Postprandial Plasma Concentrations of Individual Bile Acids and FGF-19 in Patients With Type 2 Diabetes

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Context: Bile acids regulate lipid and carbohydrate metabolism by interaction with membrane or intracellular proteins including the nuclear farnesoid X receptor (FXR). Postprandial activation of ileal FXR leads to secretion of fibroblast growth factor 19 (FGF-19), a gut hormone that may be implicated in postprandial glucose metabolism.

Objective: To describe postprandial plasma concentrations of 12 individual bile acids and FGF-19 in patients with type 2 diabetes (T2D) and healthy controls.

Design and Setting: Descriptive study, performed at the Center for Diabetes Research, Gentofte Hospital, Hellerup, Denmark.

Participants: Fifteen patients with T2D and 15 healthy matched controls with normal glucose tolerance.

Interventions: A 75-g oral glucose tolerance test and three isocaloric and isovolemic liquid meals with low, medium, and high fat content, respectively.

Main Outcome Measures: Bile acid and FGF-19 concentrations.

Results: Postprandial total bile acid concentrations increased with increasing meal fat content ($P < .05$), peaked after 1–2 hours, and were higher in T2D patients vs controls (oral glucose tolerance test, low and medium fat meals, $P < .05$; high fat meal, $P = .30$). Differences reflected mainly unconjugated and glycine-conjugated forms of deoxycholic acid (DCA) and to a lesser extent cholic acid (CA) and ursodeoxycholic acid (UDCA), whereas chenodeoxycholic acid (CDCA) concentrations were comparable in the two groups. FGF-19 concentrations tended to be lower in T2D patients vs controls, but differences were not statistically significant due to considerable variation.

Conclusion: Postprandial plasma patterns of bile acids with FXR agonistic properties (CDCA, DCA, and CA) and FXR antagonistic properties (UDCA) in T2D patients support the notion of a "T2D-bile acid-FGF-19" phenotype with possible pathophysiological implications. (***J Clin Endocrinol Metab* 101: 3002–3009, 2016**)

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Abbreviations: AUC, area under the curve; BMI, body mass index; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; EGP, endogenous glucose production; FGF-19, fibroblast growth factor 19; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1c; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; TBA, total bile acid; T2D, type 2 diabetes; TGR5, Takeda G-coupled protein receptor 5; UDCA, ursodeoxycholic acid.

Since the discovery that bile acids are ligands for the nuclear farnesoid X receptor (FXR) and the Takeda G-coupled protein receptor 5 (TGR5), studies in both animals and humans have established that bile acids are metabolic integrators involved in glucose, lipid, and energy metabolism, particularly in the postprandial state (1). Several recent reviews have addressed conditions in which TGR5 and FXR have emerged as new targets for pharmacological agents (2, 3). TGR5 activation has been linked to increased energy expenditure in muscle and brown adipose tissue, immunosuppressive effects in immune cells, and—perhaps most importantly in the present context—secretion of intestinal L-cell satiety products such as peptide YY and the incretin hormone glucagon-like peptide-1 (GLP-1) (2, 4). FXR activation by bile acids affects the expression of multiple key regulatory genes encoding components of the bile acid synthesis cascade, as well as genes acting in numerous metabolic pathways (5). Interestingly, a recent study demonstrated that FXR activation in L cells decreases GLP-1 secretion in response to glucose (6). Most of the effects of FXR and TGR5 activation have been corroborated in animal models and to a lesser degree in human studies. Alteration of the bile acid pool has been shown to induce changes of parameters that are part of the metabolic syndrome (ie, insulin resistance, hyperglycemia, hepatic steatosis, low high-density lipoprotein cholesterol levels, and cardiovascular risk) (7).

Intriguingly, drugs modulating the enterohepatic circulation of bile acids by binding bile acids in or inhibiting their absorption from the small intestine have been shown to improve glycemic control in patients with type 2 diabetes (T2D) (3, 8). The metabolic effects of bile acids may involve the gut hormone fibroblast growth factor 19 (FGF-19), which is released from the terminal ileum upon bile acid-induced FXR activation. FGF-19 exerts pleiotropic effects on hepatic bile acid metabolism as well as lipid, protein, and glucose metabolism (9). In light of the predominance of postprandial glycemia in determining overall glycemic control in T2D patients (10), as well as the accumulating evidence that bile acids play an important part in the whole-body response to nutrient ingestion (1), we hypothesized that postprandial bile acid concentrations in T2D patients could reveal a "T2D-bile acid-FGF-19" phenotype with possible pathophysiological implications. Thus, we performed a characterization of postprandial concentrations of 12 plasma bile acids and FGF-19 after various meal stimuli in patients with T2D and healthy age-, gender-, and body mass index (BMI)-matched control subjects with normal glucose tolerance (NGT).

Subjects and Methods

Participants

A detailed description of the experimental procedures and subjects was provided previously (11). In short, plasma was obtained from 15 patients with T2D (mean duration of T2D, 7.5 years [range, 6–20]; age, 59.4 ± 9.6 years [mean ± SD]; BMI, 28.0 ± 2.2 kg/m^2; hemoglobin A1c [HbA1c], 7.5 ± 1.4%) and 15 healthy, age-, gender-, and BMI-matched control subjects (age, 59.7 ± 10.0 years; BMI, 27.9 ± 2.0 kg/m^2; HbA1c, 5.2 ± 0.2%). None of the T2D patients had overt diabetic complications. Eight patients were treated with metformin, three with sulfonylurea, and four with diet only. Patients were instructed to abstain from taking blood glucose-lowering drugs for at least 1 week before the first study day.

Study design

Patients underwent four separate "meal" tests (visits were separated by 2–4 days) as follows: 75-g oral glucose tolerance test (OGTT; 75 g of water-free glucose dissolved in 300 mL water), and three isocaloric (500 kcal) and isovolemic (350 mL) liquid meals (low fat, 2.5 g fat, 107 g carbohydrate, and 13 g protein; medium fat, 10 g fat, 93 g carbohydrate, 11 g protein; and high fat, 40 g fat, 32 g carbohydrate, and 3 g protein). Written informed consent was obtained from all participants. Results on postprandial glucose metabolism, gallbladder emptying, and gut hormone secretion have been reported previously (11).

Sample collection

Arterialized blood samples were drawn 20, 10, and 0 minutes before and 15, 30, 45, 60, 90, 120, 180, and 240 minutes after ingestion of the OGTT or meals. Blood was collected into chilled tubes containing EDTA and a specific dipeptidyl peptidase 4 inhibitor (valine-pyrrolidide, final concentration of 0.01 mmol/L; a gift from Novo Nordisk) for plasma analyses of individual bile acids and FGF-19. Tubes were kept on ice, centrifuged for 20 minutes at 1200 × *g* and 4°C, and stored at −20°C until analysis. Samples were analyzed for unconjugated cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), and ursodeoxycholic acid (UDCA), as well as their corresponding glycine and taurine amidates (conjugates). Total bile acid (TBA) concentrations were determined by calculating the molar sum of all 12 bile acids. Bile acids were measured using an ultra-performance liquid chromatography ionization tandem mass spectrometry method (12) (Supplemental Data). A sandwich ELISA kit was used for colorimetric determination of FGF-19 in plasma (FGF-19 Quantikine ELISA kit, catalog no. DF1900; R&D Systems), following the manufacturer´s instructions. Intra- and interassay coefficients of variation were 6.0 and 7.5%, respectively.

Presentation of bile acid data

Bile acid concentrations are presented according to biological groupings (TBA, primary bile acids [CA, CDCA], and secondary bile acids [DCA, UDCA]) in Table 1 and Figures 1 and 2. Postprandial area under the curve (AUC) calculations for bile acids and FGF-19 concentrations are shown in Table 1, and postprandial profiles and AUCs are displayed graphically in Figures 1 and 2. Time courses for individual bile acids are found in Supplemental Figures 1–4. Fasting concentrations of bile acids and FGF-19 are presented in Supplemental Table 1. Bile acids con-

Table 1. Postprandial Bile Acids and FGF-19 Concentrations (AUC)

Bile Acids, min × μmol/L	NGT				T2D				rmANOVA		
	OGTT	Low Fat	Medium Fat	High Fat	OGTT	Low Fat	Medium Fat	High Fat	Meal	Group	Interaction
TBA	289 [202–447]	451 [432–563]	665 [367–1471]	1245 [746–1471]	562 [236–667]	776 [522–1058]	948 [640–994]	1241 [965–1589]	<0.001	<0.001	0.14
Unconjugated	48 [24–162]	77 [37–107]	76 [39–202]	137 [76–209]	105 [45–173]	205 [70–332]	170 [102–250]	216 [105–300]	<0.001	0.49	0.73
Glycine amidates	200 [140–239]	322 [285–412]	421 [243–518]	829 [591–994]	366 [157–507]	506 [370–588]	596 [358–738]	955 [714–1427]	<0.001	<0.001	0.28
Taurine amidates	30 [17–39]	52 [39–79]	64 [43–95]	140 [96–262]	60 [31–115]	89 [43–115]	95 [68–129]	152 [114–267]	<0.001	0.03	0.10
CA											
Total	58 [38–85]	93 [57–115]	119 [64–208]	239 [120–378]	109 [31–151]	123 [67–206]	164 [83–247]	181 [135–411]	<0.001	0.27	0.19
Unconjugated	18 [0–47]	12 [0–39]	11 [0–112]	18 [4–30]	9 [2–16]	9 [3–75]	19 [0–48]	21 [8–27]	0.22	0.46	0.37
Glycine amidates	33 [25–40]	58 [45–68]	71 [44–82]	162 [96–260]	67 [23–113]	84 [59–130]	106 [53–156]	152 [110–338]	<0.001	0.03	0.44
Taurine amidates	7 [4–12]	11 [7–17]	15 [6–28]	31 [17–59]	14 [5–25]	15 [8–28]	21 [10–29]	31 [16–53]	<0.001	0.09	0.39
CDCA											
Total	138 [103–232]	237 [183–351]	250 [176–413]	595 [425–803]	200 [101–288]	283 [205–358]	334 [228–450]	548 [413–660]	<0.001	0.16	0.19
Unconjugated	10 [0–31]	10 [0–39]	18 [4–29]	33 [8–65]	10 [0–23]	20 [0–48]	28 [1–43]	28 [15–52]	0.004	0.87	0.64
Glycine amidates	113 [77–159]	185 [148–277]	187 [143–345]	473 [343–595]	148 [84–215]	203 [171–256]	246 [175–355]	446 [326–596]	<0.001	0.14	0.24
Taurine amidates	17 [8–26]	30 [23–33]	38 [27–53]	89 [41–120]	21 [13–48]	37 [20–45]	39 [31–55]	69 [52–95]	<0.001	0.08	0.12
DCA											
Total	67 [49–94]	122 [60–152]	103 [72–205]	243 [168–336]	202 [71–313]	263 [126–371]	293 [166–461]	513 [276–702]	<0.001	<0.001	0.27
Unconjugated	26 [7–39]	29 [17–56]	24 [12–42]	53 [38–113]	81 [17–119]	92 [26–134]	104 [23–126]	120 [56–167]	<0.001	<0.001	0.43
Glycine amidates	29 [20–48]	57 [27–84]	64 [35–96]	114 [98–174]	92 [58–171]	130 [78–184]	153 [74–238]	298 [176–487]	<0.001	<0.001	0.31
Taurine amidates	6 [0–14]	11 [6–27]	8 [0–29]	29 [11–62]	23 [11–34]	27 [12–45]	33 [21–48]	51 [38–95]	<0.001	0.006	0.63
UDCA											
Total	5 [0–13]	9 [5–31]	11 [6–28]	24 [14–42]	20 [5–47]	38 [21–73]	43 [16–71]	43 [16–71]	<0.001	0.01	0.42
Unconjugated	2 [0–7]	1 [0–15]	0 [0–5]	5 [0–12]	5 [0–24]	13 [0–34]	16 [0–31]	19 [0–38]	0.38	<0.001	0.87
Glycine amidates	3 [0–8]	5 [0–14]	7 [0–19]	17 [5–32]	16 [5–25]	28 [9–37]	29 [13–40]	43 [16–56]	<0.001	0.002	0.57
Taurine amidates	NA	NA	NA	0 [0–1]	0 [0–2]	0 [0–2]	0 [0–1]	0 [0–2]	NA	NA	NA
FGF-19, min × ng/mL	43 [29–55]	59 [38–96]	65 [37–78]	82 [53–97]	46 [23–65]	49 [37–60]	57 [44–92]	55 [40–126]	<0.001	0.51	0.42

Abbreviations: NA, not available; rmANOVA, repeated measures ANOVA. Data are expressed as median [interquartile range]. Differences between groups were compared using two-way rmANOVA. AUC is shown for TBA, CA, CDCA, DCA, UDCA, their corresponding glycine and taurine amidates, and FGF-19 during a 75-g OGTT and three isocaloric (500 kcal) and isovolemic (350 mL) liquid meals (low fat: 2.5 g fat, 107 g carbohydrate, and 13 g protein; medium fat: 10 g fat, 93 g carbohydrate, 11 g protein; and high fat: 40 g fat, 32 g carbohydrate, and 3 g protein) in T2D patients and NGT subjects.

jugated with glycine or taurine are termed amidates in tables and figures.

Statistical analysis

Results are reported as medians and interquartile ranges (25th–75th percentiles). Baseline values are defined as the mean of the three values obtained before consumption of the meals, and AUC values were calculated using the trapezoidal rule. The effect of group and meal type was analyzed by repeated measures ANOVA in a linear mixed-effect model using group and meal as fixed effects and subjects as random effect. The assumptions of a Gaussian distribution of residuals and homogeneity of variances were assessed visually by drawing histograms, residual plots, and probability plots. If assumptions could not be met, continuous variables were transformed using Box-Cox transformations. We chose to calculate type III sums of squares for the fixed effects. Between-group differences were tested using Student's *t* test and Holm-Sidak's adjustment for multiple comparisons. Correlations were assessed with the Spearman rank correlation test. A two-sided *P* value of .05 was used to indicate significant differences. Statistical analyses were performed using GraphPad Prism version 6.0b for Windows/Mac (GraphPad Software) and R (3.2.1) for Windows/Mac (http://cran.r-project.org).

Results

Postprandial concentrations of TBA

Postprandial TBA concentrations increased with increasing meal fat content (post-test for linear trend, $P < .001$), peaked after 1–2 hours, and were higher in T2D patients vs NGT subjects (OGTT, low and medium fat meals, $P < .05$; high fat meal, $P = .30$) (Figure 1 and Table 1). Fasting concentrations of total, unconjugated, and amidated bile acids were marginally increased in T2D patients vs NGT subjects (Supplemental Table 1). A subgroup analysis revealed no differences in TBA and FGF-19 concentrations in metformin-treated patients vs patients treated with sulfonylureas and diet only (data not shown).

Postprandial concentrations of primary bile acids (CA and CDCA)

Postprandial total CA concentrations were dominated by glycine conjugates, which were higher in T2D patients vs NGT subjects (Table 1 and Figure 2) after OGTT and low and medium fat meals, but not after the high fat meal. Other CA fractions did not differ between the two groups. In comparison with the other bile acids measured, glycine-conjugated CDCA was the dominating bile acid found postprandially (Figure 2). In both groups, a clear and positive effect of meal fat (post-test for linear trend, $P < .001$) was demonstrated, but no between-group differences were present. There was a tendency to higher taurine conjugates in NGT subjects, but this difference was not statistically significant (Supplemental Figure 4). Fasting concentrations of total and unconjugated CA and CDCA were comparable between groups, whereas glycine-conjugated CA and taurine-conjugated CA and CDCA were marginally higher in T2D patients vs NGT subjects (Supplemental Table 1).

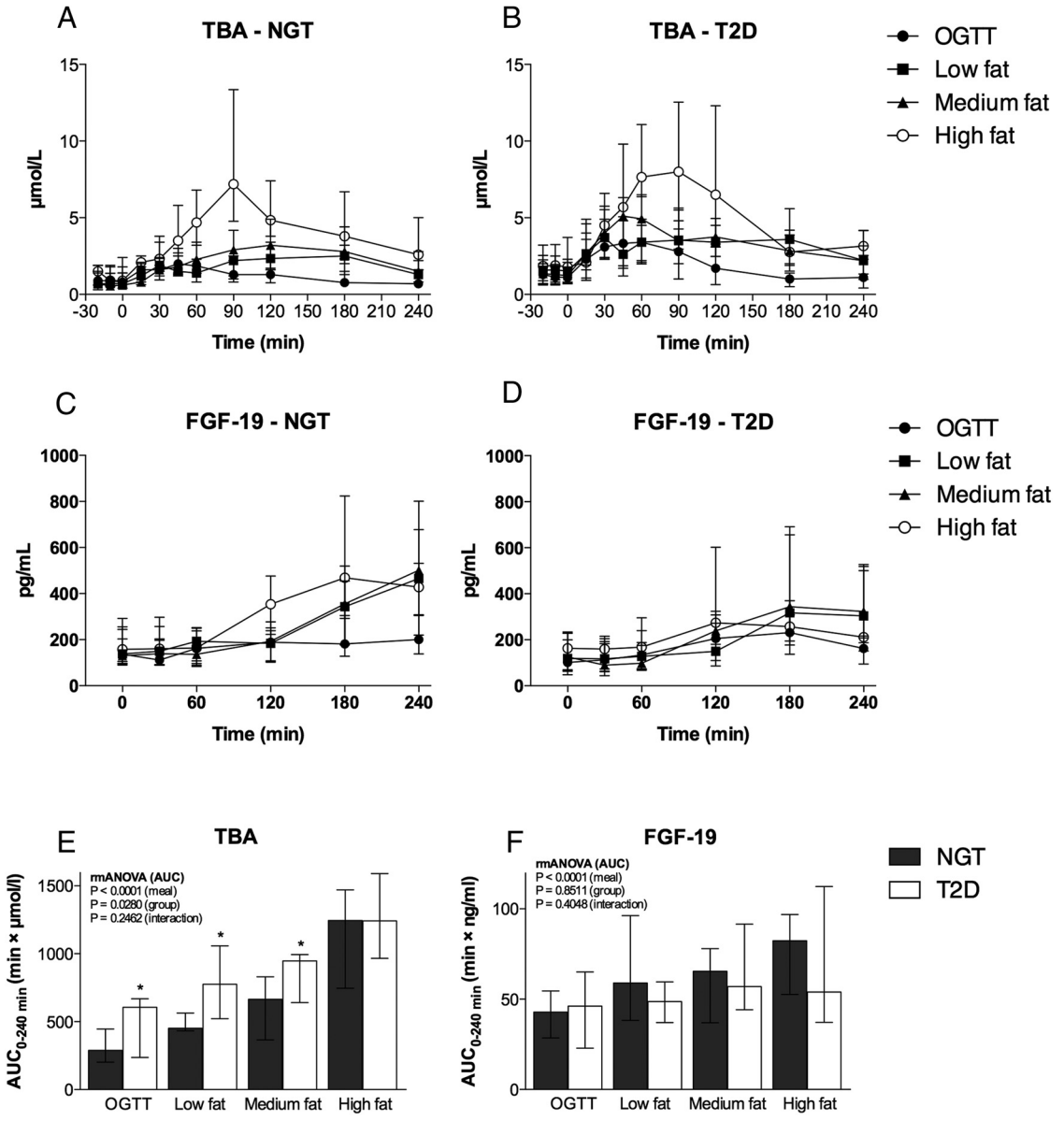

Figure 1. TBAs and FGF-19. Postprandial plasma concentrations of TBA and FGF-19 are shown during a 75-g OGTT and three isocaloric (500 kcal) and isovolemic (350 mL) liquid meals (low fat, 2.5 g fat, 107 g carbohydrate, and 13 g protein; medium fat, 10 g fat, 93 g carbohydrate, 11 g protein; and high fat: 40 g fat, 32 g carbohydrate, and 3 g protein) in NGT subjects (A and C, n = 15) and T2D patients (B and D, n = 15). AUC is shown for TBA (E) and FGF-19 (F) in NGT subjects and T2D patients. Median and interquartile range values are shown. *, Significant differences ($P < .05$) vs NGT.

Postprandial concentrations of secondary bile acids (DCA and UDCA)

Postprandial DCA concentrations were markedly higher in T2D patients after all meal stimuli. These differences reflected unconjugated and glycine-conjugated bile acids and, to a lesser extent, taurine conjugates (Table 1 and Figure 2). Although postprandial UDCA concentrations were 5- to 10-fold lower relative to the other bile acids measured, a clear increase was demonstrated in T2D patients vs NGT subjects. As for DCA concentrations, this difference reflected increased unconjugated and glycine-conjugated UDCA. However, in both groups, taurine conjugates were very low. Again, meal fat content associated positively with bile acid concentrations (post-test for linear trend, $P < .001$). Fasting concentration of total, unconjugated, and glycine and taurine conjugates of DCA and UDCA were also higher in T2D patients vs NGT subjects (Supplemental Table 1).

Postprandial concentrations of FGF-19

Both fasting and postprandial concentrations of FGF-19 varied considerably in both groups. Hence, although NGT subjects tended to have higher postprandial FGF-19 concentrations, differences were not statistically significant (Figure 1, Table 1, and Supplemental Table 1). In both groups, postprandial peak

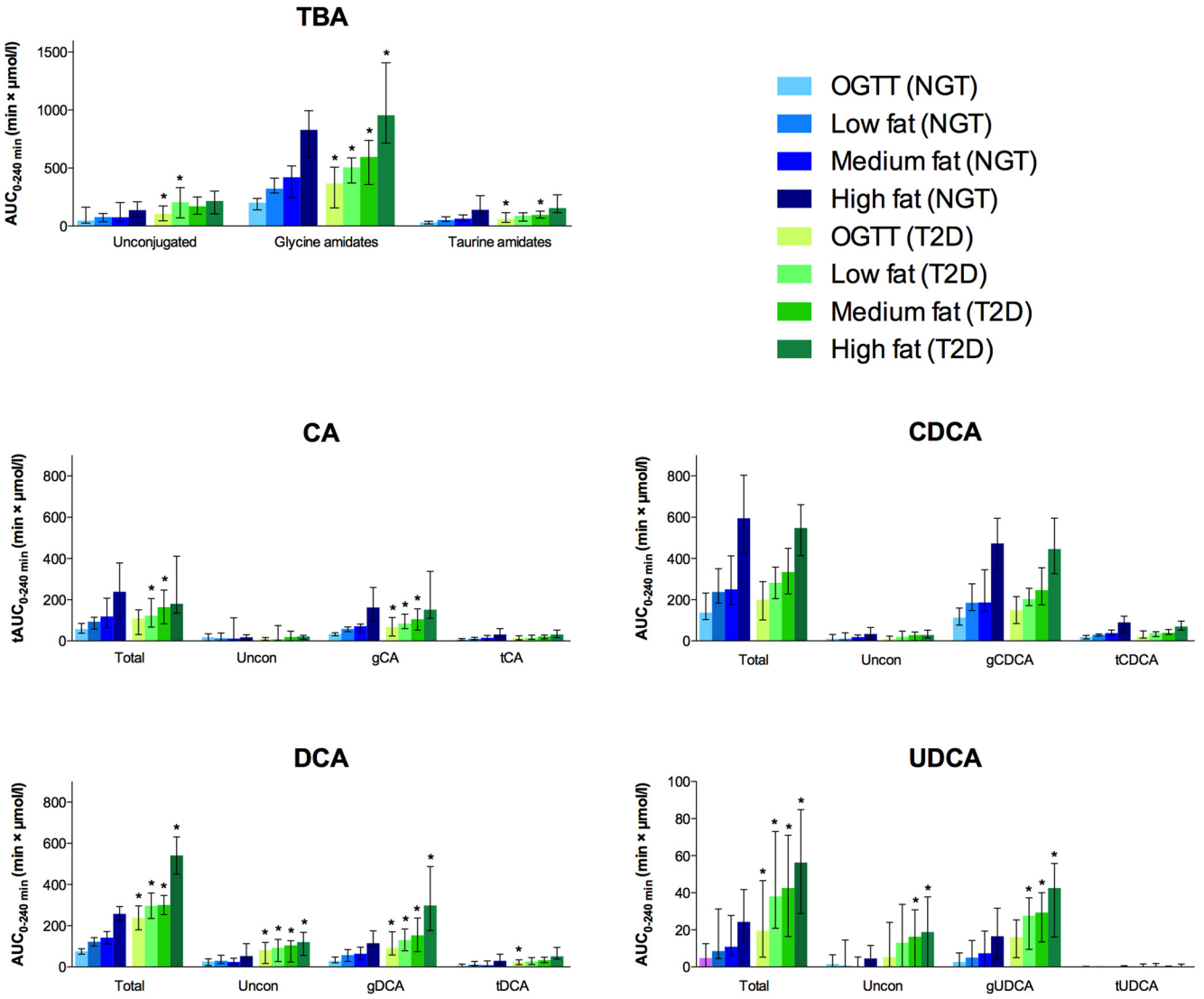

Figure 2. Individual bile acids. AUC is shown for TBA, unconjugated (uncon) CA, CDCA, DCA, and UDCA, and their corresponding glycine (g) and taurine (t) amidates during a 75-g OGTT and three isocaloric (500 kcal) and isovolemic (350 mL) liquid meals (low fat, 2.5 g fat, 107 g carbohydrate, and 13 g protein; medium fat, 10 g fat, 93 g carbohydrate, 11 g protein; and high fat, 40 g fat, 32 g carbohydrate, and 3 g protein) in T2D patients and NGT subjects. Median and interquartile range values are shown. *, Significant differences ($P < .05$) vs NGT.

concentrations occurred late (~3–4 hours after meal ingestion) and were more pronounced with increasing meal fat content (post-test for linear trend, $P < .001$). By grouping the four meal responses together, correlation analyses showed a weak, but significant, positive correlation between TBA and FGF-19 concentrations (AUCs) in both NGT subjects ($r = 0.411$; $P < .001$) and T2D patients ($r = 0.519$; $P < .001$). The association between FGF-19 and the individual bile acids (total concentrations) showed similar patterns (NGT vs T2D: CA, $r = 0.426$ and $P < .001$ vs $r = 0.471$ and $P < .001$; CDCA, $r = 0.384$ and $P = .003$ vs $r = 0.520$ and $P < .001$; DCA, $r = 0.361$ and $P = .005$ vs $r = 0.322$ and $P < .017$; UDCA, $r = 0.188$ and $P = .15$ vs $r = 0.107$ and $P = .44$). Postprandial concentrations of FGF-19 and C-peptide were inversely associated in both NGT subjects ($r = -0.266$; $P = .04$) and T2D patients ($r = -0.383$; $P = .003$), whereas the association between FGF-19 and glucose was negatively associated only in NGT subjects ($r = -0.310$; $P = .02$) vs T2D patients ($r = 0.061$; $P = .66$).

Discussion

The major findings in this study are: 1) T2D patients exhibited increased fasting and postprandial TBA concentrations after a wide range of nutritional stimuli in comparison with NGT subjects; 2) differences reflected mainly unconjugated and glycine-conjugated forms of the secondary bile acids DCA and UDCA and to a lesser extent CA, whereas postprandial CDCA concentrations were

comparable among the two groups; and 3) postprandial concentrations of taurine-conjugated bile acids were also slightly higher in T2D patients (DCA). Furthermore, both fasting and postprandial FGF-19 concentrations tended to be lower in T2D patients vs NGT subjects, but due to large variability, these differences were not statistically significant. Lastly, in both groups, a positive correlation between postprandial TBA and FGF-19 concentrations was demonstrated.

The aim of the present study was to assess postprandial bile acid concentrations in T2D patients in order to shed more light on the possible mechanisms underlying the role of bile acids in metabolic regulation and T2D pathophysiology. Recent years have seen extensive research on bile acid metabolism in T2D, but no previous study has addressed postprandial bile acid concentrations of individual bile acids together with FGF-19 after a wide range of different meals. The importance of this focus is highlighted by recent data showing that bile acids are not just luminal signaling molecules that activate TGR5 (leading to GLP-1 secretion) and FXR (leading to FGF-19 secretion) in the intestine (3). FXR and TGR5 receptors are widely expressed and may even be found on pancreatic β-cells (6, 13–15). In fact, systemic bile acids seem to stimulate TGR5 and FXR, which positions postprandial plasma bile acids suitable for the regulation of overall glucose homeostasis (1, 6, 16, 17). Such a concept could fit with the established notion that in T2D patients with a relatively low HbA1c (~7.5% [58 mmol/mol] or less), postprandial glycemia, as opposed to preprandial blood glucose, makes the predominant contribution to overall glycemic control (10). Indeed, postprandial hyperglycemia—an early defect seen in impaired glucose tolerance and T2D—is a major contributor to HbA1c and is even recognized as an independent risk factor for cardiovascular disease (18, 19)—a link that may also prove important considering the emerging evidence suggesting a role of bile acids in cardioprotection (20).

Substantial rearrangements of bile acid metabolism in T2D patients are well established, including changes in pool size, pool composition, synthesis rate, and postprandial plasma concentrations (21–27). We show that postprandial concentrations of secondary bile acids were higher in T2D patients vs NGT subjects. Indeed, on most study days, even fasting concentrations of total and secondary bile acids were higher in T2D patients vs NGT subjects. Moreover, we found increased postprandial CA concentrations (mainly glycine amidates), whereas CDCA concentrations (the predominant bile acid) were comparable among the groups. The design of the isovolemic and isocaloric meals allowed us to dissect in more detail how postprandial bile acid concentrations are affected by the fat content in the meal. Clearly, in both groups the high fat meal resulted in much higher plasma bile acid concentrations compared to the other meals—in fact, the CA and CDCA concentrations in T2D patients were equaled in the NGT group after the high fat meal (Figure 2). All four stimuli led to augmented DCA and UDCA concentrations in the T2D patients vs NGT subjects. Hence, postprandial concentrations of bile acids were clearly influenced acutely by meal composition. Most likely, this reflected increased gallbladder emptying after a larger fat stimulus (11) and increased enterohepatic circulation of all bile acid species, but it could also point toward the notion of acute effects of macronutrient composition constituting an important regulator of postprandial bile acid pool composition similar to changes seen after more extensive diet changes (28). Indeed, the meals also differed with regard to carbohydrate and protein content. Interestingly, even oral glucose resulted in increased TBA plasma concentrations in T2D patients, whereas concentrations in NGT subjects were almost unaffected. This could fit with our finding of increased gallbladder emptying in the T2D group (~30 vs ~20% in the NGT group) after the OGTT (11). Indeed, oral glucose is a minor stimulus for gallbladder contraction but—perhaps counterintuitively—a rather good stimulus for FGF-19 secretion, as recently demonstrated by Morton et al (29). However, in contrast to the data by Morton et al (29) showing that FGF-19 concentrations increase preferentially in response to carbohydrates as opposed to protein and fat, we found that FGF-19 concentrations increased with increasing fat and decreasing carbohydrate content in the meals. Notably, despite some gallbladder contraction after the OGTT (11), FGF-19 concentrations remained more or less at the basal concentration in the NGT group and increased slightly in the T2D patients (Figure 1). Thus, although the data by Morton et al (29) indicate that the increase in postprandial FGF-19 concentrations involves mechanisms additional to bile acid-induced FXR activation, our data fit with the notion of FXR dependency. However, in our T2D patients, there was a clear dissociation between postprandial concentrations of bile acids and FGF-19 (but positive correlations were still demonstrated) compared to NGT subjects, and the "dose-response" relationship between fat (gallbladder contraction) and FGF-19 was clearly reduced. This may indicate that bile acid-induced FXR activation is impaired in T2D, leading to decreased secretion of FGF-19, which could explain the higher bile acid concentrations because FGF-19 inhibits bile acid synthesis (30). Indeed, reduced FGF-19 concentrations in T2D patients have been reported in recent clinical studies (31–33). Although mechanisms independent of FXR could be at work in T2D (ie, altered synthesis, secretion, and degradation of FGF-19,

activation of the pregnane X and vitamin D receptors by bile acids) (7), the enterohepatic bile acid composition is a likely determinant of the degree of FXR activation. However, in our study, CDCA concentrations, the most potent natural ligand of FXR, were unaltered in T2D patients vs NGT subjects, whereas DCA concentrations (a much weaker FXR agonist) were higher along with CA (not an FXR ligand). Such bile acid milieu should, theoretically, favor FXR activation and subsequent FGF-19 secretion. However, postprandial UDCA concentrations were slightly higher in T2D patients vs NGT subjects. Although plasma UDCA concentrations were very low, this finding is of interest because UDCA is considered an FXR antagonist (5, 34).

Interestingly, using the murine GLUTag L cell line, human intestinal biopsies, and different mouse models, Trabelsi et al (6) demonstrated that FXR activation inhibited glycolysis and ATP production, which in turn decreased proglucagon transcription and GLP-1 secretion in response to glucose. In contrast, FXR deficiency or FXR deactivation (using bile acid sequestering agents) promoted GLP-1 production and secretion (6). Thus, this newly identified FXR/GLP-1 pathway suggests a positive effect of FXR antagonism on glucose homeostasis, which is also evidenced by the success of bile acid sequestering therapy for the treatment of T2D (3, 6). However, reduced FGF-19 concentrations, achieved with bile acid sequestration (FXR inactivation), is not likely to explain the beneficial effect of bile acid sequestering agents. Indeed, animal studies have shown that FGF-19 has insulin-like effects in the liver (9). Thus, FGF-19 promotes protein and glycogen synthesis in the liver without promoting lipogenesis—in fact, FGF-19 may even reduce triglycerides and cholesterol through currently unknown mechanisms (35, 36). A physiologically important difference is the temporal relationship between postprandial insulin and FGF-19 secretion. As has been shown in other human studies and is confirmed in our study, FGF-19 peak concentrations are segregated from insulin (~3 hours vs ~1 hour). Mice studies suggest that FGF-19—similar to insulin—is responsible for curbing postprandial endogenous glucose production, which is augmented in T2D (9). Specifically, FGF-19 may be responsible for a delayed repression of gluconeogenesis, whereas postprandial insulin works in the early postprandial phase (37). Because gluconeogenesis accounts for approximately half of the endogenous glucose production (EGP), being low for up to approximately 4 hours after a meal (38), such a temporal relationship of postprandial insulin and FGF-19 could physiologically make sense. However, our finding of very small FGF-19 perturbations (in both T2D patients and NGT subjects) after a 75-g OGTT prompts questions about the idea that FGF-19 is an important inhibitor of postprandial EGP by suppression of hepatic gluconeogenesis. However, EGP could be stimulated via bile acid-induced glucagon release from the intestine (39).

In summary, we find that T2D patients exhibit marked changes in fasting and postprandial bile acid concentrations compared to matched NGT subjects. These differences were dominated by increased unconjugated and glycine-conjugated secondary bile acids in T2D patients compared to NGT subjects, whereas primary bile acids were comparable among the two groups. In contrast, FGF-19 concentrations tended to be lower in T2D patients vs NGT subjects. Most likely, these changes arise secondary to the T2D disease, as suggested by recent studies. Theoretically, this "T2D-bile acid-FGF-19" phenotype results in altered FXR/FGF-19 signaling in the small intestine and the liver, which could potentially add to the deterioration of postprandial glycemic homeostasis in T2D.

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