

The effects of glucose-dependent insulintropic polypeptide infused at physiological concentrations in normal subjects and Type 2 (non-insulin-dependent) diabetic patients on glucose tolerance and B-cell secretion

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Summary. The effects of porcine glucose-dependent insulintropic polypeptide given by continuous intravenous infusion in normal subjects ($n=6$) and Type 2 (non-insulin-dependent) diabetic patients ($n=6$) have been investigated. The subjects were studied on 2 separate days after overnight fasts. On each day 25 g of glucose was infused from 0–30 min plus an infusion of either porcine glucose-dependent insulintropic polypeptide ($0.75 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) or control solution. During the glucose-dependent insulintropic polypeptide infusion plasma glucose values were reduced in normal subjects from 30–60 min ($p < 0.01$) and in Type 2 diabetic patients at 45 and 60 min ($p < 0.05$). In the normal subjects insulin concentrations were greater from 10–35 min ($p < 0.01$) following glucose-dependent insulintropic polypeptide infusion and peak values were increased by 123%. In the Type 2 diabetic patients following glucose-dependent insulintropic polypeptide infusion insulin levels were increased from 4–40 min ($p < 0.01$) but peak values were only increased by 27%. In the normal subjects C-peptide values

were greater from 25–45 min ($p < 0.01$) following glucose-dependent insulintropic polypeptide infusion and peak C-peptide levels were increased by 82%. In the Type 2 diabetic patients following the glucose-dependent insulintropic polypeptide infusion C-peptide levels were increased from 6–55 min ($p < 0.01$) and peak values were increased by 20%. Plasma glucose-dependent insulintropic polypeptide levels were within the physiological post prandial range during the glucose-dependent insulintropic polypeptide infusion. Glucose-dependent insulintropic polypeptide is insulintropic in normal subjects and Type 2 diabetic patients at physiological concentrations and results in improved glucose tolerance. This insulintropic effect is less marked in the diabetic patients and may represent insensitivity of the B cell to glucose-dependent insulintropic polypeptide.

Key words: Glucose, insulin, C-peptide, glucose dependent insulintropic polypeptide, normal subjects, Type 2 diabetic patients.

A much greater insulin response is evoked in response to an oral glucose load compared with an equivalent amount administered intravenously, despite a much smaller increase in the blood glucose level with the former [1, 2]. A significant proportion of the insulin secreted following an oral glucose load is dependent upon gastrointestinal factors [3–5]. The enteric hormone glucose dependent insulintropic polypeptide (GIP) is secreted by K cells situated in the upper small intestine in response to oral but not intravenous glucose [6]. Recently a high-affinity GIP receptor has been demonstrated in a hamster pancreatic B-cell line [7] which is functionally linked to cAMP production [8]. Intravenous GIP infusions have been shown to potentiate the release of insulin in the presence of hyperglycaemia in normal subjects when infused at supraphysiological dosage levels [9, 10]. An insulintropic effect at physiological concentrations has also been demonstrated [11]

but it was concluded that the effect of GIP was likely to be weak in individuals with normal glucose tolerance.

Recognition that GIP participates in the enteroinsular axis prompted investigations into its role in the pathophysiology of diabetes mellitus. Both increased and decreased GIP concentrations have been described in response to glucose, mixed meal and fat stimulation in diabetes mellitus [12–20]. Despite this large series of studies which have measured GIP concentrations in response to various stimuli, limited data is available regarding the effects of an intravenous infusion of GIP in diabetes mellitus [21, 22].

The aim of this study therefore was to investigate the metabolic responses to porcine GIP infused intravenously at a dose level which produced physiological concentrations of GIP in both normal subjects and newly diagnosed, untreated Type 2 diabetic patients.

Subjects and methods

The studies were performed on 12 subjects consisting of 6 healthy male volunteers, with body mass indexes (BMIs) between 20–28, aged 24–34 years with no family history of diabetes; and 6 newly diagnosed previously untreated Type 2 diabetic patients with BMIs between 24–34, and aged 42–64 years. The Type 2 diabetic patients all had raised fasting plasma glucose levels (Fig. 2), increased HbA_{1c} concentrations (10.6–14.2%) and were all non-ketotic (the normal range of the HbA_{1c} assay is 5.9–7.9%). All subjects were studied on 2 separate days at intervals of 1 week, during which they received an intravenous infusion of glucose (IVGTT) either with or without a concomitant infusion of porcine GIP in random order. The study was approved by the local ethical committee and informed consent was given by each subject.

After a 10-h overnight fast the subjects were admitted to a metabolic unit, where they remained on bed rest throughout the study; smoking was not permitted. An intravenous cannula (Venflon 19G) was inserted into an antecubital vein and attached via a three-way tap to a slow running infusion of saline (0.154 mmol/l). All blood samples were taken from this cannula, and at each of the sampling time points the saline infusion was stopped and 2 ml of blood was withdrawn and discarded prior to obtaining the sample for subsequent analysis. A similar cannula was inserted in the contralateral arm and this was used for the infusion of glucose and GIP. The IVGTT was performed by infusing 25 g of glucose (50 ml 50% dextrose) from 0–30 min. Porcine GIP was isolated from an acid alcohol extract of porcine small intestine by ion-exchange chromatography followed by a final purification on HPLC. The identity of the peptide was confirmed by determining its amino acid sequence [23]. The preparation used in the present study was screened for unexpected side effects in an acute toxicological study and its efficacy as an insulin releasing agent demonstrated in the pig [24]. Purified porcine GIP (Novo Research Institute, Copenhagen, Denmark) which had been sterile filtered and lyophilized with human serum albumin was dissolved in 50 ml Haemaccel (Hoechst AG, Frankfurt, FRG) to reduce adsorption to the giving set. The GIP was infused using a syringe pump and a 50 cm manometer line which had been flushed with the GIP solution for 10 min prior to being connected to the subjects via the cannula. GIP was infused intravenously from 0–30 min at a dose of 0.75 pmol·kg⁻¹·min⁻¹. During a control study a similar volume of Haemaccel was infused from 0–30 min.

Blood samples were taken at 08.30 hours and immediately prior to the infusions at 09.00 hours. Samples were then taken at 2-min intervals for 10 min then every 5 min for 60 min, with further samples taken at 90 and 120 min. At each time point plasma glucose, C-peptide, immunoreactive insulin (IRI) and GIP concentrations were measured.

The blood was taken into fluoride for plasma glucose determination (glucose oxidase) and into lithium-heparin tubes containing 1000 kallibrein inhibitory units (KIU) of aprotinin (Novo A/S, Bagsvaerd, Denmark) for the determination of C-peptide [25], insulin [26] and GIP levels [27]. All samples were collected on ice, centrifuged immediately at 4 °C and the plasma stored at –20 °C until assay. The detection limit of IRI was 0.022 nmol/l and the precision (SD within assay) was 0.009 nmol/l. The detection limit for C-peptide was 0.035 nmol/l and the precision 0.0115 nmol/l. The sequence of porcine GIP differs from human GIP at residues 18 and 24 [28]. The antiserum used in this study does not, however, discriminate between human and porcine GIP since the sequence recognised by the antiserum is identical in both molecules [23]. The GIP detection limit was 5 pmol/l and the precision 1.7 pmol/l.

Statistical analysis

The results are expressed as means ± SE and the significance of differences assessed using the Student's paired t-test. A *p* value of <0.05 was considered statistically significant.

Results

The mean venous plasma glucose, C-peptide, IRI and GIP concentrations for the normal subjects and the Type 2 diabetic patients are shown in Figure 1 and Figure 2 respectively.

In the normal subjects fasting plasma glucose levels were similar for both tests. During the IVGTT plasma glucose increased from a fasting level of 4.9 ± 0.2 to a peak of 9.9 ± 0.2 mmol/l at 30 min and returned to basal levels by 90 min. Following the intravenous infusion of glucose and GIP plasma glucose values increased from a fasting level of 4.9 ± 0.2 mmol/l to a slightly lower peak of 9.3 ± 0.3 mmol/l at 20 min. Glucose levels then started to decline and were significantly lower following the IVGTT plus GIP infusion compared with the IVGTT alone from 35–60 min (*p* < 0.05–0.01). Glucose levels returned to fasting values earlier following the GIP infusion and reached fasting concentrations by 50 min.

Following the IVGTT C-peptide levels increased from a fasting value of 0.49 ± 0.04 nmol/l to a peak level of 1.8 ± 0.2 nmol/l at 35 min. During the GIP infusion C-peptide values increased from a similar fasting value of 0.48 ± 0.04 nmol/l to a peak level of 3.28 ± 0.35 nmol/l at 30 min. C-peptide levels were significantly higher following the GIP infusion between 25 and 45 min. (*p* < 0.05–0.01).

IRI levels showed a similar pattern increasing from a fasting value of 0.08 ± 0.01 nmol/l to a peak of 0.34 ± 0.04 nmol/l at 30 min during the IVGTT. Following the GIP infusion IRI levels increased from a similar fasting level of 0.07 ± 0.01 nmol/l to a higher peak concentration of 0.76 ± 0.05 nmol/l at 25 min. During the GIP infusion IRI levels were significantly higher compared with the IVGTT alone from 10–35 min (*p* < 0.05–0.01).

The mean fasting GIP concentrations were similar for both tests and, as expected did not alter during the IVGTT, ranging between 18.4 ± 4.1 and 24.6 ± 5.2 pmol/l. The GIP infusion increased plasma GIP concentrations from a fasting level of 19.8 ± 4.0 pmol/l to a peak of 76 ± 9.4 pmol/l at 30 min thereafter falling gradually although the concentration was still above fasting levels after 120 min.

In the Type 2 diabetic patients fasting plasma glucose levels were elevated and similar for both tests. Following the IVGTT, plasma glucose increased from a fasting value of 9.2 ± 0.2 mmol/l to a peak of 14.4 ± 0.3 mmol/l at 35 min. During the GIP infusion glucose increased from a fasting value of 8.9 ± 0.2 mmol/l to a peak of 14.2 ± 0.3 mmol/l at 35 min. Glucose levels were significantly lower following the GIP infusion at 45 and 60 min (*p* < 0.05).

Following the IVGTT, C-peptide levels increased from a fasting level of 0.97 ± 0.05 nmol/l to a delayed peak value of 1.48 ± 0.2 nmol/l at 50 min. During the

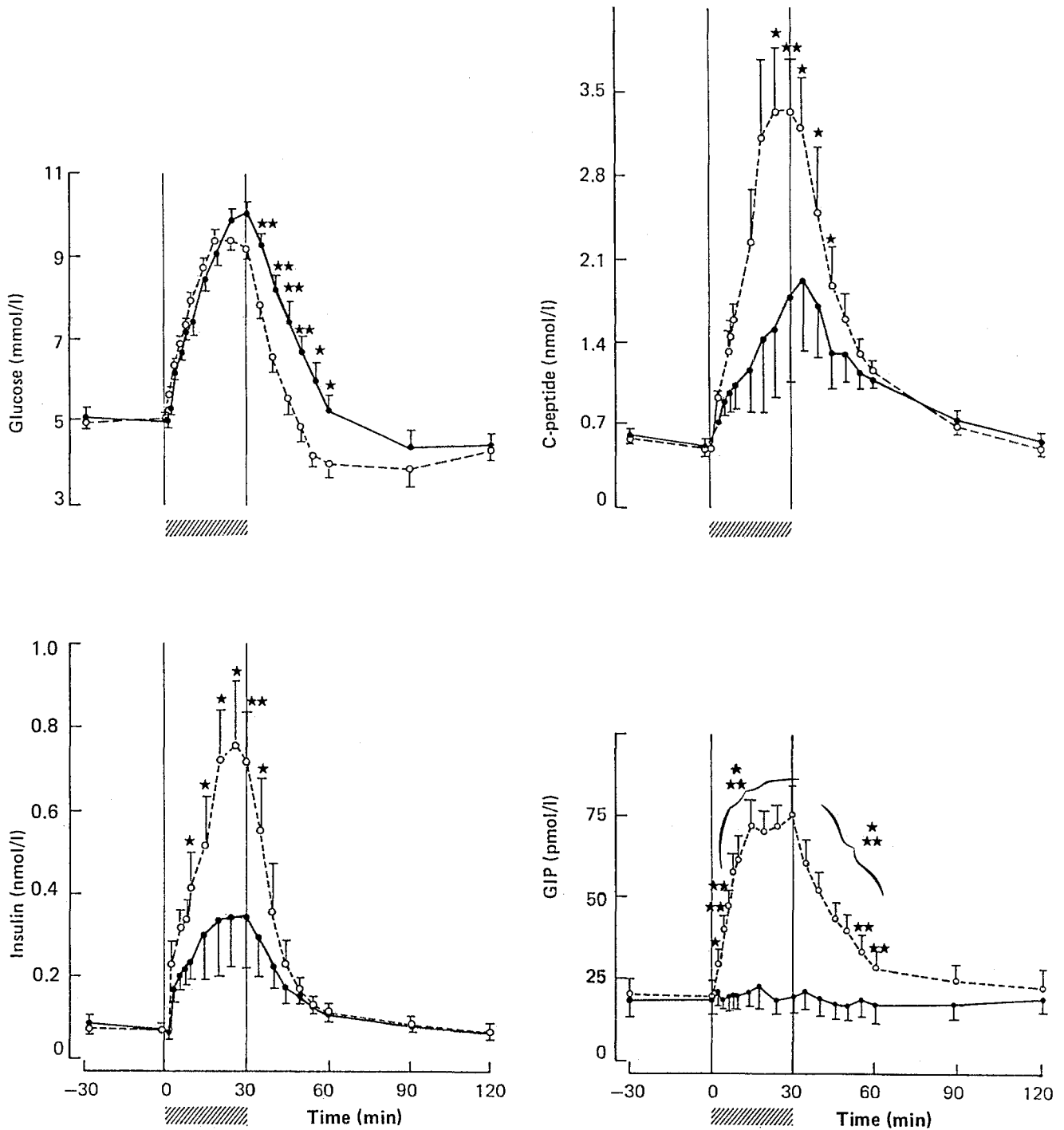


Fig. 1. Intravenous glucose tolerance test (IVGTT: 50 ml 50% dextrose) infused from 0-30 min (—) and in IVGTT plus an infusion of porcine GIP ($0.75 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) from 0-30 min (---) in normal subjects ($n=6$). Mean \pm SE concentrations of plasma glucose, insulin, C-peptide and GIP. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

GIP infusion C-peptide increased from a fasting value of $1.02 \pm 0.07 \text{ nmol/l}$ to a peak level of $1.78 \pm 0.22 \text{ nmol/l}$ at 35 min. The C-peptide values were significantly greater following the GIP infusion from 6-55 min ($p < 0.05-0.01$).

IRI concentrations increased from a fasting value of $0.14 \pm 0.01 \text{ nmol/l}$ to a peak of $0.26 \pm 0.03 \text{ nmol/l}$ at 50 min following the IVGTT. During the GIP infusion IRI increased from a value of $0.15 \pm 0.01 \text{ nmol/l}$ to an

earlier peak of $0.33 \pm 0.03 \text{ nmol/l}$ at 35 min. The IRI concentrations were significantly higher following the GIP infusion from 4-40 min ($p < 0.05-0.01$).

Fasting GIP levels were similar for both tests and did not change during the IVGTT and ranged from 17.2 ± 4.1 to $25.6 \pm 4.8 \text{ pmol/l}$. Following the GIP infusion GIP levels increased from a fasting value of $12.5 \pm 5 \text{ pmol/l}$ to a peak concentration of $104 \pm 9.6 \text{ pmol/l}$ at 35 min.

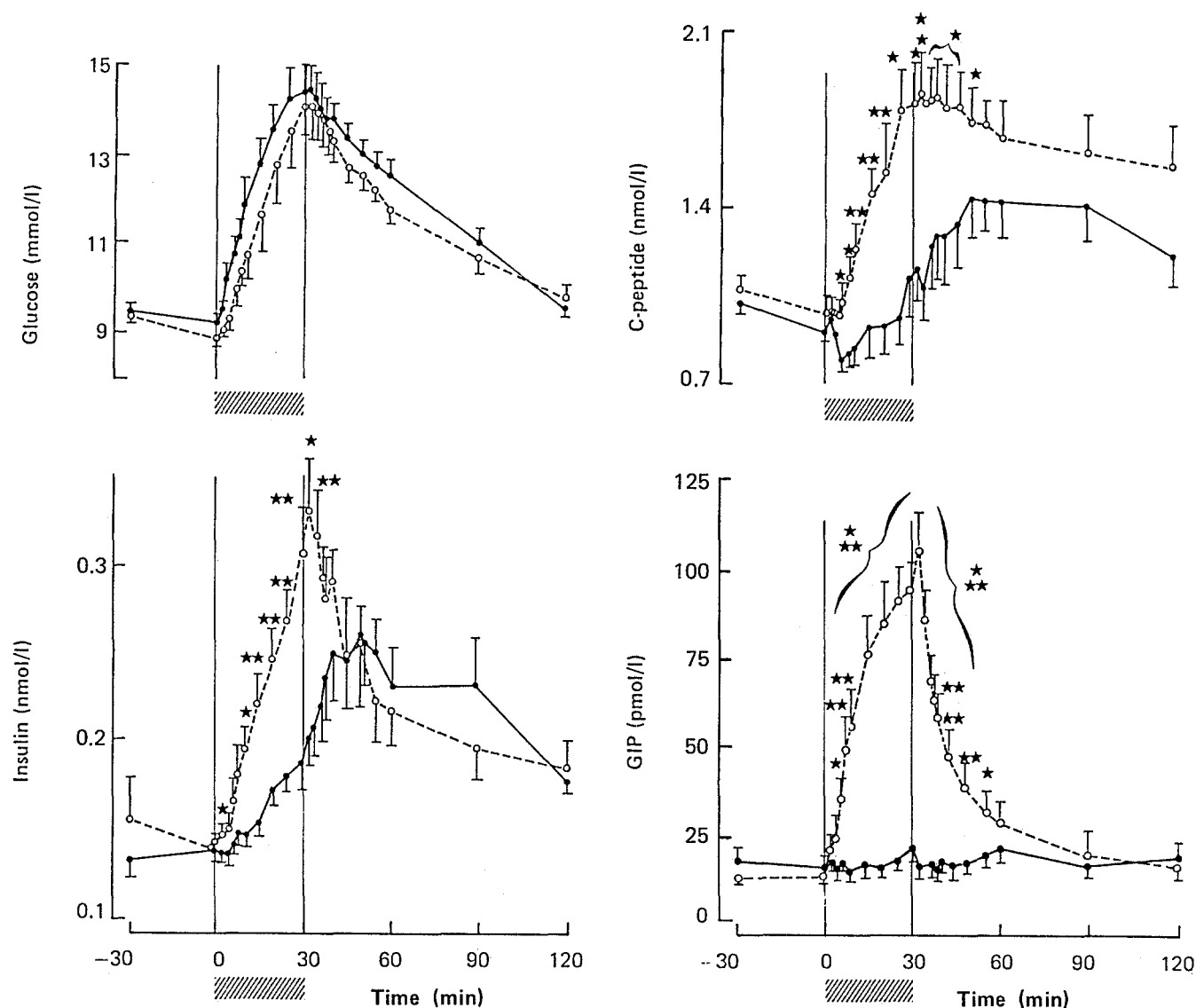


Fig. 2. Intravenous glucose tolerance test (IVGTT: 50 ml 50% dextrose) infused from 0–30 min (—) and an IVGTT plus an infusion of porcine GIP ($0.75 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) from 0–30 min (---) in Type 2 diabetic patients ($n=6$). Mean \pm SE concentrations of plasma glucose, insulin, C-peptide and GIP. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Discussion

This study clearly shows that intravenous infusion of highly purified porcine GIP, previously shown to be a strong potentiator of insulin release in the pig [24], potentiates B-cell secretion in response to intravenous glucose and results in improved glucose tolerance in both normal subjects and newly diagnosed Type 2 diabetic patients.

In the normal subjects the peak glucose values following a 25 g intravenous glucose load are similar to levels seen previously with oral glucose (75 g) or a standard test meal (500 kcal) in normal subjects [29, 30]. Similarly the glucose response in the untreated Type 2 diabetic patients approximates to the range expected following an oral glucose tolerance test or meal test [31, 32]. Following the GIP infusion in normal subjects there was a definite and highly significant im-

provement in glucose tolerance with mean glucose concentrations returning to fasting levels earlier at 50 min. In the diabetic patients the effect on glucose tolerance was less marked, with a trend for lower mean glucose values with the GIP infusion; the difference reaching significance at 45 and 60 min ($p < 0.05$).

The improvement in glucose tolerance, particularly in the normal subjects, resulted from the considerable enhancement of B-cell secretion with the addition of GIP to the intravenous glucose infusion. In the normal subjects the addition of GIP resulted in a prompt increase in plasma insulin reaching a peak of 0.76 nmol/l which represents a 123% increase above the mean peak value seen after the intravenous glucose alone. Intravenously administered GIP in the normal subjects converted the insulin response seen during the IVGTT to that normally observed in response to either an oral glucose or meal tolerance test [22, 23]. The in-

sulin level was significantly elevated as early as 10 min after the commencement of the GIP infusion, continuing for a further 25 min, falling 5 min after the cessation of GIP and intravenous glucose. A similar increase was observed in C-peptide concentrations with a prompt rise in C-peptide during the GIP infusion to a peak value 82% higher than that seen after glucose alone. These results support the earlier findings in pigs [24].

In the diabetic group there was a less marked but nevertheless significant improvement in B-cell secretory function following the infusion of GIP. From elevated basal insulin levels (~ 0.12 nmol/l) in the Type 2 diabetic patients a slow increase to a delayed peak of only 0.26 nmol/l at 50 min was seen following intravenous glucose. With the addition of GIP there was a more prompt increase in insulin levels, reaching a higher and earlier peak of 0.33 nmol/l at 35 min. This represents a 27% increase above the peak level seen after the glucose alone compared with 123% in normal subjects. A similar pattern was observed in the C-peptide response, which increased more rapidly to an earlier peak of 1.78 nmol/l at 35 min, representing a 20% increase above the maximum value achieved with the intravenous glucose. The smaller increase in insulin secretion and the fact that the diabetic group were probably more insulin resistant may account for the relatively small improvement in glucose tolerance in the Type 2 diabetic patients. These findings are consistent with those of Nauck et al. [33] who described a reduced incretin effect in Type 2 diabetic patients compared with normal control subjects. They estimated that the contribution of incretin factors to total insulin responses was $72.8 \pm 6.9\%$ in control subjects and $36.0 \pm 8.8\%$ in diabetic patients. Our results demonstrate that this difference in incretin response in Type 2 diabetic patients results mainly from a decreased response to GIP.

There are two possible explanations for this relatively small enhancement in B-cell function in response to an IVGTT by GIP in the diabetic group. Firstly B-cell function is clearly abnormal in this group with a rather slow rise in levels of insulin and C-peptide to markedly reduced peak concentrations, and it is possible that the relatively small enhancement in secretion produced by the GIP infusion represents the maximum secretory capacity of the damaged B cells.

We have recently presented data regarding the metabolic profiles in a group of Type 2 diabetic patients ($n=65$) following oral glucose and meal tolerance tests [31]. The peak insulin response in a group of similar severity following the meal test was considerably higher than the control study with intravenous glucose alone (0.57 ± 0.4 nmol/l) suggesting the B cells in these patients should have further secretory potential. An alternative explanation for decreased insulin potentiation by GIP in the Type 2 diabetic patients is that there is decreased sensitivity of the B cells to the

insulinotropic effect of GIP as suggested by Nauck et al. [33].

The GIP infusion rate in this study resulted in plasma GIP concentrations well within the post prandial range that one sees in both normal subjects and Type 2 diabetic patients [31, 32]. Porcine GIP differs from human GIP with respect to its amino acid sequence [28]. The use of porcine GIP makes it difficult to assess plasma levels and to compare, in terms of biological activity, post prandial GIP responses to those during infusions by radioimmunoassay methods. Similar radioimmunoassay readings for post prandial (endogenous human) and post infusion (exogenous porcine) GIP do not necessarily imply identical plasma concentrations with identical biological activity. This problem should, however, be minimised as the antiserum (R65) used in this study does not discriminate between porcine and human GIP, since the sequence recognised by R65 is identical in both molecules [23]. The large increase in insulin secretion particularly in the normal subjects therefore resulted from a physiological increase in the plasma GIP concentration. This indicates that GIP may be the major incretin factor in the physiological state in man.

In conclusion we have shown that GIP is insulinotropic in both normal subjects and newly diagnosed Type 2 diabetic patients at physiological plasma concentrations and results in improved glucose tolerance, although the effect is less marked in the Type 2 diabetic patients.

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