Preserved GLP-1 and exaggerated GIP secretion in type 2 diabetes and relationships with triglycerides and ALT

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Abstract

Objective: To i) compare incretin responses to oral glucose and mixed meal of diabetic patients with the normoglycaemic population and ii) to investigate whether incretin responses are associated with hypertriglyceridaemia and alanine aminotransferase (ALT) as liver fat marker.

Design: A population-based study.

Methods: A total of 163 persons with normal glucose metabolism (NGM), 20 with intermediate hyperglycaemia and 20 with type 2 diabetes aged 40–65 years participated. Participants received a mixed meal and oral glucose load on separate occasions. Glucagon-like peptide 1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and glucagon profiles were analysed as total area under the curve (tAUC) and incremental area under the curve.

Results: In diabetic patients compared with persons with NGM, we found increased GLP-1 secretion (tAUC per hour) following oral glucose (23.2 pmol/l (95% CI 17.7–28.7) vs 18.0 (95% CI 16.9–19.1), P < 0.05) but not after the mixed meal. GIP secretion among diabetic patients was increased on both occasions (82.9 pmol/l (55.9–109.8) vs 47.1 (43.8–50.4) for oral glucose and 130.6 (92.5–168.7) vs 83.2 (77.5–88.9) for mixed meal, both P < 0.05). After oral glucose, GLP-1 (tAUC per hour) was inversely related to fasting triglycerides. GIP (tAUC per hour) was positively related to fasting and postprandial triglycerides. Higher fasting GIP levels were related to higher fasting and postprandial triglyceride levels and ALT.

Conclusion: This study confirms that in type 2 diabetes, GLP-1 secretion is generally preserved and that GIP secretion is exaggerated. The mechanism underlying the divergent associations of GLP-1 and GIP metabolism with fat metabolism and liver fat accumulation warrants further study.

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Introduction

The incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released from the gut upon nutrient ingestion and are known for their insulin-stimulating effects. Among type 2 diabetic patients, a reduced incretin effect has been described (1). The reduced incretin effect has been attributed to a substantial reduction in insulinotropic effect of GIP (2, 3) while the insulinotropic efficacy of GLP-1 is largely preserved (3). Given this preserved insulinotropic efficacy of GLP-1, therapeutic interventions with GLP-1 analogues or dipeptidyl peptidase-4 inhibitors have been proven effective in controlling glucose levels in type 2 diabetic patients (4).

Despite this well-established reduced incretin effect among diabetic patients, whether a defect in incretin secretion exists is still not fully resolved (5, 6). Secretion of the incretins GLP-1 and GIP following meal intake or oral glucose has been described in a number of studies and was reviewed by Calanna et al. and Nauck et al. (5, 6). These authors concluded that patients with type 2 diabetes, in general, do not exhibit reduced GLP-1 secretion (5, 6). Nevertheless, heterogeneity was apparent between studies that used mixed meal (lower GLP-1 among diabetes) and oral glucose load (higher GLP-1). Studies that directly compared GLP-1 responses following oral glucose vs mixed meal are scarce and the available studies did not find an altered GLP-1 response after both interventions among diabetic patients (7).
GIP responses were found to be reduced (8), increased (7, 9), but mostly unchanged in patients with diabetes (10, 11, 12, 13). Possible explanations for these inconclusive findings on incretin responses among diabetic patients may relate to the assay used, the test meal, participant characteristics and probably sample size. A large study in a representative study population assessing incretin responses following meal and oral glucose would fill this gap of knowledge.

Clinical studies have demonstrated benefits of GLP-1-based therapies that go beyond the effects of incretins in insulin and glucose metabolism. These include direct cardiovascular effects (14) and effects on the liver (15). Dyslipidemia is a well-known feature of type 2 diabetic patients (16) and an acknowledged risk factor for cardiovascular disease (17). The role of incretins in lipid metabolism is of specific interest as experimental studies have demonstrated differential effects of GLP-1 and GIP on lipid metabolism. Infusion of GLP-1 analogues abrogated the rise in postprandial triglycerides after a mixed meal in healthy individuals (18). In addition, among patients with type 2 diabetes, treatment with a GLP-1 analogue or GLP-1 agonist has been demonstrated to improve blood lipid levels and cardiovascular disease risk (19, 20). Experimental studies in obesity-prone mice have shown that administration of a GLP-1 receptor agonist prevents weight gain and development of a fatty liver (21). In contrast, GIP receptors are thought to promote fat deposition and fatty acid synthesis in adipose tissue (22). Administration of a GIP receptor antagonist reverses obesity and insulin resistance in high-fat-fed mice (23). In humans, the beneficial effects of bariatric surgery in curing diabetes among obese persons were accompanied by a rapid decrease in GIP, suggesting that lowering GIP levels may play a role in the metabolic effects of such a surgery (24).

The aim of this study was twofold; first, to perform a population-based study to assess GLP-1 and GIP responses to oral glucose and a mixed meal among a study group representative for the general population. Secondly, we aimed to investigate whether GLP-1 and GIP responses are associated with markers of abnormal lipid metabolism.

**Subjects and methods**

**Study procedure**

The study population consisted of 194 persons who were randomly invited from the general population aged 40–65 years in Hoorn, The Netherlands. Another 14 patients with type 2 diabetes (not using insulin) from the Regional Diabetes Care System additionally participated to increase the number of type 2 diabetic patients. Details of the inclusion criteria were described earlier (25). After exclusion of three persons with missing data on incretin and/or glucagon response following meal, one person with uninterpretable oral glucose tolerance test (OGTT) and one person with type 1 diabetes, the number of persons for the present analysis was 203 and consisted of 20 persons with type 2 diabetes, 20 with intermediate hyperglycaemia (IH) (being isolated impaired fasting glucose, \( n = 14 \); isolated impaired glucose tolerance, \( n = 3 \); or both impaired fasting glucose and impaired glucose tolerance, \( n = 3 \) ) and 163 with normal glucose metabolism (NGM) (26). All participants signed informed consent with respect to the study and the medical ethics committee of the VU University Medical Centre approved the study.

Participants underwent a 2-h 75 g OGTT and a 4-h standardized mixed meal test (MMT) on separate occasions in random order. Tests were performed after a 10-h overnight fast, and apart from small amounts of water, participants refrained from oral glucose-lowering medication (if applicable), food, drinks and physical activity during the test. Blood samples were drawn from the antecubital vein in the fasting state and at predefined time-points during the test. Blood pressure (Collin Press-mate BP-8800, Colim, Komaki-City, Japan), weight, height, and waist and hip circumference were measured prior to the test.

The mixed meal consisted of two croissants, butter, Gouda cheese, full-fat milk and yogurt drink with soluble carbohydrates (maltose). The nutrient content was 3487 kJ, 36 energy% carbohydrates, 52 energy% fat and 12 energy% proteins.

**Laboratory analyses**

Total GLP-1 levels were determined using RIA (antisera 89390) against standards of synthetic GLP-1 7–36 amide. The assay measures the sum of intact GLP-1 and the primary metabolite, GLP-1 9–36 amide as described previously (27). Total GIP was measured using the COOH terminally directed antiserum R65, which reacts fully with intact human GIP and the NH\(_2\) terminally truncated metabolite (28). For both assays, sensitivity was below 1 pmol/l and intra-assay coefficient of variation below 6% at 20 pmol/l.

Serum total and HDL-cholesterol levels and triglycerides were measured by enzymatic colorimetric assays (Roche). Alanine aminotransferase (ALT) was measured by the IFCC method (Roche Diagnostics). Plasma glucose levels were determined by a glucose hexokinase method (Gluco-quant, Roche Diagnostics). A1c was determined using reversed-phase cation exchange chromatography (HA 8160 analyzer, Menarini, Florence, Italy) accompanied by the use of a method to standardize HbA1c across laboratories (DCCT-standardization). Serum C-peptide and insulin levels were determined by immunometric assays (ACS Centaur, Bayer Diagnostics). Glucagon levels were determined in EDTA samples by RIA, after extraction (Linco Research, St Louis, MO, USA) with inter- and intra-assay coefficients of variation of 8% and lower limit of quantitation of 2.3 pmol/l.
**Statistical analyses**

Differences in characteristics between persons with type 2 diabetes and IH when compared with those with NGM were analysed by one-way ANOVA. In case of significant F-test, comparative testing was done against persons with NGM with least significant difference post hoc test. Differences in percentages were tested by \( \chi^2 \). Skewed variables were ln-transformed before testing.

Total area under the curve (tAUC) and incremental area under the curve (iAUC) for incretin and glucagon responses were calculated as time-corrected area under the curve (AUC per hour in pmol/l). Differences in incretin and glucagon tAUC and iAUC between groups were analysed by one-way ANOVA. In case of type 2 diabetes and IH when compared with those with NGM, persons with diabetes had higher mean levels of glucagon, GLP-1 and GIP in the fasting state (33.1 kg/m\(^2\)).

Inulin sensitivity was estimated by calculation of the oral glucose insulin sensitivity index (OGIS), which was calculated from \( t = 0, 90 \) and 120 min upon OGTT (29). The OGIS is an estimate of the glucose clearance during a hyperinsulinaemic–euglycaemic glucose clamp and as such expressed in ml/min per m\(^2\) body surface area (29).

After the calculation of overall AUC for hormone responses, we aimed to investigate the postprandial patterns of these responses. Therefore, time-dependent GLP-1, GIP and glucagon profiles were analysed by linear mixed models. Linear mixed models use all available data over time and account for correlations between repeated measurements (30). The first models included the variables test occasion (being oral glucose or mixed meal), time, and glucose tolerance state and estimated regression coefficients with 95% CIs. The coefficient for test occasion estimated the effect of oral glucose in comparison with mixed meal on incretin and glucagon responses. Subsequently, we used a model that included glucose tolerance state, time and interactions between glucose tolerance state and time for both test occasions separately. A significant interaction between time and glucose tolerance state is interpreted as a stronger rate of change over time attributable to glucose tolerance state. In these analyses, adjustments were made for age and sex.

Further analyses on variables potentially explaining the GLP-1, GIP and glucagon responses to the meal were assessed in a univariate linear regression model with the tAUC of these responses as an outcome variable. Linear regression analysis was performed with fasting and tAUC of GLP-1 and GIP in relation to (postprandial) triglycerides and ALT. For these analyses, 21 persons who used lipid-lowering medication (11 of them were NGM, three IH and seven diabetic patients) were excluded. As potential confounding factors, we added age, sex and glucose tolerance state to the model. Subsequently, OGIS and glucagon responses were added to the model as potential explanatory variables of the relationship between incretins and triglycerides or ALT. Although no medication was used on the test day, metformin may have impacted on the GLP-1 response as it has been suggested to act as a GLP-1 enhancer (31). Therefore, we performed a sensitivity analysis by excluding patients who were on metformin. In all analyses, a \( P \) value < 0.05 was considered statistically significant except for interaction terms where we used \( P < 0.10 \) as statistically significant.

**Results**

**Characteristics of study participants**

Type 2 diabetic patients participating in this study had a median diabetes duration of 3.5 years; this group includes three patients who were newly diagnosed at the first study visit. Mean HbA1c among these diabetic patients was 7.1% (54.1 mmol/mol) (Table 1).

Patients with type 2 diabetes and persons with IH were significantly more overweight (BMI and waist circumference), had higher systolic blood pressure and triglyceride levels, and lower HDL-cholesterol levels than those with NGM (\( P < 0.05 \) for all comparisons). Patients with type 2 diabetes used more medication both for blood pressure and lipids (\( P < 0.05 \)). Further, persons with IH and type 2 diabetes had higher levels of ALT (\( P < 0.05 \)). When compared with persons with NGM, persons with diabetes had higher mean levels of glucagon, GLP-1 and GIP in the fasting state (\( P < 0.05 \), Table 1). Persons with IH had higher fasting GLP-1 (\( P < 0.05 \)) but not GIP (\( P = 0.29 \)) or glucagon (\( P = 0.07 \), Table 1).

**Responses following mixed meal and oral glucose**

Glucose, insulin and triglyceride responses following oral glucose and mixed meal are presented in Fig. 1. GLP-1, GIP and glucagon responses are presented in Fig. 2. Among the total study population, the OGTT elicited a higher GLP-1 response (\( P < 0.05 \)), a non-significantly lower GIP response (\( P = 0.58 \)) and a lower glucagon response (\( P < 0.05 \)) than the mixed meal (assessed by linear mixed models, Fig. 2).

The response in GLP-1 following oral glucose (t120) but especially following mixed meal (t15 and t90, t120, t180 and t240) was lower among patients with type 2 diabetes (\( P \) value interaction for time×type 2 diabetes state < 0.10, Fig. 2D). GLP-1 tAUC per hour among diabetic patients was similar after the mixed meal (\( P = 0.32 \)) and higher after oral glucose (\( P < 0.01 \)), in comparison with normoglycaemic persons (Fig. 3). Despite higher tAUC after the mixed meal, the iAUC...
per hour of GLP-1 was lower for diabetic patients following mixed meal \((P<0.05)\) but not oral glucose \((P=0.63)\). **Supplementary Figure 1**, see section on supplementary data given at the end of this article). The GLP-1 response upon mixed meal was similar for metformin and non-metformin users (data not shown).

Persons with IH had similar tAUC GLP-1 response to those with NGM both following oral glucose and mixed meal \((P=0.12)\) and in comparison with non-diabetic persons \((P=0.10)\) in comparison with non-diabetic persons with IH (Fig. 3).

The GIP response was more pronounced in diabetic patients at t15, t30, t60 and t90 min after oral glucose and at t30, t60, t90 and t120 after mixed meal \((P=0.05)\) but not oral glucose \((P=0.10)\) in comparison with non-diabetic persons. Interestingly, the GIP response was also elevated among persons with IH after oral glucose \((t30 and t60)\) and after the mixed meal at t30 also elevated among persons with IH after oral glucose \((t30 and t60)\) (Fig. 2) and after the mixed meal at t30 also elevated among persons with IH after oral glucose \((t30 and t60)\) (Fig. 2) and after the mixed meal at t30 also elevated among persons with IH after oral glucose \((t30 and t60)\) (Fig. 2) and after the mixed meal at t30 also elevated among persons with IH after oral glucose \((t30 and t60)\) (Fig. 2). Total and incremental AUC per hour of GIP was significantly higher for diabetic patients following both oral glucose and mixed meal \((P<0.05)\) by ANOVA (continuous variables) or \(\chi^2\) test (percentages) against NGM. ALT, alanine aminotransferase; IH, intermediate hyperglycaemia; NGM, normal glucose metabolism; OGIS, oral glucose insulin sensitivity index; T2DM, type 2 diabetes.

**Table 1** Characteristics of study participants. Values represent means (s.d.) or medians (interquartile range) in case of skewed distribution.

<table>
<thead>
<tr>
<th></th>
<th>NGM</th>
<th>IH</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n)</td>
<td>163</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.1 (6.8)</td>
<td>56.6 (5.3)</td>
<td>53.3 (6.7)</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>47.2</td>
<td>60.0</td>
<td>60.0</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>26.7 (3.7)</td>
<td>28.5 (3.7)</td>
<td>33.1 (7.3)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>92.7 (10.6)</td>
<td>102.7 (11.0)*</td>
<td>113.8 (17.2)*</td>
</tr>
<tr>
<td>Use of anti-hypertensives (%)</td>
<td>15.3</td>
<td>25.0</td>
<td>60.0*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134 (14)</td>
<td>143 (22)*</td>
<td>140 (16)*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76 (9)</td>
<td>80 (10)</td>
<td>78 (9)</td>
</tr>
<tr>
<td>Use of lipid-lowering medication (%)</td>
<td>6.7</td>
<td>15.8</td>
<td>35.0*</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.1 (1.0)</td>
<td>5.5 (1.0)</td>
<td>4.6 (0.9)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.42 (0.38)</td>
<td>1.21 (0.24)*</td>
<td>1.12 (0.42)*</td>
</tr>
<tr>
<td>Fasting triglycerides (mmol/l)</td>
<td>1.2 (0.9–1.6)</td>
<td>1.7 (1.3–2.3)*</td>
<td>1.8 (1.4–2.3)*</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>10 (7–14)</td>
<td>15 (10–21)*</td>
<td>19 (14–24)*</td>
</tr>
<tr>
<td>OGIS (ml/min per m(^2))</td>
<td>424 (57)</td>
<td>326 (49)*</td>
<td>306 (43)*</td>
</tr>
<tr>
<td>Fasting glucagon (pmol/l)</td>
<td>9.7 (2.4)</td>
<td>10.9 (3.5)</td>
<td>13.9 (4.7)*</td>
</tr>
<tr>
<td>Fasting GLP-1 (pmol/l)</td>
<td>10.8 (4.0)</td>
<td>13.9 (6.8)*</td>
<td>16.7 (6.0)*</td>
</tr>
<tr>
<td>Fasting GIP (pmol/l)</td>
<td>7.0 (5.6)</td>
<td>8.6 (5.9)</td>
<td>14.1 (9.5)*</td>
</tr>
<tr>
<td>HbA1c (%/mmol per mol)</td>
<td>–</td>
<td>–</td>
<td>7.1 (1.4)/54.1 (15.2)</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>–</td>
<td>–</td>
<td>3.2 (1.2–5.4)</td>
</tr>
<tr>
<td>Diabetes treatment (diet only/metformin only/metformin and SU/SU only)</td>
<td>–</td>
<td>–</td>
<td>(n=5) (n=9) (n=5) (n=1)</td>
</tr>
</tbody>
</table>

*Significant difference \((P<0.05)\) by ANOVA (continuous variables) or \(\chi^2\) test (percentages) against NGM. ALT, alanine aminotransferase; IH, intermediate hyperglycaemia; NGM, normal glucose metabolism; OGIS, oral glucose insulin sensitivity index; T2DM, type 2 diabetes.

Patients with diabetes had a higher tAUC per hour of GLP-1 after oral glucose and a higher tAUC per hour of GIP when compared with non-diabetic persons with similar BMI (Fig. 4). There were no differences in tAUC per hour of GLP-1 or GIP between subgroups of BMI among non-diabetic individuals (Fig. 4).

**Determinants of GLP-1, GIP and glucagon responses**

Univariate associations with GLP-1, GIP and glucagon tAUC per hour to the meal are presented in Table 2. None of the variables studied were associated with the GLP-1 response. Glucose tolerance state (both IH and diabetes) and the glucagon response were associated with higher GIP response and OGIS was associated with lower GIP response. BMI, waist circumference and glucose tolerance state (both IH and diabetes) were associated with a higher glucagon response to meal and male gender and OGIS with a lower glucagon response to meal (Table 2).

**Associations with triglycerides and ALT**

Results of linear regression analyses with fasting and postprandial triglycerides and ALT as dependent variables are shown in Table 3. Fasting GLP-1 levels were not associated with fasting or postprandial triglycerides or ALT (Table 3). Fasting levels of GIP were associated with...
fasting and postprandial triglycerides and ALT, also after full adjustment for glucose tolerance state, OGIS and fasting glucagon (model 3, Table 3).

GLP-1 tAUC per hour after oral glucose was inversely related to fasting but not postprandial triglycerides in some models (Table 3). GIP-tAUC per hour after oral glucose was positively related to fasting and postprandial triglycerides also after full adjustment (Table 3). The GLP-1 and GIP responses to oral glucose were unrelated to ALT. GLP-1 and GIP-tAUC per hour after mixed meal was not associated with triglycerides or ALT (Table 3).

Discussion

We found an increased GLP-1 secretion following oral glucose but not after mixed meal, together with an overall increased GIP response among diabetic patients when compared with persons with NGM. After oral glucose, GLP-1 (tAUC per hour) was inversely related to fasting triglycerides, while GIP (tAUC per hour) was positively related to fasting and postprandial triglycerides. Further, higher fasting GIP levels were related to higher fasting and postprandial triglycerides and ALT.

Strengths and limitations of the study

The size of the study together with the population-based design is strength. The sample size had a number of advantages. First, it enabled us to assess multiple factors associated with incretin responses. Secondly, it allowed linear mixed model analyses to fully explore time effects. Another strength of the study is that we measured oral glucose and mixed meal responses among persons with NGM, IH and type 2 diabetes. As such, the study is more comprehensive than existing studies. A limitation is the cross-sectional nature of the study, not enabling us to study incretin responses along the natural history of type 2 diabetes development. Another limitation is that the subgroup of 20 persons with IH consisted of persons with impaired fasting glucose and/or impaired glucose tolerance that may(32) or may not (33) differ from each other in incretin secretion patterns. Because of limited numbers, we were unable to separate these subgroups in the analysis. However, the majority of persons in this subgroup had impaired fasting glucose, and therefore results can be interpreted as valid for this subgroup.

Glucose tolerance state and other determinants of incretin and glucagon responses

Although the reduced incretin effect among diabetic patients has been well characterized for some time (1), a potential defect in incretin secretion may only be present in patients with long-standing diabetes (5, 6). This study demonstrates higher GLP-1 response (tAUC) after oral glucose among patients with diabetes when compared with persons with NGM, while no altered GLP-1 response after mixed meal is observed. This is in

Figure 1 Glucose, insulin and triglyceride responses (mean±S.E.M.) to oral glucose tolerance test (OGTT) (A, B, and C) and mixed meal test (MMT) (D, E, and F) among 163 persons with normal glucose metabolism (NGM) (black circles), 20 with intermediate hyperglycaemia (IH) (open circles) and 20 with type 2 diabetes (T2DM) (black triangles).
indicating a significant different change from t0 among T2DM compared with NGM. Significant interaction between time and IH in linear mixed models, indicating a significant different change from t0 among IH compared with NGM.

Figure 2 GLP-1, GIP and glucagon responses (mean ± s.e.m.) to oral glucose tolerance test (OGTT) (A, B, and C) and mixed meal test (MMT) (D, E, and F) among 163 persons with normal glucose metabolism (NGM) (black circles), 20 with intermediate hyperglycaemia (IH) (open circles) and 20 with type 2 diabetes (T2DM) (black triangles). *Significant interaction between time and T2DM in linear mixed models, indicating a significant different change from t0 among T2DM compared with NGM. **Significant interaction between time and IH in linear mixed models, indicating a significant different change from t0 among IH compared with NGM.

line with earlier meta-analyses showing that diabetic state impacts differentially on GLP-1 response to oral glucose and mixed meal (5, 6). While the GLP-1 response after oral glucose (tAUC) was increased in diabetic patients, we observed a preserved total GLP-1 response (tAUC) but an almost 50% diminished iAUC after the mixed meal among diabetic patients. This implies that if a diabetes-related defect in GLP-1 secretion exists, it would be a defect in response to nutrients other than glucose, which has been suggested earlier (34). More complex carbohydrates may play a role here (35). Alternatively, fat is known as a potent stimulus for GLP-1 secretion (36, 37) but whether GLP-1 response to fat is different among diabetic patients would be a topic for further study. Another explanation for the higher GLP-1 response after oral glucose vs the solid mixed meal would be an accelerated gastric emptying that has been described for early diabetic patients during a liquid meal (38, 39).

In line with earlier studies, GLP-1 response among persons with IH was unaltered (7, 8, 10, 40, 41). This suggests that a defect in GLP-1 secretion is unlikely to precede type 2 diabetes development, which is consistent with earlier findings among first-degree diabetes relatives, and in induced insulin resistance among healthy subjects (27, 42).

Next to diabetes, several other factors have been described to influence GLP-1 secretion, i.e. obesity, diabetes duration, rate of gastric emptying, gall bladder kinetics and metformin use (43). We were unable to address them all, but we found no association with obesity, age, gender, OGIS or glucagon response. In addition, BMI-stratified analyses clearly showed no effect of BMI on incretin responses in the present population.

The increased GIP response among persons with diabetes and IH observed after both oral glucose and mixed meal adds to the existing literature. Earlier studies among diabetic patients mostly report no change in GIP response after a meal or oral glucose (10, 11, 12, 13), a higher response (7, 9, 41) or a slightly lower response (8, 44). Similarly, GIP response among persons with IH has been reported unaltered (8, 41), decreased (40) or increased (7). Obesity has been reported to be associated with higher GIP responses (43), but we did not find a relationship between BMI and GIP meal response. Instead, OGIS was related to lower GIP response. We may speculate that GIP secretion is increased as a compensation for the well-known reduced GIP effect in diabetes (3). Alternatively, the relatively well-controlled diabetes and short diabetes duration of these patients may explain the high GIP response upon oral glucose and mixed meal.

The observation that glucagon was suppressed after oral glucose but increased following mixed meal may relate to the incretin patterns associated with these
GLP-1 is known to suppress, while GIP and also GLP-2 stimulate glucagon secretion (45). The lower GLP-1 and the higher GIP response after the mixed meal when compared with oral glucose may explain the relatively higher glucagon secretion.

The cause of increased fasting GLP-1 and GIP levels in type 2 diabetic patients is unknown so far. Elevated fasting levels of GLP-1 as found among type 2 diabetic patients in this study and in an earlier study may indicate chronic elevated GLP-1 (8). In isolated perfused porcine ileum, a feed-forward mechanism was described where high plasma glucose enhanced fasting GLP-1 (46). We suggest that higher fasting incretin levels may reflect a compensatory mechanism to overcome the loss in islet response, which has been described as an early sign of development of hyperglycaemia (43). Indeed, fasting GLP-1 was already increased among persons with IH (this study) and may therefore develop gradually along the line of hyperglycaemia.

The finding that fasting GIP was related to fasting and postprandial triglycerides and ALT is novel. Fasting GIP was reported to increase upon high-fat feeding for 5 days in healthy individuals and was as such suggested to mediate the increase in insulin secretion that compensated for the increase in hepatic insulin resistance (47). Levels of ALT were not measured in that study, but along with the increase in GIP, an increase in aspartate aminotransferase, another marker of liver fat accumulation, was reported (47). In animal models, long-term administration of a GIP receptor antagonist decreased

**Fasting incretins in relation to triglycerides and ALT**

![Figure 3](image1.png)

**Figure 3** Total area under the curve (tAUC) of GLP-1 (A), GIP (B) and glucagon (C) (mean ± s.e.m.) following oral glucose tolerance test (OGTT) and mixed meal test (MMT) calculated over the first 2 h of the test in 163 persons with normal glucose metabolism (NGM), 20 with IH and 20 with type 2 diabetes. *Significant difference by ANOVA against NGM. #Significant difference by ANOVA against IH.

tests. GLP-1 is known to suppress, while GIP and also GLP-2 stimulate glucagon secretion (45). The lower GLP-1 and the higher GIP response after the mixed meal when compared with oral glucose may explain the relatively higher glucagon secretion.

![Figure 4](image2.png)

**Figure 4** Total AUC of GLP-1 (A) and GIP (B) following oral glucose tolerance test (OGTT) and mixed meal test (MMT) stratified by BMI and type 2 diabetes. *Significant difference by ANOVA.
adipose tissue mass and triglyceride deposition in liver and muscle (23). So the notion of GIP adversely related to lipid metabolism and fatty liver is in line with earlier findings, but the mechanisms underlying increased fasting GIP levels warrant further study.

**Incretin responses in relation to triglycerides and ALT**

The oral glucose responses of GLP-1 and GIP showed opposite directions of association with triglycerides. The inverse relationship between GLP-1 response (tAUC) after oral glucose load and fasting triglycerides is consistent with clinical study results among patients with type 2 diabetes, showing that treatment with a GLP-1 analogue or GLP-1 agonist improves blood lipid profile (19, 20). The positive relationship between tAUC GIP after oral glucose and (postprandial) triglycerides adds to findings from animal studies that indicate an adverse role of GIP in lipid metabolism (48).

**Implications of the study**

As the MMT reflects habitual hormone patterns more closely than does an oral glucose load, the GLP-1 response upon solid mixed meal in diabetic patients and

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Univariate associations with GLP-1, GIP and glucagon responses to the meal (tAUC) by univariate regression analysis among the total study population (n=203).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression coefficient (95% CI)</td>
<td>GLP-1-tAUC</td>
</tr>
<tr>
<td>Age (years)</td>
<td>−0.007 (−0.030 to 0.015)</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>0.239 (0.057 to 0.534)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>−0.010 (−0.042 to 0.022)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>−0.005 (−0.016 to 0.006)</td>
</tr>
<tr>
<td>Glucose tolerance state</td>
<td></td>
</tr>
<tr>
<td>IH</td>
<td>0.030 (−0.152 to 0.811)</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>0.248 (−0.245 to 0.741)</td>
</tr>
<tr>
<td>OGIS (ml/min per m²)</td>
<td>0.001 (−0.001 to 0.003)</td>
</tr>
<tr>
<td>Glucagon tAUC per hour (pmol/l)</td>
<td>−0.036 (−0.186 to 0.113)</td>
</tr>
</tbody>
</table>

OGIS, oral glucose insulin sensitivity index; tAUC, total area under the curve; IH, intermediate hyperglycaemia. Regression coefficient indicates increase in SD tAUC per hour per unit as indicated (years for age, etc.); *P<0.05.

Table 3 | Linear regression analyses of incretins with fasting triglycerides, triglyceride responses and ALT (n=182). |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Standardized regression coefficient (95% CI)</td>
<td>Fasting triglycerides</td>
</tr>
<tr>
<td>Fasting GLP-1</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.02 (−0.13 to 0.17)</td>
</tr>
<tr>
<td>Model 2</td>
<td>−0.11 (−0.26 to 0.05)</td>
</tr>
<tr>
<td>Model 3</td>
<td>−0.09 (−0.24 to 0.06)</td>
</tr>
<tr>
<td>Fasting GIP</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.17 (0.03 to 0.31)*</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.11 (−0.04 to 0.25)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.13 (0.00 to 0.27)*</td>
</tr>
<tr>
<td>Post oral glucose GLP-1-tAUC</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>−0.08 (−0.23 to 0.07)</td>
</tr>
<tr>
<td>Model 2</td>
<td>−0.15 (−0.31 to 0.00)*</td>
</tr>
<tr>
<td>Model 3</td>
<td>−0.11 (−0.25 to 0.03)</td>
</tr>
<tr>
<td>Post oral glucose GIP-tAUC</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.20 (0.05 to 0.36)*</td>
</tr>
<tr>
<td>Model 2</td>
<td>0.14 (−0.02 to 0.30)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.15 (0.004 to 0.30)*</td>
</tr>
<tr>
<td>Post mixed meal GLP-1-tAUC</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>−0.04 (−0.20 to 0.12)</td>
</tr>
<tr>
<td>Model 2</td>
<td>−0.08 (−0.24 to 0.07)</td>
</tr>
<tr>
<td>Model 3</td>
<td>−0.02 (−0.17 to 0.12)</td>
</tr>
<tr>
<td>Post mixed meal GIP-tAUC</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>0.05 (−0.12 to 0.22)</td>
</tr>
<tr>
<td>Model 2</td>
<td>−0.04 (−0.22 to 0.13)</td>
</tr>
<tr>
<td>Model 3</td>
<td>0.01 (−0.16 to 0.17)</td>
</tr>
</tbody>
</table>

ALT, alanine aminotransferase; tAUC, total area under the curve; OGIS, oral glucose insulin sensitivity index; triglycerides-tAUC, tAUC following mixed meal test (for fasting and post mixed meal incretins) or oral glucose (for post oral glucose incretins). *P<0.05. Model 1, including age and sex; Model 2, including age, sex and glucose tolerance state; Model 3, including age, sex, glucose tolerance state, OGIS and glucagon (fasting, post oral glucose and post mixed meal glucagon for respective GLP-1 and GIP values).
especially linked the potential relevance of a diminished IAUC warrants further attention in future studies. Further, the present data indicate that the consequences of altered GLP-1 and GIP secretion may be more comprehensive than yet understood, including effects on lipid metabolism and liver fat.

Conclusions

This study confirms earlier findings of a generally preserved GLP-1 secretion in type 2 diabetes and shows exaggerated GIP secretion in this population. The associations of GLP-1 and GIP secretion with (postprandial) triglycerides and ALT suggest that these hormones may reflect risk for dyslipidemia and liver fat accumulation in an opposite way.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-13-0487.

Declaration of interest

J M Rijkelijkhuizen, J J Holst, T. Teerlink, P G Scheffer, E M W Eekhoff, A Gastaldelli, A Mari, L M ’t Hart and G Nijpels have nothing to declare. M Alsem is currently employed by Unilever Research and Development. J M Dekker received an unrestricted investigator-initiated grant of Merck & Co., Inc.

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