The hepatic response to FGF19 is impaired in patients with nonalcoholic fatty liver disease and insulin resistance

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Academic Medical Center, Departments of †Gastroenterology and Hepatology, §Surgery, and ¶Radiology, and ∥AMC Liver Center, Amsterdam; ‡Department of Gastroenterology and Hepatology, Liver Unit, Vrije Universiteit Medical Center, Amsterdam, The Netherlands; and ¶Institute of Clinical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles University in Prague, Czech Republic

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Schreuder TC, Marsman HA, Lenicek M, van Werven JR, Nederveen AJ, Jansen PL, Schaap FG. The hepatic response to FGF19 is impaired in patients with nonalcoholic fatty liver disease and insulin resistance. Am J Physiol Gastrointest Liver Physiol 298: G440–G445, 2010. First published January 21, 2010; doi:10.1152/ajpgi.00322.2009.—Intestinal FGF19 has emerged as a novel endocrine regulator of hepatic bile salt and lipid metabolism. In patients with nonalcoholic 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 [homeostasis model of assessment (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 vs. 0.18 ± 0.09 ng/ml, respectively, P = 0.94). Postprandial FGF19 levels in both controls and NAFLD patients peaked between 3–4 h 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 impaired 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.

enterohepatic signaling; postprandial response; 7α-hydroxy-4-cholesten-3-one; CYP7A1; bile salt uptake

INCREASED HEPATIC FAT ACCUMULATION underlies the development of nonalcoholic fatty liver disease (NAFLD). The spectrum of liver abnormalities in NAFLD ranges from simple steatosis to nonalcoholic 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 toward 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 whether 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 feedforward 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, 20 patients between the ages of 18 and 75 yr were recruited for participation in this study. Initial diagnosis
of NAFLD was based on elevated alanine aminotransferase levels (≥ 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 men and more than 1 unit/day in women, were excluded. Following study inclusion, all NAFLD patients underwent assessment of hepatic fat content by magnetic resonance spectroscopy (1H-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 g cream (35 g 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 h 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 h.

**Determination of hepatic fat content.** The fat content in the liver of NAFLD patients was determined by 1H-MRS. All measurements were performed on a 3.0-T Philips Intera scanner (Philips Healthcare, Best, The Netherlands). A voxel of 20 × 20 × 20 mm was positioned in the right hepatic lobe, avoiding vascular, biliary, and extrahepatic structures. Spectra were acquired by use of first order iterative shimming, a PRESS sequence with echo time/repetition time = 35/2,000 ms, and 64 signal acquisitions. The water (4.7 ppm) and fat (1.3 ppm) resonance peaks were integrated by use of 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 (T2water = 34 ms, T2fat = 68 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 h). 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 (Sanguin, 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 h after oral fat intake as previously described (19). C4 levels were expressed relative to total cholesterol, because their ratio was shown to be a more accurate marker of bile salt synthesis (15).

**Determination of plasma FGF19.** Plasma FGF19 levels were determined by using an in-house developed sandwich ELISA described in detail elsewhere (F. G. Schaap, unpublished observations). 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% Tween-20. Captured antigen was detected with biotinylated goat anti-human FGF19 antibody (BAF969, R&D Systems) and streptavidin-horseradish peroxidase with tetramethylbenzidine as chromogenic substrate.

**Data and statistical analysis.** Area under the postprandial curve (AUC) was calculated by use of baseline-subtracted values with GraphPad Prism (GraphPad Software, 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 h) in the sampling scheme of the controls were omitted for AUC calculations (denoted as AUC0–6 h). Descriptive statistics are expressed as means ± 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, 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 (body mass index >30). Hyperglycemia and hyperinsulinemia in the NAFLD population were 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 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 HOMAbased 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 compared 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 farnesoid X 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 Fig. 1.

Packaging of digested dietary lipids into chylomicrons and their release into lymph causes an increase in plasma TG levels (Fig. 1A).
Table 1. Characteristics and baseline values of controls, NAFLD patients, and the HOMA score-based subgroups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls</th>
<th>All</th>
<th>HOMA &lt;2.5</th>
<th>HOMA ≥2.5</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>15/0</td>
<td>14/6</td>
<td>6/2</td>
<td>8/4</td>
<td>1.00</td>
</tr>
<tr>
<td>Age, yr</td>
<td>50 ± 8</td>
<td>49 ± 11</td>
<td>47 ± 9</td>
<td>51 ± 12</td>
<td>0.51</td>
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<tr>
<td>BMI, kg/m²</td>
<td>26.4 ± 3.5</td>
<td>30.6 ± 3.7</td>
<td>29.8 ± 4.5</td>
<td>31.3 ± 3.2</td>
<td>0.39</td>
</tr>
<tr>
<td>% hepatic fat</td>
<td>n.d.</td>
<td>16.0 ± 11.4</td>
<td>13.4 ± 11.8</td>
<td>17.9 ± 11.2</td>
<td>0.42</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>n.d.</td>
<td>6.3 ± 2.3</td>
<td>5.5 ± 0.8</td>
<td>6.8 ± 2.9</td>
<td>0.18</td>
</tr>
<tr>
<td>Insulin, µmol/l</td>
<td>n.d.</td>
<td>118.6 ± 127.2</td>
<td>35.6 ± 13.3</td>
<td>173.8 ± 139.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<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>
</tr>
<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>
</tr>
<tr>
<td>γGT, IU/l</td>
<td>n.d.</td>
<td>102 ± 87</td>
<td>83 ± 44</td>
<td>114 ± 107</td>
<td>0.65</td>
</tr>
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<td>CRP</td>
<td>n.d.</td>
<td>3.6 ± 3.1</td>
<td>3.3 ± 3.1</td>
<td>3.9 ± 3.2</td>
<td>0.52</td>
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<tr>
<td>TC, mmol/l</td>
<td>n.d.</td>
<td>5.10 ± 0.69</td>
<td>4.86 ± 1.01</td>
<td>4.78 ± 0.47</td>
<td>0.77</td>
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<tr>
<td>TG, mmol/l</td>
<td>n.d.</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>
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<tr>
<td>Bile salts, µmol/l</td>
<td>n.d.</td>
<td>2.2 ± 1.4</td>
<td>4.2 ± 2.2</td>
<td>2.6 ± 0.3</td>
<td>5.3 ± 0.7</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>
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<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>
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</tbody>
</table>

All values are expressed as means ± 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). NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; n.d., not determined; HOMA-IR, homeostasis model of assessment for insulin resistance; ALT, alanine aminotransferase; γGT, γ-glutamyltransferase; CRP, C-reactive protein; TC, total cholesterol; TG, triglycerides; C4, 7α-hydroxy-4-cholesten-3-one.

As reported earlier for the volunteer group (26), a postprandial increase in TG was first noted after 2 h. TG levels peaked after 3 h in controls and between 3 and 4 h in NAFLD patients. The total area under the postprandial TG curve (AUC_{TG 0–6h}) was elevated in NAFLD patients (4.1 ± 3.2 vs. 2.1 ± 1.4 mmol·l⁻¹·h⁻¹ in controls, P = 0.013). During the ascending phase of the postprandial TG excursion, TG production (i.e., chylomicron synthesis) predominates. This notion is supported when ascending, descending and total AUCTG values for the control and the latter appears in line with impaired TG clearance in insulin-resistant subjects (23). This notion is supported when ascending, progressing, and descending AUCTG values for the control and the HOMA-based NAFLD subgroups are compared. This analysis revealed that total and postprandial AUCTG values are significantly elevated in the NAFLD HOMA ≥2.5 subgroup only (data not shown). Thus the observed difference in AUCTG 0–6h 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 biliary salt levels is apparent after 1 h in NAFLD patients, with levels reaching a maximum after 3–4 h (Fig. 1B). In control subjects, which were first sampled after 2 h, biliary salt levels peak after 2 h and return to baseline levels after 6 h. In contrast, bile salts remain elevated at this and at the final time point in NAFLD patients (Figs. 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 spillover and is depending on the Na⁺/taurocholate cotransporting protein (NTCP) (13). As can be appreciated from Fig. 1B, the postprandial biliary salt excursion is different in controls and NAFLD patients. Bile salt levels were significantly higher in NAFLD subjects at time points T = 0 (P = 0.004), T = 4 (P = 0.015), and T = 6 h (P = 0.001), and this was entirely attributable to NAFLD patients with HOMA score ≥2.5 (data not shown). The area under the postprandial biliary salt curve (AUC_{BHS 0–6h}) tended to be elevated in NAFLD patients (67.3 ± 47.7 vs. 47.1 ± 29.4 µmol·l⁻¹·h⁻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·h⁻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 compared with controls (2.1 ± 1.6 vs. 0.9 ± 0.6 µg/ml, respectively, P = 0.005, Table 1).

Following fat ingestion, a slight drop in plasma FGF19 level occurs after 1 h in NAFLD patients. Plasma FGF19 was significantly elevated after 2 h in control subjects and after 3 h in NAFLD patients (Fig. 1C). Postprandial FGF19 levels peaked between 3–4 h 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 compared 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 postprandial FGF19 AUC (AUC_{FGF19 0–6h}) was, however, significantly lower in NAFLD patients (1.4 ± 1.3 vs. 1.9 ± 1.4 ng·ml⁻¹·h⁻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 (Fig. 2A) and had indistinguishable AUC_{BS 0–7h} values (61.2 ± 17.8 vs. 85.1 ± 69.6 µmol·l⁻¹·h⁻¹ in the HOMA <2.5 and HOMA ≥2.5 subgroups, respectively, P = 0.47). The postprandial FGF19
response in HOMA-based NAFLD subgroups is shown in Fig. 2B. Plasma FGF19 is significantly elevated above baseline levels after 3 h in the HOMA <2.5 subgroup (P = 0.018) and, albeit with borderline significance, in the HOMA ≥2.5 subgroup (P = 0.051). Postprandial FGF19 levels peaked at 3 and 4 h in the HOMA <2.5 and HOMA ≥2.5 subgroups, respectively. Mean FGF19 levels in the NAFLD subgroups were not different at any of the individual time points. The AUC_{FGF19 0–7h} was similar in both NAFLD subgroups (1.6 ± 1.3 vs. 1.4 ± 1.5 ng·ml⁻¹·h⁻¹ in the HOMA <2.5 and HOMA ≥2.5 groups, respectively, P = 0.49). In addition, analysis of variance indicated that AUC_{FGF19 0–6h} values were not significantly different in controls and the two NAFLD subgroups (ANOVA P = 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 h following an oral fat challenge. Despite similar postprandial elevation of FGF19 in both NAFLD subgroups (Fig. 3A), C4 levels declined after 5 h only in the HOMA <2.5 group (∼30%, P = 0.015) while remaining unchanged in the HOMA ≥2.5 group (+10%, P = 0.22) (Fig. 3B).

DISCUSSION

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 transscriptional 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 h, followed by an increase in plasma FGF19 level after 2–3 h (Fig. 1). In line with an earlier study in volunteers receiving regular meals (21), FGF19 levels show a postprandial peak after 3–4 h. Healthy volunteers and NAFLD patients had comparable fasted FGF19 levels (0.26 ± 0.28 vs. 0.18 ± 0.09 ng/ml, respectively, \( P = 0.94 \)) and displayed a similar postprandial FGF19 response. Peak postprandial FGF19 levels were 3.0-fold higher than baseline values in both groups. The AUC\(_{FGF19 \ 0–6h} \) appeared somewhat lower in NAFLD patients compared with the volunteers when considering the entire NAFLD group (1.4 ± 1.3 vs. 1.9 ± 1.4 ng·ml\(^{-1} \)·h\(^{-1} \)) 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 (Fig. 3B). Interestingly, such decline in C4 levels was not observed in insulin-resistant (i.e., HOMA score \( \geq 2.5 \)) NAFLD patients. Because 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 spillover of bile salts and consequently prolonged circulation times. Proinflammatory cytokines released from inflamed adipose tissue have been implicated in the development of hepatic insulin resistance (22), and among these IL-1β 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/short heterodimer partner (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, bypassing 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). Taking 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 \( \geq 2.5 \).

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 an 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, whereas 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 Fgf4 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 whether this impaired response contributes to the pathology in NAFLD.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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