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Degradation of Endogenous and Exogenous Gastric Inhibitory Polypeptide in Healthy and in Type 2 Diabetic Subjects as Revealed Using a New Assay for the Intact Peptide*

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ABSTRACT

Gastric inhibitory polypeptide (GIP) is susceptible to degradation, but only recently has dipeptidyl peptidase IV been identified as the enzyme responsible. Most RIAs recognize both intact GIP-(1–42) and the noninsulinotropic N-terminally truncated metabolite, GIP-(3–42), hampering measurement of plasma concentrations. The molecular nature of GIP was examined using high pressure liquid chromatography and a newly developed RIA specific for the intact N-terminus of human GIP. In healthy subjects after a mixed meal, intact GIP (N-terminal RIA) accounted for 37.0 ± 2.5% of the total immunoreactivity determined by C-terminal assay. High pressure liquid chromatographic analysis of fasting samples by C-terminal assay revealed one major peak (73.8 ± 2.9%) coeluting with GIP-(3–42). One hour postprandially, two major peaks were detected, corresponding to GIP-(3–42) and GIP-(1–42) (58.1 ± 2.7% and 35.7 ± 4.2%, respectively). GIP-(3–42) was not detected by N-terminal assay; the major peak coeluted with intact GIP (86.4 ± 5.8% and 81.3 ± 0.9%, 0 and 1 h, respectively). After iv infusion, intact GIP constituted 37.1 ± 4.1% and 41.3 ± 3.4% of the total immunoreactivity in healthy and type 2 diabetic subjects, respectively. The plasma $t_{1/2}$ was shorter ($P < 0.0001$) when determined by N-terminal compared with C-terminal assay (7.3 ± 1.0 *vs.* 16.8 ± 1.6 and 5.2 ± 0.6 *vs.* 12.9 ± 0.9 min, healthy and diabetic subjects, respectively), and both $t_{1/2}$ were shorter in the diabetic group ($P < 0.05$). We conclude that dipeptidyl peptidase IV is important in GIP metabolism in humans *in vivo*, and that an N-terminally directed assay is required for determination of plasma concentrations of biologically active GIP. (*J Clin Endocrinol Metab* **85:** 3575–3581, 2000)

GASTRIC INHIBITORY polypeptide (GIP; also referred to as glucose-dependent insulinotropic peptide) is secreted from K cells in the upper small intestine in response to the presence of nutrients, especially fats (1). Like the structurally related peptide, glucagon-like peptide-1 (GLP-1), which is released from the intestinal L cell, GIP is considered to be an incretin hormone (2–4).

It has recently been shown that GLP-1 is rapidly degraded *in vivo* by the ubiquitous enzyme dipeptidyl peptidase IV (DPP IV) (5, 6), forming a metabolite, GLP-1-(9–36)amide, that is truncated by two amino acids from the N-terminus. This metabolite can still bind to the GLP-1 receptor, but lacks efficacy because of loss of the N-terminal histidine residue (7, 8). The development of specific analytical techniques capable of distinguishing between intact GLP-1 and the metabolite has been crucial to revealing the key role of DPP IV in determining the biological activity of GLP-1 *in vivo* (5).

Unlike GLP-1, it has been known for a number of years that GIP is susceptible to enzymatic degradation. The fragment GIP-(3–42) was first identified as a minor component in a preparation of GIP and was reported to have insignificant insulinotropic activity (9). It was suggested that the metabolite was formed by secondary processing by dipeptidyl peptidases or related enzymes in the intestine (10). However, it was not until more recently that DPP IV was specifically identified as the enzyme responsible for GIP degradation in human serum *in vitro* (11, 12). Studies in rats (6, 13) have indicated that DPP IV also cleaves GIP *in vivo*.

To date, it has been difficult to fully assess the role of DPP IV in the metabolism of GIP in humans. This is largely due to the lack of suitable methodology. All existing assays for GIP are centrally or C-terminally directed and, as such, are incapable of distinguishing between the intact biologically active peptide and the truncated metabolite. Because of this, the results of earlier studies, which rely on these assays, should be interpreted with caution with regard to the biological activity and metabolism of the hormone. Both the intact peptide and the metabolite have similar chromatographic characteristics, but high pressure liquid chromatography (HPLC) methods can be developed that will separate them. However, the relative insensitivity and labor intensity of this approach preclude the routine determination of the relative concentrations of the endogenous peptides, particularly when samples at serial time points are taken.

For this reason, we developed a specific assay directed

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toward the intact N-terminus of GIP. This assay is free from cross-reaction with GIP-(3–42) and allows, for the first time, direct determination of the levels of endogenous and exogenous intact, biologically active GIP in small plasma samples. This assay was used to determine the fate of human GIP in healthy subjects and in patients with type 2 diabetes.

Materials and Methods

Degradation of GIP in vitro

Blood was collected from four healthy volunteers [all women; age, 31.8 ± 3.6 yr; body mass index (BMI), 21.2 ± 1.0 kg/m^2] into chilled heparinized tubes, and the plasma was separated by centrifugation. Plasma (2.7 mL) was incubated at 37 C with synthetic human GIP (Peninsula Laboratories, Europe Ltd., St. Helens, UK; final concentration, 200 pmol/L) for 0, 30, 60, 120, 180, and 240 min. A specific DPP IV inhibitor (valine-pyrrolidide; 0.01 mmol/L, final concentration; a gift from Drs. R. D. Carr and L. B. Christiansen, Novo Nordisk A/S, Bagsværd, Denmark) was added to an additional sample, which was incubated for 240 min. After incubation, the samples were placed in an ice bath, and valine-pyrrolidide (0.01 mmol/L, final concentration) was added. Aliquots (700 μL) were immediately extracted with ethanol (70%, vol/vol; final concentration) for direct RIA measurement. The remaining 2 mL were acidified with trifluoroacetic acid (TFA; Rathburn, Walkerburn, Scotland; 200 μL; 10%, vol/vol) and extracted on Sep-Pak C_{18} cartridges (Waters-Millipore Corp., Milford, MA) eluted with acetonitrile (AcN; Rathburn) in 0.1% TFA for later analysis by HPLC, as described below.

Endogenous GIP peptides in vivo

Two blood samples (5 mL) were collected from five healthy volunteers with no personal or family history of diabetes or gastrointestinal disease (two men and three women; age, 37.6 ± 5.1 yr; BMI, 22.1 ± 1.2 kg/m^2) after an overnight fast and 15, 30, 45, 60, 90, 120, 150, and 180 min after a mixed meal (~500 Cal, in which 58% of the calories were derived from carbohydrate, 33% from fat, and 9% from protein). Two extra samples (40 mL) were taken at 0 and 60 min. Samples were collected into chilled tubes containing ethylenediamine tetraacetate (7.4 mmol/L; final concentration), aprotinin (500 kallikrein inhibitory units/mL blood), and valine-pyrrolidide (0.01 mmol/L; final concentration); plasma was separated by centrifugation at 4 C and stored at −20 C until extraction.

Aliquots were extracted with ethanol, as described above, for direct RIA measurement using the N-terminally and C-terminally directed assays for GIP described below. Aliquots from samples collected at 0 and 60 min (15 and 5 mL plasma, respectively) were extracted on Sep-Pak C_{18} cartridges and analyzed by HPLC and RIA as described below.

Fate of exogenous GIP in vivo

The study was approved by the ethics committee of the Medical Faculty of the Ruhr University (Bochum, Germany) in April 1998, and written consent was obtained from all participants after the nature and possible risks of the study had been explained to them. The study was carried out in 10 nondiabetic, healthy subjects with no personal or family history of diabetes or gastrointestinal disease (6 men and 4 women; age, 48.6 ± 5.4 yr; BMI, 25.7 ± 1.1 kg/m^2) and in 10 age- and weight-matched patients with type 2 diabetes (7 men and 3 women; age, 51.7 ± 2.8 yr; BMI, 28.6 ± 1.6 kg/m^2; duration of diabetes, 3.4 ± 1.0 yr). Five of the diabetic subjects were treated with diet alone, 1 received a sulfonyl urea compound, 1 received metformin, and 3 were treated with acabose.

All antidiabetic medication was continued until the evening before the study, which was performed in the morning after an overnight fast. The study examined the fate of GIP given as a continuous iv infusion (nominally 2 pmol/kg·min). The peptide (lot C-0229, Polypeptide Laboratories GmbH, Wolfenbuttel, Germany) was dissolved in sterile 0.9% saline solution containing in addition 1% human serum albumin (Behring, Marburg, Germany), filtered through 0.2-μm pore size nitrocellulose filters (Sartorius, Gottingen, Germany) and stored at −28 C until use. Four basal blood samples were taken at 15-min intervals, after which the infusion was started, and additional samples were collected every 15 min. After 1 h, the infusion was stopped, and an additional two blood samples were taken. Blood samples were taken into chilled tubes containing ethylenediamine tetraacetate (3.9 mmol/L) and aprotinin (500 kallikrein inhibitory units/mL) and were kept on ice to prevent *in vitro* degradation of GIP. The plasma was separated by centrifugation at 4 C and was stored at −20 C until analysis using the C-terminally and N-terminally directed assays described below. Samples from the infusate were collected for later analysis to allow calculation of the actual infusion rates.

Chromatography

Sep-Pak eluates were lyophilized, reconstituted in 0.1% TFA (400 μL), and subjected to analytical reverse phase HPLC on a Vydac C_{18} column (300 Å, 5 μm; Mikrolab Aarhus A/S, Højbjerg, Denmark). The column was eluted at a flow rate of 1 mL/min, with stepwise linear gradients of AcN in 0.1% TFA (0–26% AcN over 5 min, followed by 26–33% over 20 min and 33–75% over 3 min). Fractions (250 μL) were collected and assayed for GIP immunoreactivity using the two GIP assays described below. Calibration of the entire procedure (Sep-Pak extraction, HPLC, and RIA) gave an overall recovery of 61.6 ± 6.6% and a detection limit of approximately 50 fmol. The column was calibrated with synthetic human GIP-(1–42) and GIP-(3–42). GIP-(3–42) was prepared by incubating GIP-(1–42) with purified human DPP IV (a gift from Dr. S. Branner, Novo Nordisk A/S), and the structure was confirmed by amino acid sequencing and mass analysis (performed by Dr. A. H. Johnsen, Department of Clinical Biochemistry, Rigshospital, Copenhagen, Denmark).

Hormonal analysis

Total GIP was measured using the C-terminally directed antiserum R65 (14, 15), which reacts fully with intact GIP and the N-terminally truncated metabolite, GIP-(3–42), but not with the so-called 8-kDa GIP, whose chemical nature and relation to GIP secretion are uncertain. In this assay, addition of synthetic human GIP-(1–42) to plasma gives a recovery of 85%. Recovery of the metabolite was assessed using GIP-(3–42) prepared by incubating GIP-(1–42) with DPP IV (see above) and was found to be 81.4 ± 7.8%. The assay has a detection limit of less than 2 pmol/L and an intraassay variation of approximately 6%.

Intact, biologically active GIP was measured using a newly developed assay. Antibodies were raised by immunizing rabbits with the synthetic sequence, GIP-(1–10)-Cys [Genosys Biotechnologies (Europe) Ltd., Cambridge, UK] coupled to keyhole limpet hemocyanin using *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester, as described by Dyrberg and Kofod (16). Antiserum 98171 could be used in a final dilution of 1:40,000 and endows the assay with a detection limit of approximately 5 pmol/L and an ED_{50} of 47.5 ± 2.3 pmol/L. The assay is specific for the intact N-terminus of GIP and cross-reacts less than 0.1% with GIP-(3–42) or with the structurally related peptides GLP-1-(7–36)amide, GLP-1-(9–36)amide, GLP-2-(1–33), GLP-2-(3–33), or glucagon at concentrations of up to 100 nmol/L. Plasma samples were extracted with ethanol (70%, vol/vol; final concentration), giving recoveries of synthetic GIP-(1–42) added to plasma of 84.8 ± 1.4%. Intraassay variation was less than 6%, and interassay variations were approximately 8% and 12% for 20 and 80 pmol/L standards, respectively. Valine-pyrrolidide (0.01 mmol/L, final concentration) was added to the assay buffer to prevent N-terminal degradation of GIP during the assay incubation.

For both assays, human GIP (Peninsula Laboratories, Europe Ltd.) was used as standard, and radiolabeled GIP was obtained from Amersham Pharmacia Biotech Ltd. (Aylesbury, UK). Separation of bound from free peptide was achieved using plasma-coated charcoal (17).

Calculations and statistical analysis

The area under the curve (AUC) for endogenous and exogenous GIP was calculated for each assay using the trapezoidal method after subtraction of the basal concentrations. The *in vivo* plasma $t_{1/2}$ was calculated by $\log_e$-linear regression analysis of peptide concentrations in samples collected after termination of the GIP infusion, after subtraction of endogenous fasting concentrations. The MCR of GIP was calculated from the actual infusion rate for each subject divided by the plateau

plasma concentration, after subtraction of basal values. The distribution space (DS) was calculated using the formula DS = MCR/k, where k is the fractional clearance rate (=0.693/$t_{1/2}$). Results are expressed per kg BW.

Data are expressed as the mean ± SEM and were analyzed using InStat software, version 1.13 (GraphPad Software, Inc., San Diego, CA), and Statistica software (Stat Soft, Inc., Tulsa, OK). Two-factor ANOVA for repeated measures with *post-hoc* analysis was used to analyze time-course curves. The $t_{1/2}$, MCR, and DS were compared using ANOVA and two-tailed t tests for paired and nonpaired data as appropriate. $P < 0.05$ was considered significant.

Results

Degradation of GIP in human plasma

When human plasma was incubated at 37 C with synthetic human GIP, concentrations measured with the C-terminally directed assay were unchanged, whereas concentrations determined with the N-terminally directed assay decreased with time, giving a $t_{1/2}$ of 75 ± 4 min (Fig. 1). HPLC analysis (Fig. 2) of these samples revealed the time-dependent disappearance of intact GIP-(1–42) (detected equally with both GIP assays) and the concomitant formation of a second immunoreactive peak (detected only with the C-terminally directed assay). The elution position of this second peak corresponded exactly to the N-terminally truncated metabolite, GIP-(3–42), and was formed with a $t_{1/2}$ of 62 ± 5 min, which was not significantly different from that calculated after direct assay of the samples. No other immunoreactive peaks were detected. After incubation for 240 min in the presence of the DPP IV inhibitor, valine-pyrrolidide (0.01 mmol/L), concentrations determined with the N-terminally directed RIA (106 ± 4 pmol/L) were not significantly different from preincubation values (123 ± 13 pmol/L). Analysis of these samples by HPLC revealed that the inhibitor largely prevented degradation of the intact peptide (70.5 ± 3.8% intact GIP in the presence of valine-pyrrolidide compared to 8.5 ± 0.7% after 240 min in the absence of inhibitor; $P < 0.001$).

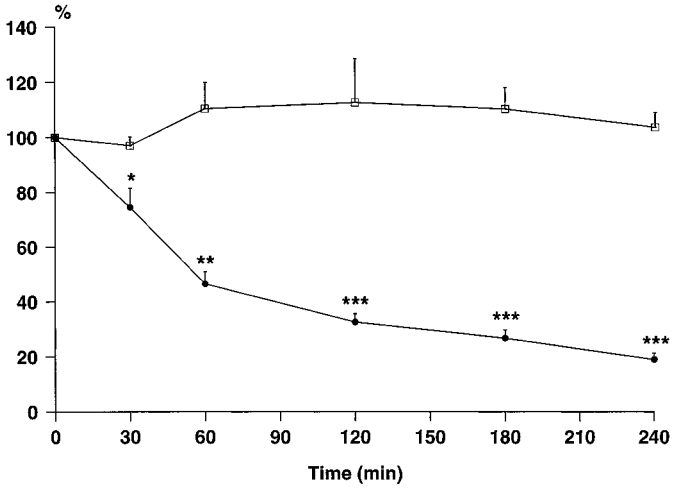

FIG. 1. Human plasma was incubated at 37 C with 200 pmol/L GIP. Aliquots were extracted with ethanol (70%, vol/vol) and assayed using C-terminal (□) and N-terminal (●) RIAs for GIP. Due to the presence of endogenous GIP [GIP-(1–42) and GIP-(3–42)], which causes different initial concentrations to be determined with each antiserum, results for each assay are expressed as percentages of the concentrations measured at 0 min (162 ± 9 and 123 ± 13 pmol/L, C- and N-terminal assays, respectively). Values are given as the mean ± SEM (n = 4). C-Terminal values were not significantly different from the 0 min value. For N-terminal values: *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$ (compared to 0 min).

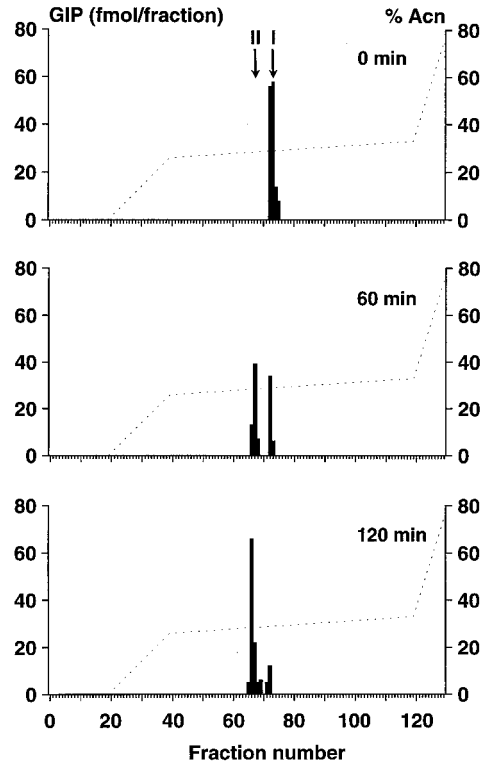

FIG. 2. Human plasma was incubated at 37 C with 200 pmol/L GIP and analyzed by reverse phase HPLC on a Vydac C_{18} column, eluted with gradients of AcN in 0.1% TFA. The elution positions of the intact peptide (I) and the N-terminally truncated fragment (II) are indicated. In the *upper panel*, the reaction was stopped immediately after addition of the peptide. The *middle* and *lower panels* show the effects of incubation for 60 and 120 min. Fractions were assayed using the C-terminally directed RIA for GIP.

Endogenous GIP

After a mixed meal, endogenous GIP concentrations measured in plasma using the C-terminally directed assay were higher than those determined with the N-terminal assay (Fig. 3). When the AUCs (0–180 min) for each assay were calculated, the amount determined by the N-terminal assay (11,333 ± 1,190 pmol·min/kg) was 37.0 ± 2.5% of that determined with the C-terminal assay (30,969 ± 3,507 pmol·min/kg; significant difference between AUC, $P < 0.001$).

When the 0 min plasma samples were analyzed using a combination of HPLC and the C-terminal RIA, the major immunoreactive peak (73.8 ± 2.9% of the total C-terminal immunoreactivity) eluted at the position of GIP-(3–42) (28.7% AcN; fraction 69). A second peak (21.8 ± 2.5%) eluted at the position of GIP-(1–42) (29.3% AcN; fraction 75), and, in addition, a third minor peak, constituting 4.1 ± 1.7% of the total C-terminal immunoreactivity, was seen (27.3% AcN; fraction 52). When the same fractions were assayed using the N-terminal assay, a single major peak (86.4 ± 5.8% of the total N-terminal immunoreactivity) eluting at the position of GIP-

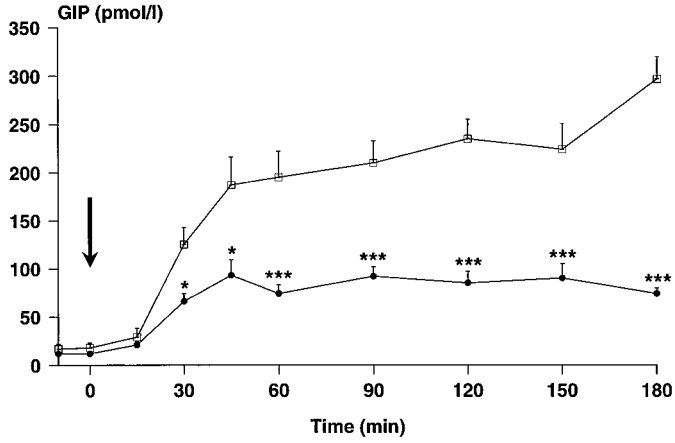

FIG. 3. Increase in plasma concentrations of GIP after ingestion of a mixed breakfast (*arrow*) in healthy subjects (n = 5). Samples were measured using C-terminally (□) and N-terminally (●) directed assays for GIP. Comparison between assays: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.

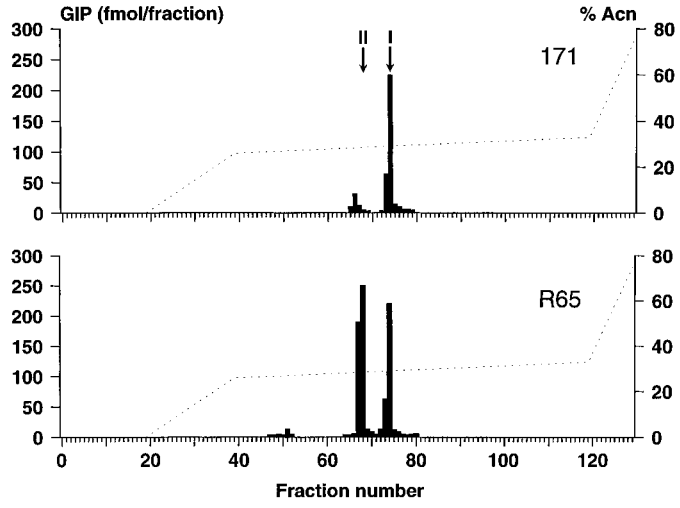

FIG. 4. Plasma collected 60 min after ingestion of a mixed breakfast was analyzed by reverse phase HPLC on a Vydac C_{18} column, eluted with gradients of AcN in 0.1% TFA. The elution positions of the intact peptide (I) and the N-terminally truncated fragment (II) are indicated. Fractions were assayed using N-terminally (antiserum 98171; *upper panel*) and C-terminally (antiserum R65; *lower panel*) directed RIAs for GIP.

(1–42) was seen, together with a minor peak constituting 13.6 ± 5.8% of the total N-terminal immunoreactivity (28.5% AcN; fraction 67). HPLC analysis of the 60 min plasma samples (Fig. 4) using the C-terminal RIA revealed two major immunoreactive peaks eluting at the positions of GIP-(1–42) and GIP-(3–42) (35.7 ± 4.2% and 58.1 ± 2.7% of the total C-terminal immunoreactivity, respectively), and the same minor peak (6.4 ± 1.9% of the total C-terminal immunoreactivity. The N-terminal assay revealed a single major peak, eluting at the position of GIP-(1–42) and the same second peak (81.3 ± 0.9% and 18.7 ± 0.9% of total N-terminal immunoreactivity, respectively).

GIP administration

The actual GIP infusion rate was significantly lower than the nominal rate of 2 pmol/kg·min, indicating that major losses occurred, probably during the sterile filtration step. However, the infusion rate, calculated using the C-terminal assay (0.80 ± 0.05 pmol/kg·min) did not differ from that determined using the N-terminal assay (0.77 ± 0.05 pmol/kg·min), indicating that GIP in the infusate was not degraded during the course of the experiment.

During iv infusion of GIP in both healthy and diabetic subjects, plasma concentrations reached a stable plateau when determined using the N-terminal assay, but not using the C-terminal assay. Concentrations of the intact, biologically active peptide, measured using the N-terminally directed assay, were consistently lower than those measured using the C-terminally directed assay (Fig. 5). When expressed as a percentage, the intact peptide (AUC) accounted for 37.1 ± 4.1% of the total immunoreactivity determined with the C-terminal assay for controls and 41.3 ± 3.4% for the diabetics (no significant difference between controls and diabetics). Once the infusion was halted, the N-terminal immunoreactivity disappeared more rapidly than the C-terminal immunoreactivity [$t_{1/2}$, 7.4 ± 0.8 *vs.* 16.6 ± 1.6 min for controls ($P < 0.0001$); 5.2 ± 0.6 *vs.* 12.6 ± 0.8 min for diabetics ($P < 0.0001$)]. Both C- and N-terminal $t_{1/2}$ were shorter in the diabetic group than in the controls ($P < 0.05$). The MCRs, determined using the C-terminal assay (8.7 ± 0.7 and 9.3 ± 1.3 mL/kg·min for control and diabetic subjects, respectively), were significantly ($P < 0.001$) shorter than the corresponding values determined using the N-terminal assay (23.3 ± 3.2 and 22.1 ± 3.1 mL/kg·min), but there was no difference between the two groups of subjects. The distribution space for GIP was the same regardless of which assay was used and did not differ between the two groups (208 ± 27 and 236 ± 43 mL/kg for control subjects *vs.* 164 ± 20 and 172 ± 36 mL/kg for the patients, C-terminal and N-terminal assays, respectively).

Discussion

In this study we have shown that both endogenous and exogenous GIP are rapidly degraded from the N-terminus, leading to the formation of a metabolite truncated by two amino acids, consistent with the action of the enzyme DPP IV. This is analogous with the situation for GLP-1, where DPP IV has been shown to be the most important enzyme for the inactivation of GLP-1 (5). However, compared to GLP-1, GIP appears to be somewhat less susceptible to DPP IV action, which is a little surprising because in the first nine N-terminal amino acids, the two peptides differ only at positions 1 and 7. The residue at position 1 (histidine in GLP-1 and tyrosine in GIP) is involved in the interaction between substrate and enzyme, but it is also likely that other sites in the peptide (affecting, for example, the tertiary structure) may influence the rate of cleavage by the enzyme (11). In plasma, where DPP IV is probably the only enzyme that contributes significantly to the degradation of both peptides (12), GIP is more stable, with an *in vitro* $t_{1/2}$ about 4 times longer than that of 20 min for GLP-1 (5). GIP also appears to be less susceptible to degradation *in vivo*. More of the intact peptide survives during an iv infusion (>40% for GIP compared to around 20% for GLP-1) (18), and this is reflected in

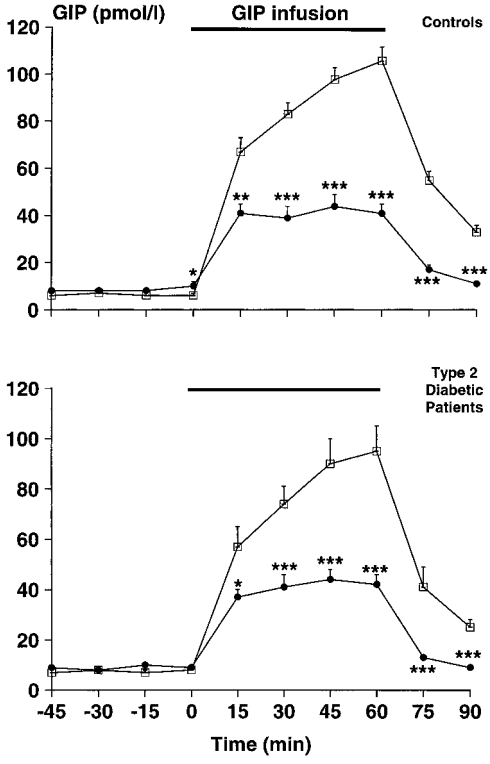

FIG. 5. Increase in plasma concentrations of GIP during infusion of synthetic human GIP (0.8 pmol/kg·min) in healthy subjects (n = 10; *upper panel*) and subjects with type 2 diabetes (n = 10; *lower panel*). Samples were measured using C-terminally (□) and N-terminally (●) directed assays for GIP. The *horizontal bar* indicates the period of the infusion. Comparison between assays: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.

the longer plasma $t_{1/2}$ for intact GIP (~5-fold longer than the 1–1.5 min for intact GLP-1) (19, 20). Previous studies reported a $t_{1/2}$ of approximately 20 min for GIP in man (21, 22). These earlier studies used assays that do not distinguish between intact GIP and the metabolite and are in good agreement with the value found in the present study using the C-terminally directed assay. However, because of their cross-reactivity with GIP-(3–42), such assays overestimate the levels of the intact peptide and consequently overestimate the $t_{1/2}$ of the biologically active form. As was the case for GLP-1, the $t_{1/2}$ for intact GIP is much shorter when determined using the N-terminal assay, underlining the role of DPP IV in GIP metabolism *in vivo* and emphasizing the need for specific assays for the measurement of the biologically active peptide. The N-terminally directed assay reported here is specific for the intact N-terminus of GIP, showing no cross-reactivity with the truncated metabolite. Its sensitivity is such that concentrations of biologically active GIP can be determined directly, and therefore removes the requirement for a prior separation step.

The mixed meal used in the present study proved to be a potent stimulus for GIP secretion, and the concentrations determined with the C-terminally directed assay are in good agreement with previous reports using the same antiserum (23). However, concentrations determined with the N-terminally directed assay are significantly lower, and HPLC analysis confirms that this is because much of the endogenous peptide circulates as the N-terminally truncated peptide, GIP-(3–42). Thus, endogenous GIP is found in the plasma in two major forms, corresponding to the intact peptide and the N-terminally truncated metabolite, which together account for most of the total GIP immunoreactivity. After HPLC, the C-terminally directed assay detected one additional minor immunoreactive peak, constituting about 5% of the total immunoreactivity. This component was not detected with the N-terminal assay. However, an additional component, not detected with the C-terminal assay, was identified with the N-terminal assay. In preliminary studies this component was shown to elute on HPLC with a similar retention time as a larger molecular weight form that can be extracted from human small intestine (data not shown). It has previously been reported that the C-terminal antiserum (R65) employed in the present study does not detect the so-called 8-kDa GIP, whose chemical nature and relation to GIP secretion are uncertain (14, 15). It is, therefore, possible that the additional peak detected with the N-terminal assay may be this component, but the extent of the cross-reaction is, at present, unknown. Alternatively, it has been reported that neutral endopeptidase 24.11 is capable of degrading GIP, albeit that GIP is a poor substrate (24), so it may be that this immunoreactive peak is a metabolite arising from neutral endopeptidase 24.11 degradation. Further studies, aimed an isolating and identifying this material should resolve these issues. However, based on the HPLC analysis, this component constitutes only a minor proportion of the overall N-terminal immunoreactivity and, thus, does not negate the conclusion that the major route of GIP metabolism *in vivo* is mediated by DPP IV.

To date, there have been few reports concerning the metabolism of GIP *in vivo*. The MCR for GIP calculated in the present study using the C-terminal assay is in good agreement with previously reported values using assays of similar specificity (21, 22). However, our finding that the MCR is almost 3 times faster when determined using the N-terminally directed assay together with the differences in $t_{1/2}$ calculated with the two assays support the idea that N-terminal degradation of GIP occurs *in vivo*. Recent investigations of the stability of GLP-1 and GIP have indicated that both peptides are degraded by DPP IV (5, 6, 11), with a thorough *in vitro* study concluding that DPP IV is the most important enzyme in plasma and that other proteases play only a minor secondary role (12). Only one study, performed in rats, has attempted to assess the importance of DPP IV in terms of GIP metabolism *in vivo* (6). In that study the conversion of radiolabeled porcine GIP-(1–42) to GIP-(3–42) was monitored by HPLC analysis, revealing a $t_{1/2}$ for the conversion of approximately 2 min. This is shorter than the 5–7 min found in the present study and may partly reflect the different analytical approaches used. Moreover, direct measurement of plasma DPP IV activity reveals greater activity in the rat (6) compared to man (11), indicating that species differences in this respect are likely to account for most of the difference. It has recently been shown that DPP IV inhibition amplifies the insulin response and improves glucose tolerance in insulin-resistant rats and is associated with an increase in the proportion of intact biologically active GLP-1

(25). As GIP is also degraded by DPP IV, it is likely that preservation of intact GIP contributes to the effect.

Elevated GIP concentrations are found in patients with renal failure (26) or uremia (27), indicating that the kidneys may be involved in GIP clearance. This is further supported by the demonstration of a renal arterio-venous concentration difference in both man (28) and dog (27), suggesting removal of GIP by the kidneys. Hepatic extraction of GIP could not be demonstrated in dogs or rats (29, 30). However, this does not preclude the occurrence of DPP IV-mediated degradation of GIP in the liver, as is the case for GLP-1 (19), as it is likely that the assays used in these two earlier studies would not have distinguished between intact GIP and the metabolite.

In our previous study of GLP-1 metabolism (18), there was an indication that GLP-1 may be degraded more rapidly in the diabetic group compared to that in nondiabetic subjects, although because the groups were not matched in terms of age and BMI, direct comparison was not possible. In the present study the plasma $t_{1/2}$ for intact GIP was shorter in the diabetic group, which may suggest that there was greater DPP IV activity in this group. However, the $t_{1/2}$ for total peptide was also significantly shorter, which, together with the fact that the percentage of peptide that remained intact was similar in both groups, suggests that factors other than DPP IV are involved. The kidney is a major site of clearance of both intact GLP-1 and its metabolite (19); moreover, it is known that the glomerular filtration rate may be elevated in some patients with type 2 diabetes (31–33). This raises the possibility that a difference in the renal handling of GIP in the diabetic patients may be the explanation for the shorter $t_{1/2}$ of both intact and total GIP in the present study. Moreover, the shorter survival time of intact GIP in the diabetic group may be one factor (albeit a minor one, given that no significant differences in MCR were found), contributing to the reduced GIP effect seen in type 2 diabetic patients (34, 35). Further investigations using methodologies specifically designed to address this question should clarify this point.

In conclusion, the use of a novel assay, specific for the intact N-terminus of GIP-(1–42), has revealed that the predominant molecular form of endogenous GIP in healthy human subjects is the noninsulinotropic metabolite GIP-(3–42), indicating a major role of the enzyme DPP IV in GIP metabolism *in vivo*. Furthermore, exogenous GIP is also degraded by DPP IV in both healthy and type 2 diabetic subjects. As most, if not all, existing assays for GIP fail to distinguish between the intact peptide and the metabolite, previously reported GIP levels will be considerably overestimated in terms of concentrations of the biologically active peptide.

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