Reduction of hepatic insulin clearance after oral glucose ingestion is not mediated by glucagon-like peptide 1 or gastric inhibitory polypeptide in humans

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Meier JJ, Holst JJ, Schmidt WE, Nauck MA. Reduction of hepatic insulin clearance after oral glucose ingestion is not mediated by glucagon-like peptide 1 or gastric inhibitory polypeptide in humans. Am J Physiol Endocrinol Metab 293: E849–E856, 2007. First published July 3, 2007; doi:10.1152/ajpendo.00289.2007.—Changes in hepatic insulin clearance can occur after oral glucose or meal ingestion. This has been attributed to the secretion of incretin hormones, such clearance effects may be important for the future treatment of type 2 diabetes. Therefore, we determined insulin clearance in response to endogenously secreted and exogenously administered GIP and GLP-1. Insulin clearance was estimated from the molar C-peptide-to-insulin ratio calculated at basal conditions and from the respective areas under the curve after glucose, GIP, or GLP-1 administration. Oral glucose administration led to an ~60% reduction in the C-peptide-to-insulin ratio (P < 0.0001), whereas intravenous glucose administration had no effect (P = 0.09). The endogenous secretion of GIP or GLP-1 was unrelated to the changes in insulin clearance. The C-peptide-to-insulin ratio was unchanged after the intravenous administration of GIP or GLP-1 in the fasting state (P = 0.27 and P = 0.35, respectively). Likewise, infusing GLP-1 during a meal course did not alter insulin clearance (P = 0.87). An inverse nonlinear relationship was found between the C-peptide-to-insulin ratio and the integrated insulin levels after oral and during intravenous glucose administration. Insulin clearance is reduced by oral but not by intravenous glucose administration. Neither GIP nor GLP-1 has significant effects on insulin extraction. An inverse relationship between insulin concentrations and insulin clearance suggests that the secretion of insulin itself determines the rate of hepatic insulin clearance.

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obtained from all participants. Parts of these studies related to the effects of GIP and GLP-1 on insulin secretion have been published previously (22, 25, 28). In the present study, the data were reanalyzed to examine the changes in insulin clearance under these conditions.

Study design. To estimate the rate of hepatic insulin clearance, molar ratios of C-peptide and insulin were calculated from fasting plasma concentrations and from the total area under the concentration curves determined under the following conditions: 1) under basal fasting conditions, over 240 min following oral glucose ingestion (75 g), and over 240 min during an “isoglycemic” intravenous infusion of glucose (28); 2) under basal fasting conditions and over 180 min during and after the intravenous infusion of GIP (1 pmol·kg⁻¹·min⁻¹) and GLP-1 (0.5 pmol·kg⁻¹·min⁻¹), each administered over 30 min on separate occasions (25); and 3) Under basal fasting conditions and during the intravenous infusion of GLP-1 (1.2 pmol·kg⁻¹·min⁻¹) or placebo, each administered over a period of 390 min during the ingestion of a mixed test meal (250 kcal; 44% carbohydrates, 19% fat, 37% protein) at time (t) = 30 min (22).

Subjects. Details of the subject characteristics have been described elsewhere (22, 25, 28). Briefly, the following subjects were included in the studies: 1) 16 nonobese first-degree relatives of patients with type 2 diabetes (4 male, 12 female, age 50 ± 12 yr, body mass index (BMI) 26.1 ± 3.8 kg/m², fasting glucose concentrations: 95 ± 2 mg/dl) and 10 healthy controls (negative family history, 6 male, 4 female, age 45 ± 13 yr, 26.1 ± 4.2 kg/m², 99 ± 3 mg/dl). Detailed characteristics have been described previously (28); 2) 10 healthy nonobese subjects (4 female, 6 male, age: 44 ± 12 yr, BMI: 24.9 ± 3.4 kg/m², fasting glucose concentrations: 102 ± 10 mg/dl). Detailed characteristics have been described previously (25), and 3) 14 healthy male volunteers (24.2 ± 2.0 yr, BMI 24.7 ± 2.2 kg/m², fasting glucose concentrations: 94 ± 7 mg/dl). Detailed characteristics have been described previously (22).

Experimental procedures. The tests were performed in the morning after an overnight fast. A Teflon canula (Moskito 123, 18 gauge; Vygon, Aachen, Germany) was inserted in one or two forearms veins and kept patent using 0.9% NaCl (for blood sampling and for peptide/placebo administration, respectively). The experiments were started after withdrawal of two basal blood samples each. For the meal study, a standard test meal (one egg, two slices of white bread, 5 go of margarine, 150 ml of water; 250 kcal) was served 30 min after starting the infusion of GLP-1 or placebo. Details of the study procedures have been described elsewhere (22, 25, 28).

Peptides. Synthetic GIP was purchased from PolyPeptide Laboratories (Wolfenbüttel, Germany) and processed for intravenous infusions as described (25). Synthetic GLP-1 was a kind gift from Restogen and processed for intravenous infusions as described (22).

Blood specimens. Venous blood was drawn into chilled tubes containing EDTA and aprotinin (Trasylol, 20,000 KIU/ml, 200 μl/kg/ml blood; Bayer, Leverkusen, Germany) and kept on ice. After centrifugation at 4°C, plasma for hormone analyses was kept frozen at −28°C. This procedure has previously been shown to effectively prevent DPP-4 degradation (4).

Measurements. Glucose was measured as described (23) using a glucose oxidase method with a Glucose Analyzer 2 (Beckman Instruments, Munich, Germany).

Insulin was measured using an insulin microparticle enzyme immunoassay (IMx Insulin; Abbott Laboratories, Wiesbaden, Germany).

C-peptide was measured using an enzyme-linked immunoabsorbent assay (ELISA) from DAKOP (Cambridgshire, UK). Intra-assay coefficient of variation was 3.5–5.7%, and interassay variation was 4.6–5.7%. Human insulin and C-peptide were used as standards.

GIP immunoreactivity was determined using two different assays specific for either the COOH terminus or the NH2 terminus of the peptide (5). The COOH-terminal assay using antiserum R65 fully reacts with intact GIP-(1–42) and the truncated metabolite (3–42), but not with the so-called 8-kDa GIP, of which the chemical nature and relation to GIP secretion is uncertain. The assay has a detection limit of <2 pmol/l and an intra-assay variation of ~6%. The NH2-terminal assay measures the concentration of intact GIP-(1–42), using antiseraum 98171. The cross-reactivity with GIP-(3–42) was <0.1%. The lower detection limit of the assay is ~5 pmol/l. Intra-assay variation was <6%, and interassay variations were ~8 and 12% for 20 and 80 pmol/l standards, respectively. For both assays, human GIP (Peninsula Laboratories) was used as standard, and radiolabeled GIP was obtained from Amersham Pharmacia Biotech (Aylesbury, UK).

GLP-1 immunoreactivity was determined using two different assays. The COOH-terminal assay measures the sum of the intact peptide plus the primary metabolite GLP-1-(9–36) amide using the antisera 89390 and synthetic GLP-1-(7–36) amide as standard. This assay cross-reacts <0.01% with COOH terminally truncated fragments and 83% with GLP-1-(9–36) amide. The detection limit was 3 pmol/l. Intra-assay and interassay coefficients of variation were <6 and 15%, respectively, at 40 pmol/l. Intact GLP-1 was measured using untruncated GLP-1 using an ELISA (6). The assay is a two-site sandwich assay using two monoclonal antibodies, GLP-1F5 as catching antibody (COOH terminally directed) and Mah26.1 as detecting antibody (NH2 terminally directed). It reacts <0.1% with GLP-1 precursors extended from the NH2 terminus and NH2 terminally truncated peptides, including GLP-1-(9–36) amide. The detection limit was 0.5 pmol/l, and intra- and interassay variations at 16 pmol/l were <5 and <10%, respectively.

Calculations and statistics. Results are reported as mean ± SE. For estimating insulin clearance, the molar ratio of C-peptide over insulin was calculated from the mean plasma levels determined in the basal state after an overnight fast and from the total areas under the respective concentration curves (baseline not subtracted). The following assumptions underlying these calculations: 1) the C-peptide clearance by the liver is negligible (33). 2) The renal clearance of C-peptide is constant after oral glucose administration and during the intravenous infusion of glucose, GIP, and GLP-1. Although the former assumption has been validated in previous experiments (33), changes in the renal clearance of C-peptide under the influence of incretin hormones cannot be excluded with certainty. However, based on the studies available so far, there is no reason to expect such effects.

Insulin resistance was calculated using the homeostasis model assessment (HOMA insulin resistance score), as described previously (19).

All statistical calculations were carried out using paired ANOVA or Student’s t-test, as appropriate, using Statistica version 5.0 (Statsoft Europe, Hamburg, Germany). A two-sided P value <0.05 was taken to indicate significant differences. Nonlinear regression analyses were carried out using the equation: y = A × x/(B + x) + C × x/(D + x), using Graph Pad Prism, version 3 (San Diego, CA).

RESULTS

Oral and isoglycemic intravenous glucose administration. Following oral glucose ingestion, plasma concentrations of glucose, insulin, and C-peptide rose significantly (P < 0.001) and returned to baseline levels within the 240-min study period (28). GIP plasma levels increased from baseline levels of 11 ± 2 pmol/l to peak levels of 69 ± 5 pmol/l after 60 min (P < 0.001), and total GLP-1 concentrations increased from 13 ± 1 to 29 ± 2 pmol/l (P < 0.001; Fig. 1A). The molar C-peptide-to-insulin ratio was reduced significantly from 15.6 ± 1.7 in the basal state (~15 to 30 min) to 6.0 ± 0.5 following glucose ingestion [area under the curve at 2–240 min (AUC₀–240 min); P < 0.001; Fig. 1E].

The pattern of plasma glucose concentrations determined after oral glucose ingestion was exactly copied by the respective isoglycemic intravenous glucose infusion (P = 0.99; see Ref. 28). Insulin and C-peptide concentrations increased during
intravenous glucose administration ($P < 0.001$), but to a lesser extent than after oral glucose ingestion ($P < 0.001$; Fig. 1D). In contrast to the experiments with oral glucose administration, the C-peptide-to-insulin ratio was similar in the basal state ($-15$ to $0$ min) and during intravenous glucose infusion ($AUC_{0-240}$ min; $P = 0.09$; Fig. 1F). The C-peptide-to-insulin ratio was significantly lower after oral than during intravenous glucose administration ($P < 0.001$).

The C-peptide-to-insulin ratio was similar in first-degree relatives of patients with type 2 diabetes and healthy controls in the euglycemic fasting state ($16.1 \pm 2.3$ vs. $14.9 \pm 2.3$, respectively; $P = 0.74$), as well as after oral ($6.6 \pm 0.7$ vs. $5.2 \pm 0.6$; $P = 0.15$) and during intravenous glucose administration ($15.1 \pm 1.9$ vs. $13.5 \pm 1.4$; $P = 0.57$).

The secretion of GIP and GLP-1, as judged from the integrated plasma levels, was not related to the C-peptide-to-insulin ratio after glucose ingestion ($r^2 = 0.11, P = 0.10$ for GIP, $r^2 = 0.083, P = 0.15$ for GLP-1; details not shown). Likewise, fasting GIP and GLP-1 levels were unrelated to insulin clearance ($r^2 = 0.001, P = 0.87$ for GIP, $r^2 = 0.05, P = 0.27$ for GLP-1; details not shown). However, when the estimated insulin clearance was expressed in relation to the respective plasma insulin concentrations, an inverse nonlinear relationship was apparent both in the fasting state as well as after oral and during intravenous glucose administration (Fig. 2, A and B). These analyses imply decreasing rates of hepatic insulin clearance in response to high insulin concentrations. A similar relationship was found between the basal C-peptide-to-insulin ratio and the HOMA insulin resistance (Fig. 2C).

**Intravenous administration of GIP and GLP-1 in the euglycemic fasting state.** During the exogenous infusion of GIP in the fasting state, peak GIP plasma concentrations of $86 \pm 12$ pmol/l (total GIP) and $31 \pm 4$ pmol/l (intact GIP) were reached (Fig. 3A). Insulin and C-peptide concentrations increased by $\sim 40\%$ during GIP administration (25 and Fig. 3B). The C-peptide-to-insulin ratio was $14.5 \pm 3.4$ in the basal state and $15.7 \pm 3.7$ following GIP administration ($P = 0.27$; Fig. 3C).

Infusing GLP-1 raised plasma concentrations to peak levels of $114 \pm 12$ pmol/l (total GLP-1) and $34 \pm 5$ pmol/l (intact GLP-1; Fig. 4A). Insulin and C-peptide levels were increased by approximately twofold during GLP-1 infusion ($P < 0.001$; Fig. 4B). The C-peptide-to-insulin ratio was similar in the basal state and following GLP-1 infusion ($P = 0.35$; Fig. 4C).

**Intravenous administration of GLP-1 and placebo during a meal course.** During the exogenous infusion of GLP-1 over a meal course, steady-state plasma concentrations of $\sim 150$ pmol/l (total GLP-1) and $\sim 25$ pmol/l (intact GLP-1) were reached, whereas meal ingestion only marginally raised endogenous GLP-1 levels (Fig. 5, A and B; see Ref. 22). Insulin and C-peptide levels rose significantly after the test meal in the placebo experiments ($P < 0.001$; Fig. 5C). In contrast, during GLP-1 administration, insulin and C-peptide concentrations increased during GLP-1 infusion before meal ingestion, but were even lowered after the meal, most likely as a consequence of delayed gastric emptying ($P < 0.05$; Fig. 5D). Meal ingestion did not lead to a

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**Fig. 1.** Plasma concentrations of total gastric inhibitory polypeptide (GIP; A and B; left y-axes; filled circles) and glucagon-like peptide 1 (GLP-1; A and B; right y-axes; ◊) as well as insulin (C and D; left y-axes; ●) and C-peptide (C and D; right y-axes; □) following oral ingestion of 75 g glucose (A and C) and during isoglycemic intravenous glucose infusion (B and D) in 26 nondiabetic subjects (16 first-degree relatives of patients with type 2 diabetes and 10 controls). E and F: molar C-peptide-to-insulin ratios calculated from the respective plasma concentrations in the euglycemic fasting state and from the areas under the curves [AUC; time $(t) = 0$ to $240$ min]. $P$ values were determined by paired Student’s $t$-test.
significant reduction in the C-peptide-to-insulin ratio either during GLP-1 or placebo administration (Fig. 5, E and F). Likewise, there was no difference in insulin clearance between the experiments with the administration of GLP-1 and placebo (P = 0.87).

DISCUSSION

The introduction of the first generation of GLP-1 analogs and DPP-4 inhibitors has broadened the therapeutic options in the management of type 2 diabetes (7, 24). In particular, their glucose-dependent insulinotropic action has made the incretin hormones an attractive target for the development of novel antidiabetic drugs. However, although most studies so far have focused on the effect of GLP-1 and GIP on β-cell mass and function (9, 16, 30), little is known about their potential actions on hepatic insulin clearance. Because the rate of hepatic insulin extraction largely determines systemic insulin levels (8, 27, 34), we addressed the potential role of GIP and GLP-1 in this process.

To examine the effects of endogenously secreted incretin hormones on insulin clearance, we first compared the molar C-peptide-to-insulin ratios after oral glucose ingestion and during isoglycemic intravenous glucose infusion. Consistent with prior reports (18, 29, 40), we found a significant reduction in insulin clearance following oral, but not during intravenous, glucose administration. However, these changes were unrelated to the endogenous secretion of incretin hormones, thereby arguing against a role of GIP and GLP-1 in this process.

Fig. 2. Relationship between the fasting insulin levels and the basal C-peptide-to-insulin ratio (A) and (B) relationship between the integrated insulin plasma concentrations following oral glucose ingestion or during iv glucose administration and the C-peptide-to-insulin ratio calculated from the areas under the concentration curves in 26 nondiabetic subjects (16 first-degree relatives of patients with type 2 diabetes and 10 controls) studied at two separate occasions [oral glucose tolerance test (1); iv glucose infusion (•); B]. AUCO₋240 min, area under the curve from 0 to 240 min; Insulin0₋240 min, insulin infusion over 0 to 240 min. C: relationship between the basal C-peptide-to-insulin ratio and the homeostasis model assessment (HOMA) insulin resistance determined in 16 first-degree relatives of patients with type 2 diabetes (●) and 10 controls ( ). r² Values were calculated using nonlinear regression analysis using the following equation: \( y = A \times x^B + C \times x^D \times x \), where A, B, C, and D are estimated constants.

Fig. 3. Plasma concentrations of total (●) and intact (•) GIP (A) as well as of insulin (B; left y-axis; ●) and C-peptide (B; right y-axis; •) during iv administration of GIP (1 pmol·kg⁻¹·min⁻¹ from 0–30 min) in 10 healthy nondiabetic subjects. C: molar C-peptide-to-insulin ratios calculated from the respective plasma concentrations in the euglycemic fasting state and from the areas under the curves (t = 0–180 min). The P value was determined by paired Student's t-test.
We next examined the effects of exogenous GIP and GLP-1 on insulin extraction. Because GLP-1 analogs have just become available for the treatment of type 2 diabetes, pharmacological doses of GLP-1 were chosen, whereas for GIP a rather physiological dose was administered. However, the C-peptide-to-insulin ratio was not altered by the intravenous administration of either GIP or GLP-1 at fasting conditions (Figs. 3 and 4). In addition, when insulin clearance was studied over a meal course, no differences were found between the experiments with GLP-1 and placebo administration (Fig. 5).

The present findings are at variance with some (1, 12, 15, 36), but not all (2, 13, 20), previous reports suggesting a reduction in hepatic insulin clearance under the influence of GIP and GLP-1. It is therefore important to bear in mind the methodological differences between these studies. In fact, direct assessment of hepatic insulin extraction would require concomitant catheterization of the portal vein, the hepatic artery, and the hepatic vein and is therefore not feasible in humans. Therefore, indirect approaches are warranted to estimate insulin clearance. Because C-peptide is cosecreted along with insulin at equimolar amounts, but, unlike insulin, passes the liver without considerable extraction (8, 34), some investigators have drawn conclusions on insulin clearance from changes in the molar C-peptide-to-insulin ratios determined at single or multiple time points (15, 36). However, because the plasma half-life of C-peptide exceeds that of insulin by ~10-fold, (34) this approach bears the risk of underestimating insulin clearance, particularly in situations of increased insulin secretion or during non-steady-state clamp conditions (36). Thus any stimulation of insulin secretion during non-steady-state conditions will necessarily lead to disproportionate rises in insulin and C-peptide levels independent of any changes in insulin clearance. To take into account the different in vivo kinetics of insulin and C-peptide, Polonsky and Rubenstein (34) suggested to estimate hepatic insulin clearance based on the C-peptide-to-insulin ratio from the areas under the respective plasma concentration curves. It is important to emphasize that this ratio will only give an accurate estimate of hepatic insulin clearance if baseline values of both insulin and C-peptide are reached after a period of stimulated secretion. Consistent with these theoretical considerations, we observed disproportionate increments in insulin concentrations during the initial rise in GIP- or GLP-1- induced insulin secretion, whereas calculation of the C-peptide-to-insulin ratios from the areas under the respective concentration curves revealed no changes in overall insulin clearance. Furthermore, such estimation of insulin clearance implies that the elimination rates of C-peptide are constant throughout the experiments.

The absence of GIP and GLP-1 effects on hepatic insulin clearance observed in this study is also consistent with the lack of GIP or GLP-1 receptor expression on hepatocytes (3, 43). It has previously been suggested that reduced hepatic insulin clearance compensates for abnormal insulin secretion in first-degree relatives of patients with type 2 diabetes (14, 36). In contrast, no differences in insulin clearance were found between first-degree relatives and control subjects in the present as well as in other previous studies (20, 31). Most likely, these discrepancies are the result of the different subject characteristics in the studies. In fact, reduced rates of insulin clearance were found when more insulin-resistant cohorts of first-degree relatives were studied (14, 36), whereas insulin clearance was

Rather, the variations in insulin clearance could be attributed to the respective amounts of insulin secreted and the degree of insulin resistance, as estimated by HOMA analysis (Fig. 2). Such inverse relationship between overall insulin secretion and insulin clearance is consistent with previous studies (8, 10, 27, 32, 41, 42) and explains the absence of a reduction in insulin clearance after intravenous glucose administration, since, under these conditions, insulin secretion was stimulated to a much lesser extent than after oral glucose ingestion. Most likely this reduction of insulin clearance upon increasing levels of insulin secretion is explained by the finite number of insulin receptors on hepatocytes (17), which leads to saturation of receptor-mediated endocytosis. Such reasoning is also consistent with recent studies showing that hepatic insulin clearance changes minute by minute as a function of the fluctuations in portal venous insulin concentrations (27). Thus the hepatic uptake of insulin seems to vary as a function of the amount of insulin to which the liver is exposed, whereas the importance of exogenously secreted GIP and GLP-1 for the regulation of hepatic insulin clearance appears to be negligible.

Fig. 4. Plasma concentrations of total (●) and intact (○) GLP-1 (A) as well as of insulin (B; left y-axis; ●) and C-peptide (B; right y-axis; ○) during iv administration of GLP-1 (0.5 pmol·kg \(^{-1}\)·min \(^{-1}\) from 0 to 30 min) in 10 healthy nondiabetic subjects. C: molar C-peptide-to-insulin ratios calculated from the respective plasma concentrations in the euglycemic fasting state and from the areas under the curves \((t = 0–180\) min). The \(p\) value was determined by paired Student’s \(t\)-test.

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almost normal in first-degree relatives with no obvious impairment in insulin sensitivity (20, 31). Given the close relationship between insulin resistance and insulin clearance (Fig. 2), reduced insulin clearance rates in some first-degree relatives of patients with type 2 diabetes may develop as a consequence of hyperinsulinemia.

Even though in the present experiments with infusion periods between 30 and 360 min, GIP and GLP-1 had no effects on insulin clearance, it is theoretically possible that prolonged elevations in incretin levels would have been more effective in this regard. Against this, previous studies have shown that changes in insulin clearance occur within minutes, thereby allowing for rapid responses to changing metabolic demands (27). It is therefore unlikely that longer exposure times of GIP and GLP-1 would have had elicited a more pronounced effect on insulin clearance. Moreover, given the glucose dependency of the insulinoctropic actions of GIP and GLP-1, one might argue that any clearance effects might be present at hyperglycemia only, too. However, because the glucose dependency seems to be restricted to the effects at the islet level, but does not affect the other actions of GIP and GLP-1, e.g., on gastric emptying and energy homeostasis (11, 21), one would not expect any differences in the hepatocyte response to incretin hormones at various glucose levels. Furthermore, in previous studies, we did not observe a reduction of insulin clearance by GIP at hyperglycemia either (20).

In conclusion, insulin clearance is reduced after oral glucose ingestion but not during isoglycemic intravenous glucose infusion. Neither the endogenous secretion nor exogenous administration of GIP and GLP-1 has a significant influence on insulin clearance. An inverse relationship between insulin plasma concentrations and insulin clearance suggests that the secretion of insulin itself determines the rate of hepatic insulin clearance, probably through saturation of receptor-mediated endocytosis. Therefore, changes in insulin clearance are unlikely to contribute to the antidiabetic effects of GLP-1 and GIP.
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