Decrease in circulating fibroblast growth factor 21 after an oral fat load is related to postprandial triglyceride-rich lipoproteins and liver fat

Niina Matikainen1,2, Marja-Riitta Taskinen1, Sanna Stennabb1, Nina Lundbom3, Antti Hakkarainen3, Kirsi Vaaralahti4 and Taneli Raivio4,5
1Division of Cardiology, 2Division of Endocrinology, Department of Medicine, Helsinki University Central Hospital, University of Helsinki, PO Box 700, FIN-00029 Helsinki, Finland, 3Department of Radiology, 4Children’s Hospital, Helsinki University Central Hospital and 5Institute of Biomedicine/Physiology, Biomedicum Helsinki, University of Helsinki, FIN-00029 Helsinki, Finland

Abstract

Background: Elevated levels of circulating fibroblast growth factor 21 (FGF21) are commonly encountered in type 2 diabetes, dyslipidemia, and non-alcoholic fatty liver disease, all of which share exaggerated postprandial lipemia as a common proatherogenic feature. How FGF21 responds to an oral fat load in man is unknown.

Methods: We measured liver fat contents and subcutaneous and visceral fat volumes in 47 healthy subjects, who also underwent an oral fat load with measurements of plasma FGF21 and free fatty acid (FFA). Triglyceride (TG), apolipoprotein B-48 (apoB-48), and apoB-100 concentrations were measured in triglyceride-rich lipoprotein (TRL) fractions.

Results: When compared with fasting levels, the concentration of FGF21 decreased significantly at 4 h (P<0.05) and tended to return to fasting levels at 8 h after an oral fat load. Fasting and postprandial FGF21 correlated significantly with liver fat as well as with TRLs in the chylomicron and especially in very low-density lipoprotein 1 (VLDL1) and VLDL2 fractions representing remnant particles, but not with FFA. Subjects with increased liver fat (>5%, n=12) showed impaired suppression of FGF21 at 4 h (P<0.05) and at 8 h (P=0.01) and demonstrated higher postprandial TG area under the curve in plasma and TRL fractions (P<0.032) compared with those with normal liver fat (%<5%, n=35).

Conclusions: We observed a significant decrease of FGF21 concentration after an oral fat load. Fasting and postprandial FGF21 levels were closely related to large VLDL and remnants, but not to plasma FFA. Our pilot findings suggest that the postprandial accumulation of TRL remnants and liver fat may modulate postprandial FGF21 levels.
liver disease (NAFLD) (8, 10). Likewise, in subjects with
dyslipidemia and coronary heart disease, elevated
centersations of triglycerides (TGs) and apolipoprotein A1 (apoA1) independently predicted elevated FGF21.
A common feature for these conditions is prolonged and exaggerated postprandial lipemia (11). So far, the
relationship between TG metabolism and FGF21 has been poorly characterized.

Increases in adipose tissue lipolysis and, consequently, in FFA concentration during fasting, induce
FGF21 expression (4). During alimentary lipemia, fatty acids from the meal are carried as TGs within
triglyceride-rich lipoproteins (TRLs) together with hepatic-derived particles, both contributing to the rise
in chylomicron, very low-density lipoprotein 1 (VLDL1), and VLDL2 particles (i.e. TRLs) for hours. We
hypothesized that ingestion of fat in a form of mixed
meal could be reflected to postprandial FGF21 levels
expressed as total abdominal fat.

Subjects and methods

We recruited 47 previously healthy family members of
our dyslipidemic cohort taking part in our previous studies. Of the 54 volunteers screened for the study,
we excluded one because of diabetes and six subjects
declined to participate in the study because of personal reasons. The subject characteristics are shown in
Table 1. To detect possible changes in postprandial FGF21, we included both normolipidemic and mildly
dyslipidemic subjects who were otherwise healthy.

Further inclusion criteria included patients aged
18–65 years, non-smokers, and those with no history
of cardiovascular or other disease. The subjects were
screened to confirm they had no secondary conditions affecting the lipid levels or abnormalities in thyroid and kidney function or in basic blood count. Severe liver
diseases including non-alcoholic steatohepatitis were excluded by clinical examination and measurement of liver enzymes. Subjects were asked to discontinue possible lipid-lowering therapy 1 month before the
study. We performed an oral fat load and determined the liver fat content and the amount of subcutaneous and visceral fat for each study subject. The study was
approved by the Ethical Committee of Helsinki University Central Hospital and the study subjects
gave their informed consent.

As the liver fat content is a predictor of both hepatic
VLDL overproduction (12, 13) and postprandial lipemia
(11), we compared postprandial FGF21 responses in
two subgroups with normal (≤5%) and high (>5%) liver fat determined by magnetic resonance (MR)
spectroscopy (Table 2). This cut point is based on a population study (14).

**Determination of liver fat content and abdominal fat volumes**

All subjects underwent an oral fat load test and measurements of subcutaneous and visceral fat depots were measured by MR imaging and liver fat content was
measured by MR spectroscopy as described (13). The
calculated sum of visceral and subcutaneous fat was expressed as total abdominal fat.

**Oral fat load**

After a 12-h fast, the subjects received a mixed meal consisting of bread, butter, cheese, skinned milk, and tea
or coffee (50 g carbohydrates, 72 g fat (P:S ratio 0.08), and
35 g protein) in the morning. Blood samples were drawn
before and 3, 4, 6, 8, and 10 h after the meal. During this
time, only water was served _ad libitum_. The subjects remained physically inactive during the whole study.

**Separation of lipoproteins and biochemical methods**

Blood samples obtained during the oral fat load study were separated into lipoprotein fractions with gradient

**Table 2** Metabolic characteristics and body composition of the study subjects according to liver fat ≤5% or >5%. Data are
mean ± s.d. or interquartile range.

<table>
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<tr>
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<th>LF ≤5%</th>
<th>LF &gt;5%</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>43.3 ± 12.8</td>
<td>41.0 ± 8.3</td>
</tr>
<tr>
<td>Gender (males/females)</td>
<td>15/20</td>
<td>7/5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.0 ± 3.9</td>
<td>28.6 ± 3.6†</td>
</tr>
<tr>
<td>Liver fat (%)</td>
<td>1.5 (1.98)</td>
<td>12.0 (8.9)†</td>
</tr>
<tr>
<td>Subcutaneous fat (cm²)</td>
<td>3429 (1893)</td>
<td>5425 (3342)†</td>
</tr>
<tr>
<td>Visceral fat (cm²)</td>
<td>1215 (1153)</td>
<td>2310 (658)†</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.1 ± 0.6</td>
<td>5.6 ± 0.5*</td>
</tr>
<tr>
<td>Glycohemoglobin A1C (%)</td>
<td>5.5 ± 0.36</td>
<td>5.6 ± 0.35</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.45 (0.61)</td>
<td>3.28 (1.57)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.48 ± 0.35</td>
<td>1.23 ± 0.44</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.17 (0.83)</td>
<td>2.42 (2.39)†</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>28 (16)</td>
<td>98 (35)†</td>
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*P<0.05; †P<0.01.
ultracentrifugation. Concentrations of TG, apoB-48 and apoB-100, and cholesterol were measured from whole plasma and/or chylomicron (Sf > 400), VLDL1 (Sf 60–400), VLDL2 (Sf 20–60), and intermediate density lipoprotein (IDL) (Sf 12–20) fractions at each time point as described (13). FGF21 concentrations were analyzed from serum samples at 0, 4, and 8 h. The serum samples for FGF21 analysis were prepared by centrifugation after blood collection and stored at −80 °C until analyzed. FGF21 concentrations were measured by ELISA kit (BioVendor GmbH, Heidelberg, Germany) according to the manufacturer’s instructions. In statistical analyses, the values below the sensitivity of the assay were assigned a value 0.1 pg/ml. Intra-assay coefficient of variation (CV) was 3% and inter-assay CV was 12.6%. Plasma FFA and apoA1 concentrations were measured as described previously (13) and β-hydroxybutyrate was measured by an enzymatic method (DiaSys Diagnostic Systems GmbH, Holzheim, Germany).

**Statistical analysis**

Statistical comparisons of data were performed by SPSS Statistical Package (version 19.0, SPSS, Inc., Armonk, NY, USA). Data are presented as mean ± S.D. of S.E.M. and as medians and ranges, when appropriate. The area under curve (AUC) for postprandial variables was calculated according to the trapezoid rule. The withinsubject change from baseline during postprandial period was analyzed with Greenhouse–Geisser corrected repeated measures ANOVA. We used Shapiro–Wilk’s test to explore the normality of variable distribution. Variables were compared between subjects with low liver fat (≤ 5%) content and subjects with high liver fat (> 5%) content using Student’s t-test or Mann–Whitney U test when assumptions of normal distribution were not met. Correlations were calculated with Spearman’s rank correlations method. If significant change was detected, Bonferroni correction was applied in the analysis. A multivariable regression analysis was performed by a stepwise method. We considered a two-tailed P value < 0.05 as statistically significant.

**Results**

**Fasting correlations between FGF21 and metabolic parameters**

Fasting FGF21 concentrations demonstrated a large range from undetectable concentrations to 832.7 pg/ml. Fasting FGF21 displayed only a borderline correlation with fasting plasma TG (r = 0.308, P = 0.06), but a significant correlation existed with TG in chylomicron and VLDL1 fractions (r = 0.424, P = 0.008 and r = 0.382, P = 0.018 respectively). No significant relationship existed between fasting FGF21 and baseline FFA, β-hydroxybutyrate, apoA1, or apoB concentrations. Fasting FGF21 concentration was strongly related to liver (r = 0.395, P < 0.01) and visceral (r = 0.342, P < 0.05) fat, but not to subcutaneous fat.

**Postprandial FGF21 and TRL responses**

The FGF21 response to the oral fat load is shown in Fig. 1. Postprandial FGF21 concentration decreased in 46 of the 47 subjects at 4 h (P = 0.045). The concentration returned toward the fasting levels by the end of the study at 8 h. As shown in Fig. 1, FGF21 and TRLs in VLDL1 demonstrated reciprocal patterns after the oral fat load. Besides elevation of TG in plasma and lipoprotein fractions (chylomicron, VLDL1, and VLDL2), significant increases occurred in concentrations of apoB-48 and apoB-100 in each of these TRL fractions (P<0.01 by repeated measures ANOVA), indicating that both intestinal and hepatic TRL particles contributed to postprandial lipemia (Fig. 1).

Postprandial TG, apoB-48, and apoB-100 measurements at 3, 4, 6, and 8 h correlated with FGF21 concentrations (Table 3) during the postprandial period (data not shown). To avoid multiple testing, we calculated the correlations between postprandial apoB-48 and apoB-100 AUC values in VLDL1 and VLDL2 fractions and fasting FGF21 and the change of FGF21 from 0 to 4 h (ΔFGF21, 0–4 h). Although FGF21 levels decreased and TRL levels increased after the oral fat load, the FGF21 was used and therefore the correlations presented in Table 3 are positive. Table 4 reports the multivariable analysis.
Table 3 Spearman’s correlation coefficients for postprandial apolipoprotein B-48 (apoB-48) and apoB-100 responses (area under curve (AUC)) in very low-density lipoprotein 1 (VLDL1) and VLDL2 fractions and for fasting fibroblast growth factor 21 (FGF21; 0 h) and for the change of FGF21 from 0 to 4 h (Δ 0–4 h).

<table>
<thead>
<tr>
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<th>Spearman’s ρ for AUC</th>
<th>DeltaDelta FGFG21 G21 (0–4 h)</th>
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<tbody>
<tr>
<td>VLDL1</td>
<td>0.374a</td>
<td>0.398b</td>
</tr>
<tr>
<td>VLDL2</td>
<td>0.497b</td>
<td>0.554b</td>
</tr>
<tr>
<td>B-100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL1</td>
<td>0.389b</td>
<td>0.407b</td>
</tr>
<tr>
<td>VLDL2</td>
<td>0.431b</td>
<td>0.457b</td>
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*aCorrelation is significant at the 0.05 level (two-tailed).

**Postprandial FGF21 and TRL responses between subjects with high vs low liver fat**

We observed increased FGF21 levels in subjects with high liver fat compared with those with low liver fat (Table 2 and Fig. 2). These subjects with high liver fat also exhibited significantly higher postprandial responses of apoB-48 and apoB-100 in VLDL1 fraction. Besides, the AUC values for plasma TG (P=0.008), chylomicron TG (P=0.032), VLDL2 TG (P=0.016), and chylomicron apoB-100 (P=0.007) were increased in high liver fat subjects.

**Postprandial FGF21 responses and measures of liver, visceral, and subcutaneous fat**

Postprandial FGF21 levels and Δ-change of FGF from 0 to 4 h correlated positively with liver and visceral fat, but not with subcutaneous fat in the whole study population (data not shown).

**Postprandial FGF21 and other metabolic parameters**

Postprandial plasma FFA concentrations demonstrated only insignificant changes, which were opposite to those of TG with slight decrease at 4 h and increase at 8 h. FGF21 concentrations followed the pattern of FFA concentrations but were not related to FFA at 4 h or to the Δ-changes of FFA from 0 to 4 h. The 8-h FFA concentration correlated with the FGF21 nadir at 4 h (r=−0.346, P=0.019). We observed no correlations between FGF21 and β-hydroxybutyrate levels at 4 or 8 h (data not shown).

**Discussion**

Our study is the first to report a decrease in postprandial FGF21 concentration in response to an oral fat load in humans. Although FFAs are known regulators of fasting FGF21 expression (4), we did not detect this association during the postprandial period. Our findings indicate that both fasting and postprandial chylomicron and VLDL particles are better predictors of FGF21 concentrations than fasting or postprandial plasma FFAs.

Specifically, the concentrations of FGF21 both in fasting and after oral fat load were positively correlated with postprandial TRL areas in chylomicron, VLDL1, and VLDL2 fractions, i.e. with TRLs and their remnants. The association applied to both apoB-48 and apoB-100 AUC values, which implies that both intestinal and hepatic-derived particles may reflect FGF21 secretion. In multivariate analysis, TG and apoB-48 AUC in VLDL2 fraction, i.e. small TRL remnants, were the best predictors of fasting FGF21 levels and postprandial FGF21 change from fasting to 4 h. It is possible that the suppression of FGF21 regulates postprandial lipid metabolism and permits a better clearance of TRLs. This obviously remains speculative due to our observational study design and due to antecedent lack of data as to whether FGF21 regulates lipoprotein lipase activity, remnant removal through hepatic receptors, or fatty acid uptake by hepatocytes in humans. Irrespective of the specific mechanism, the decrease in FGF21 following oral fat load was closely related to an increase in postprandial intestinal and hepatic TRLs and their remnants, but to find whether a feedback regulation exists between TRLs and postprandial FGF21, further research is warranted.

The postprandial decrease in FGF21 was a nearly uniform phenomenon among the 47 study participants. Previously, lipid–heparin infusion to healthy men induced a threefold rise in FFA and eightfold rise in TG concentrations, but the FGF21 levels remained unchanged (15). The differences between these findings and ours include the route of fat administration (i.v. vs oral) and the use of heparin, a potent inductor of lipolysis, resulting in considerable rise in plasma FFAs. Contrary to the findings of Mai et al. (15), we observed a significant physiological rise in TRLs but only a minor

<table>
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<th>FGF21 (0 h)</th>
<th>ΔFGF21 (0–4 h)</th>
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<tr>
<td>Best predictors</td>
<td>VLDL2 apoB-48 AUC</td>
<td>VLDL2 apoB-48 AUC</td>
</tr>
<tr>
<td>VLDL2 TG AUC</td>
<td>VLDL2 TG AUC</td>
<td></td>
</tr>
<tr>
<td>liver fat</td>
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Adj. ρ²: 0.394; P<0.001

0.338; P<0.001
decrease in plasma FFA following an oral fat load, which may explain differences in outcomes. Animal data on metabolic regulation of FGF21 are conflicting and not directly applicable to humans. In rats, however, hepatic Ppara and Fgf21 expression and also FGF21 serum levels increased significantly in response to carbohydrate and fat feeding (16) and Fgf21 gene expression was inversely related to hepatic TG concentration (17). Our results may thus imply that FGF21 levels may not follow FFA changes, but reflect the rise in TRL concentrations in the postprandial period and liver fat content, since these two are closely related (13).

Subjects with low levels of postprandial TRL remnants and low liver fat demonstrated low FGF21 levels 4 and 8 h after an oral fat load. Instead, in apparently healthy subjects with slightly increased liver fat (> 5%) and more enhanced postprandial lipemia, FGF21 also decreased, but remained significantly higher at postprandial time-points suggesting impaired suppression of postprandial FGF21. Furthermore, multivariate analysis of the whole study population indicated that liver fat content predicted fasting FGF21 levels and postprandial ΔFGF21 change from fasting to 4 h. This finding is in line with a recent finding that fasting FGF21 rises progressively with increasing liver fat content (18). Previous studies demonstrated elevated fasting FGF21 levels in metabolic conditions such as obesity, dyslipidemia, and type 2 diabetes mellitus, which are all associated with increased liver fat and NAFLD (6, 10, 19). Importantly, these conditions also share excessive postprandial lipemia as a common feature and a risk for cardiovascular disease. Especially, high levels and prolonged residence time of small TRL remnants provide a link to atherosclerosis, allowing vascular endothelium to

load with excess cholesterol and promoting low-grade inflammation and thrombosis (20). To determine whether impaired postprandial suppression of FGF21 clusters occurs with other markers of atherosclerosis warrants further studies.

The relevance of FGF21 in human metabolism is currently under extensive research activity, and lately FGF21 has been implicated in various clinical conditions including insulin resistance (8, 9), NAFLD (8, 9, 21), mitochondrial diseases (22), anorexia nervosa (23), and as a biomarker predicting type 2 diabetes (24). These apparently heterogenic clinical conditions reflect the fact that the role of FGF21 is poorly characterized in human physiology. Therefore, our results are not readily applicable to the clinic, but expand the significance of FGF21 to postprandial lipid metabolism possibly modified by liver fat content.

Our study population was rather small and it did not receive a weight-based amount of oral fat. Furthermore, correlation analyses do not reflect any cause-and-effect relationship. As we did not measure insulin sensitivity in our subjects, we are unable to estimate whether variation in postprandial FGF21 is accounted for by different degrees of insulin sensitivity, which is the case in the fasting state (9). There is a lack of a fasting control group, but insignificant daytime variations of FGF21 levels have been demonstrated previously (5).

Taken together, the results from this pilot study imply that postprandial TRLs and their remnants are related to FGF21 levels after a fat load. Subjects with increased liver fat exhibit diminished suppression of postprandial FGF21. Our findings suggest that the postprandial accumulation of TRL remnants and liver fat modulate postprandial FGF21 levels.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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