sampling points after oral fat intake. Baseline interleukin-6 (IL-6) levels were determined by sandwich ELISA (Sanquin, Amsterdam, The Netherlands). 7α-hydroxy-4-cholesten-3-one (C4) levels, a plasma marker for bile salt synthesis, were determined at baseline and at 4 and 5 hours after oral fat intake as previously described (19). C4 levels were expressed relative to total cholesterol, as their ratio was shown to be a more accurate marker of bile salt synthesis (15).

**Determination of plasma FGF19**

Plasma FGF19 levels were determined using an in-house developed sandwich ELISA described in detail elsewhere (Schaap *et al.*, manuscript in preparation). Briefly, microtiter
plates were coated with goat anti-human FGF19 antibody (AF969, R&D Systems, Minneapolis, MN). Samples and recombinant FGF19 standards (R&D Systems) were diluted in PBS containing 1.0% casein and 0.05% Tween-20. Captured antigen was detected with biotinylated goat anti-human FGF19 antibody (BAF969, R&D Systems) and streptavidin-HRP using tetramethylbenzidine as chromogenic substrate.

Data and Statistical analysis
Area under the postprandial curve (AUC) was calculated using baseline-subtracted values employing GraphPad Prism (GraphPad Software Inc., La Jolla, CA). For comparison with the historical control population, data derived from time points that were missing (i.e. T=1, 5 and 7 hrs) in the sampling scheme of the controls were omitted for AUC calculations (denoted as AUC0-6 hrs). Descriptive statistics are expressed as mean ± standard deviation. Within each group, a paired t test was used to evaluate changes from baseline during the oral fat challenge. Differences between groups (AUC or individual time points of the postprandial curves) were evaluated by Student's t test, Mann-Whitney U test or one-way ANOVA with Bonferroni post-hoc testing. Statistical analyses were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL). Statistical significance was accepted at P < 0.05.
**Results**

**Patient characteristics**

Table 1 shows the characteristics of the controls and the NAFLD groups. As expected, NAFLD patients were obese (BMI >30). Hyperglycemia and hyperinsulinemia in the NAFLD population was attributable to patients with HOMA score ≥2.5. Nine NAFLD patients fulfilled the American Diabetes Association criteria for type 2 diabetes mellitus (2). The majority of these patients (6 out of 9) was treated with metformin. Five diabetic patients had dyslipidemia (TG >1.7 mmol/L and/or HDL-cholesterol <0.9 mmol/L (1)). Two patients were treated with statins.

**Plasma FGF19 levels in NAFLD patients**

Baseline FGF19 levels in NAFLD patients (0.18±0.09 ng/mL) were comparable to values in the studied controls (0.26±0.28 ng/mL, \( P=0.94 \)) and to values recently reported for an unrelated control population (0.28±0.20 ng/mL) (28). Furthermore, baseline plasma FGF19 levels were identical (\( P=1.00 \)) in the HOMA-based NAFLD subgroups. Baseline FGF19 levels in NAFLD patients showed a weak inverse correlation (Spearman's \( r = -0.47, P=0.038 \)) with baseline plasma levels of 7α-hydroxy-4-cholesten-3-one (C4), a marker for bile salt synthesis. This appears in line with FGF19's role in negative regulation of bile salt synthesis. Baseline FGF19 levels showed no correlation with hepatic fat content or HOMA score (data not shown).

Baseline bile salt levels were elevated in NAFLD patients with a HOMA score ≥2.5 in comparison with controls (\( P<0.001 \)) and NAFLD patients with a HOMA score <2.5 (\( P=0.006 \)) (Table 1).

**Postprandial responses in NAFLD patients**
Entry of dietary fat in the duodenum causes gallbladder contraction and inflow of bile salts into the intestinal lumen (13). Reabsorption of bile salts in the distal part of the small intestine activates the bile salt receptor FXR resulting in enhanced transcription and portal release of FGF19 (16). The postprandial excursions of TG, bile salts and FGF19 in control subjects and NAFLD patients are depicted in Figure 1.

Packaging of digested dietary lipids into chylomicrons and their release into lymph, causes an increase in plasma TG levels (Figure 1A). As reported earlier for the volunteer group (26), a postprandial increase in TG was first noted after 2 hrs. TG levels peaked after 3 hrs in controls and between 3 and 4 hrs in NAFLD patients. The total area under the postprandial TG curve \( (\text{AUC}_{\text{TG }0-6\text{hrs}}) \) was elevated in NAFLD patients \( (4.1\pm3.2 \text{ vs. } 2.1\pm1.4 \text{ mmol.L}^{-1}\cdot\text{hr}^{-1} \text{ in controls, } P=0.013) \). During the ascending phase of the postprandial TG excursion, TG production \( (\text{i.e. chylomicron synthesis}) \) predominates. The area under the ascending part of the curve \( (\text{AUC}_{\text{TG }0-3\text{hrs}}) \) was not different \( (1.4\pm1.3 \text{ vs. } 1.0\pm0.6 \text{ mmol.L}^{-1}\cdot\text{hr}^{-1} \text{ in NAFLD patients and controls, resp., } P=0.079) \). In the descending phase of the postprandial TG curve, clearance of TG-rich lipoproteins prevails. The area under the descending part of the curve \( (\text{AUC}_{\text{TG }3-6\text{hrs}}) \) was elevated in NAFLD patients \( (2.7\pm2.1 \text{ vs. } 1.1\pm1.0 \text{ mmol.L}^{-1}\cdot\text{hr}^{-1} \text{ in volunteers, } P=0.002) \). The latter appears in line with impaired TG clearance in insulin-resistant subjects (23). This notion is supported when ascending, descending and total AUC\(_{\text{TG}} \) values for the control and the HOMA-based NAFLD subgroups are compared. This analysis revealed that total and descending AUC\(_{\text{TG}} \) values are significantly elevated in the NAFLD HOMA \( \geq 2.5 \) subgroup only (data not shown). Thus, the observed difference in AUC\(_{\text{TG }0-6\text{hrs}} \) is likely due to impaired TG clearance in insulin-resistant NAFLD patients. This also indicates that gastrointestinal passage and handling of ingested lipids is similar in controls and both NAFLD subgroups.

Following an oral fat challenge, an incline in plasma bile salt levels is apparent after 1 hr in NAFLD patients, with levels reaching a maximum after 3-4 hrs (Figure 1B). In control subjects, which were first sampled after 2 hrs, bile salt levels peak after 2 hrs and return to
baseline levels after 6 hrs. In contrast, bile salts remain elevated at this and at the final time
point in NAFLD patients (Figures 1B and 2A). After gallbladder contraction-induced entry into
the duodenum, bile salts are efficiently reclaimed from the small intestinal lumen and released
into the portal circulation. First-pass clearance of bile salts by the liver is highly efficient with
little systemic spill-over and is depending on the Na\(^+\) taurocholate co-transporting protein
(NTCP) (13). As can be appreciated from Figure 1B, the postprandial bile salt excursion is
different in controls and NAFLD patients. Bile salts levels were significantly higher in NAFLD
subjects at time points T=0 (P=0.004), T=4 (P=0.015) and T=6 hrs (P=0.001) and this was
entirely attributable to NAFLD patients with HOMA score ≥2.5 (data not shown). The AUC\(_{BS}
0-6\) hrs tended to be elevated in NAFLD patients (67.3±47.7 vs. 47.1±29.4 µmol.L\(^{-1}\).hr\(^{-1}\) in
controls, \(P=0.09\)). NAFLD patients with HOMA score ≥2.5 (75.8±59.9 vs. 54.5±14.9 µmol.L\(^{-1}\).
hr\(^{-1}\) in the HOMA<2.5 subgroup, \(P=0.43\)) largely accounted for this tendency. Since these
results suggest impaired hepatic bile salt uptake we measured interleukin-6 (IL-6), a known
regulator of NTCP expression (3). NAFLD patients with HOMA score ≥2.5 showed elevated
IL-6 levels in comparison with controls (2.1±1.6 vs. 0.9±0.6 pg/mL, resp., \(P=0.005\), Table 1).

Following fat ingestion, a slight drop in plasma FGF19 level occurs after 1 hr in NAFLD
patients. Plasma FGF19 was significantly elevated after 2 hrs in control subjects, and after 3
hrs in NAFLD patients (Figure 1C). Postprandial FGF19 levels peaked between 3-4 hrs in
controls and NAFLD patients and remained elevated in both groups until the last sampling
point. Peak postprandial FGF19 levels were 3.0 fold higher in comparison with baseline levels
in both controls and in NAFLD patients. The postprandial excursion of FGF19 closely
followed that of bile salts, although some lagging was apparent. This is in line with bile salt-
mediated induction of ileal FGF19 expression (14,16). When analyzing individual time points,
no significant differences in plasma FGF19 level were apparent between controls and NAFLD
patients. The AUC\(_{FGF19\ 0-6\ hrs}\) was however significantly lower in NAFLD patients (1.4±1.3 vs.
1.9±1.4 ng.mL\(^{-1}\).hr\(^{-1}\) in controls, \(P=0.032\)).
Influence of insulin-resistance on the postprandial FGF19 response in NAFLD patients

Both NAFLD subgroups displayed similar postprandial bile salt excursions (Figure 2A) and had indistinguishable AUC\textsubscript{BS 0-7hrs} values (61.2±17.8 vs. 85.1±69.6 µmol.L\textsuperscript{-1}.hr\textsuperscript{-1} in the HOMA<2.5 and HOMA\geq2.5 subgroups, resp., P=0.47). The postprandial FGF19 response in HOMA-based NAFLD subgroups is shown in Figure 2B. Plasma FGF19 is significantly elevated above baseline levels after 3 hrs in the HOMA<2.5 subgroup (P=0.018) and, albeit with borderline significance, in the HOMA\geq2.5 subgroup (P=0.051). Postprandial FGF19 levels peaked at 3 and 4 hrs in the HOMA<2.5 and HOMA\geq2.5 subgroups, respectively. Mean FGF19 levels in the NAFLD subgroups were not different at any of the individual time points.

The AUC\textsubscript{FGF19 0-7hrs} was similar in both NAFLD subgroups (1.6±1.3 vs. 1.4±1.5 ng.mL\textsuperscript{-1}.hr\textsuperscript{-1} in the HOMA<2.5 and HOMA\geq2.5 groups, resp., P=0.49). In addition, analysis of variance indicated that AUC\textsubscript{FGF19 0-6hrs} values were not significantly different in controls and the two NAFLD subgroups (ANOVA P\textsubscript{ALL}=0.081).

Elevation of plasma FGF19 is expected to result in diminished bile salt synthesis through downregulation of hepatic CYP7A1 mRNA (14,16). Plasma C4, a marker for bile salt synthesis, was determined in NAFLD patients at baseline, and at 4 and 5 hrs following an oral fat challenge. Despite similar postprandial elevation of FGF19 in both NAFLD subgroups (Figure 3, upper panel), C4 levels declined after 5 hrs only in the HOMA<2.5 group (-30%, P=0.015) while remaining unchanged in the HOMA\geq2.5 group (+10%, P=0.22) (Figure 3, lower panel).
Recent findings indicate a role for the endocrine factor FGF19 in the regulation of hepatic lipid metabolism (6,11,32). Altered intestinal FGF19 production and/or altered hepatic responsiveness to FGF19 may accordingly contribute to the dysregulation of lipid homeostasis encountered in NAFLD. In this study, we evaluated the postprandial FGF19 response following an oral fat challenge in healthy volunteers and in NAFLD patients with and without insulin resistance. The major novel finding of this study is that the response of the liver to elevated plasma FGF19 levels is impaired in insulin-resistant NAFLD patients.

FGF19 is an integral part of a regulatory mechanism by which bile salts negatively regulate their own synthesis (14,16). Having fulfilled their function in digestion and absorption of dietary lipids in the proximal parts of the small intestine, bile salts are reclaimed in the terminal ileum (13). This results in activation of the bile salt-activated transcription factor FXR and transcriptional induction of FGF19, an ileal FXR target gene (16). Binding of FGF19 to its cell surface receptor on hepatocytes results in repression of bile salt synthesis via downregulation of CYP7A1 (14,16). In this study we used a physiological stimulus, viz. a fatty meal, to induce gallbladder contraction and accordingly stimulate intestinal FGF19 production. The functional consequence of postprandial elevation of FGF19 levels was assessed by measurement of plasma C4, a marker for bile salt synthesis.

A standardized oral fat challenge resulted in elevation of bile salt levels after 1 hr, followed by an increase in plasma FGF19 level after 2-3 hrs (Figure 1). In line with an earlier study in volunteers receiving regular meals (21), FGF19 levels show a postprandial peak after 3-4 hrs. Healthy volunteers and NAFLD patients had comparable fasted FGF19 levels (0.26±0.28 ng/mL vs. 0.18±0.09 ng/mL, resp., \(P=0.94\)) and displayed a similar postprandial FGF19 response. Peak postprandial FGF19 levels were 3.0 fold higher than baseline values in both groups. The AUC_{FGF19 0-6hrs} appeared somewhat lower in NAFLD patients in comparison with
the volunteers when considering the entire NAFLD group \((1.4\pm1.3 \text{ vs. } 1.9\pm1.4 \text{ ng.mL}^{-1}.\text{hr}^{-1})\) in controls, \(P=0.032\), but was indistinguishable when comparing the volunteers and the two HOMA-based NAFLD subgroups by ANOVA \((P_{\text{ALL}}=0.081)\).

Postprandial elevation of plasma FGF19 is expected to result in repression of bile salt synthesis. Indeed, plasma C4 levels were decreased following postprandial peaking of FGF19 levels in NAFLD patients with a normal HOMA score (Figure 3B). Interestingly, such decline in C4 levels was not observed in insulin-resistant \((i.e. \text{ HOMA score } \geq 2.5)\) NAFLD patients. As postprandial FGF19 levels in the HOMA-based NAFLD subgroups were similar at all individual time points, this leaves the possibility that the response of the liver to FGF19 is impaired in insulin-resistant NAFLD patients.

In addition to an apparently impaired hepatic response to FGF19 in NAFLD patients with a HOMA score \(\geq 2.5\), we noted an altered postprandial bile salt excursion in this patient group. Similar postprandial FGF19 excursions in controls and both NAFLD patient groups suggests that ileal bile salt reclamation proceeds similarly in these groups, it is thus tempting to speculate that the hepatic uptake of bile salts is reduced in NAFLD patients with HOMA score \(\geq 2.5\). Reduced first-pass clearance of bile salts by the liver would result in a higher systemic spill-over of bile salts and consequently prolonged circulation times. Pro-inflammatory cytokines released from inflamed adipose tissue have been implicated in the development of hepatic insulin-resistance (22), and among these IL-1\(\beta\) and IL-6 are known to reduce NTCP expression (3,10). Elevated IL-6 levels in NAFLD patients with HOMA score \(\geq 2.5\) (Table 1) may thus have contributed to the altered postprandial bile salt excursion in this patient group.

Activation of the hepatic FXR/SHP axis by bile salts has been implicated in the regulation of bile salt synthesis (7,27). Could reduced hepatic uptake of bile salts account for the absence of a postprandial decline in plasma C4 levels in NAFLD patients with HOMA score \(\geq 2.5\)? Although this cannot be ruled out in the present study, several lines of evidence implicate the intestinal FXR/FGF19 axis as the principal mediator of bile salt-mediated repression of bile
salt synthesis. Firstly, by-passing the small intestine through direct infusion of taurocholate in the portal or systemic circulation, failed to downregulate Cyp7a1 expression in the rat (24). Secondly, in mice lacking intestinal Fxr the expression of Cyp7a1 is unaffected by FXR agonists whereas in mice lacking hepatic Fxr, Cyp7a1 is effectively repressed following FXR agonism (18). Moreover, bile salt-mediated negative feedback control of Cyp7a1 expression was lost in mice deficient for either Fgf15 or its receptor Fgfr4 (16). Thirdly, postprandial increases in plasma FGF19 levels rather than postprandial increases in bile salt levels are followed by a decline in plasma C4 levels (21). Taken into account the above findings, it is unlikely that reduced hepatic bile salt uptake underlies the absence of a postprandial decline in plasma C4 levels in NAFLD patients with HOMA score ≥2.5.

What mechanism could underlie the apparently abrogated hepatic response to FGF19 in insulin-resistant NAFLD patients? Altered expression of the FGF19-receptor FGFR4 and/or the obligate signaling cofactor βKlotho may be a underlying factor. A recent study revealed that expression of hepatic Fgfr4 mRNA was reduced after prolonged fasting as well as in streptozotocin-induced diabetic mice, while insulin-treatment induced hepatic Fgfr4 mRNA expression in mice (29). Hepatic expression of βKlotho was affected by none of these treatments. No information is available on FGFR4 expression in the insulin-resistant human liver, although microarray analysis suggests that Fgfr4 expression is somewhat reduced in the liver of insulin-resistant, leptin-deficient mice (20). Apart from altered signal transduction at the level of the plasma membrane, intracellular relay of the FGF19 signal may be affected in the insulin-resistant liver. FGF19 was recently shown to reduce insulin-stimulated fatty acid synthesis and lipogenic gene expression in hepatocytes (6). This suggests that FGF19 signaling interferes with insulin signaling. Whether such interference is mutual, and whether it is maintained in the insulin-resistant state is unknown. Adding complexity to a possible cross-talk between FGF19 and insulin signaling pathways, recent studies in mice indicate that FGF19 signals -at least in part- through the insulin-activated PI3K pathway (29).
In conclusion, a reduced response of the liver to FGF19 in NAFLD patients with insulin resistance may result in further derangement of hepatic lipid homeostasis. Further studies will be required to address the mechanisms by which insulin-resistance affects the hepatic response to FGF19 and if this impaired response contributes to the pathology in NAFLD.

Acknowledgements

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References


31. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH and Dobbins RL. Magnetic resonance spectroscopy to measure hepatic

Figure 1. Plasma levels of triglycerides, bile salts and FGF19 increase after an oral fat challenge. Following overnight fasting, control subjects (open symbols) and NAFLD patients (closed symbols) received a standardized oral fat challenge. Serial blood samples were drawn at the indicated time points, and plasma was assayed for triglycerides (A), total bile salts (B) and FGF19 (C). Data points are depicted as mean±SD. Asterisks denote significant changes from baseline values (T=0) in the respective groups.

Figure 2. Postprandial bile salt and FGF19 excursions in NAFLD patients are not affected by insulin-resistance. The postprandial responses in NAFLD patients (Figure 1) were analyzed separately in each HOMA-based subgroup. Postprandial excursions of both bile salts and FGF19 are similar in NAFLD patients with (HOMA ≥2.5, black symbols) and without (HOMA <2.5, grey symbols) insulin-resistance. Data points are depicted as mean±SD. Asterisks and number signs denote significant changes from baseline values (T=0) in the HOMA<2.5 and HOMA≥2.5 groups, respectively.

Figure 3. Postprandial elevation of FGF19 in insulin-resistant NAFLD subjects is not accompanied by reduction of C4 levels. Plasma FGF19 (upper panel) and C4 (bottom panel) levels were determined at baseline (white bars) and at 4 hrs (grey bars) and 5 hrs (black bars) after an oral fat challenge. In NAFLD patients with normal HOMA score (HOMA<2.5), the postprandial increase in FGF19 levels was accompanied by an expected decline in C4 levels, a marker for bile salt synthesis. In contrast, C4 levels were unaffected in insulin-resistant NAFLD patients (HOMA≥2.5) despite a similar postprandial elevation of FGF19 level. Data points are depicted as mean±SD.
A

Triglycerides (mmol/L)

- controls
- NAFLD

B

Bile Salts (μmol/L)

C

FPGR1 (ng/mL)

hours after oral fat challenge

* indicates statistical significance.
Table 1. Characteristics and baseline values of controls, NAFLD patients and the HOMA score-based subgroups.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>NAFLDALL</th>
<th>NAFLD HOMA&lt;2.5</th>
<th>NAFLD HOMA ≥2.5</th>
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<td>Male/Female</td>
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<td>6/2</td>
<td>8/4</td>
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<td>Age (yrs)</td>
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<td>49 ± 11</td>
<td>47 ± 9</td>
<td>51 ± 12</td>
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<td>BMI (kg/m²)</td>
<td>26.4 ± 3.5</td>
<td>30.6 ± 3.7</td>
<td>29.8 ± 4.5</td>
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<td>% hepatic fat</td>
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<td>13.4 ± 11.8</td>
<td>17.9 ± 11.2</td>
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<td>Glucose (mmol/L)</td>
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<td>6.3 ± 2.3</td>
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<td>6.8 ± 2.9</td>
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<td>Insulin (μmol/L)</td>
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<td>&lt;0.001</td>
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<td>HOMA-IR</td>
<td>n.d.</td>
<td>6.0 ± 10.9</td>
<td>1.2±0.5</td>
<td>9.2±13.3</td>
<td>&lt;0.001</td>
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<tr>
<td>ALT (IU/L)</td>
<td>n.d.</td>
<td>74 ± 37</td>
<td>69 ± 22</td>
<td>78 ± 44</td>
<td>0.62</td>
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<td>γGT (IU/L)</td>
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<td>102 ± 87</td>
<td>83 ± 44</td>
<td>114 ± 107</td>
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<td>CRP</td>
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<td>TC (mmol/L)</td>
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<td>TG (mmol/L)</td>
<td>1.13 ± 0.35</td>
<td>1.88 ± 1.13</td>
<td>1.63 ± 1.01</td>
<td>2.05 ± 1.21</td>
<td>0.42</td>
</tr>
<tr>
<td>bile salts (μmol/L)</td>
<td>2.2 ± 1.4</td>
<td>4.2 ± 2.4</td>
<td>2.6 ± 0.3</td>
<td>5.3 ± 0.7</td>
<td>0.009</td>
</tr>
<tr>
<td>C4 (ng/mg cholesterol)</td>
<td>n.d.</td>
<td>12.2 ± 9.7</td>
<td>16.1 ± 3.6</td>
<td>9.7 ± 5.2</td>
<td>0.24</td>
</tr>
<tr>
<td>FGF19 (ng/mL)</td>
<td>0.26 ± 0.28</td>
<td>0.18 ± 0.09</td>
<td>0.18 ± 0.09</td>
<td>0.18 ± 0.09</td>
<td>1.00</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.9 ± 0.6</td>
<td>1.8 ± 1.5</td>
<td>1.5 ± 1.4</td>
<td>2.1 ± 1.6</td>
<td>0.40</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SD. *P-values are for comparison between the HOMA <2.5 and HOMA ≥2.5 subgroups. Part of the data in the control group has been published previously (26). Abbreviations: n.d. = not determined, ALT = alanine aminotransferase, γGT = γ-glutamyltransferase, TG = triglycerides, TC = total cholesterol, CRP = C-reactive protein, C4 = 7-hydroxy-4-cholesten-3-one.