Glucose-dependent Insulinotropic Polypeptide Augmentation of Insulin

Physiology or Pharmacology?

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SUMMARY

Glucose-dependent insulinotropic polypeptide (GIP) is said to be a major physiologic factor in the augmentation of the insulin response to oral glucose. Whether GIP promotes insulin release at physiologic concentrations of glucose or GIP, however, is questionable. To investigate this further, volunteers were infused with 10, 20, or 40 g intravenous (i.v.) glucose, with or without simultaneous GIP infusion, to produce plasma levels of GIP or glucose similar to those seen after oral glucose. The effect of 40 g i.v. glucose with three times the original dose of GIP was also investigated.

No significant enhancement of glucose-stimulated insulin secretion was seen when GIP was infused with 10 or 20 g i.v. glucose; however, with 40 g a doubling of the insulin response occurred. The higher dose of GIP caused a further increase in insulin response (30-min increment, 972 ± 191 pmol/L; compared with glucose alone, 356 ± 100 pmol/L, $P < 0.01$; and compared with low GIP, 602 ± 247 pmol/L, $P < 0.02$). The glucose increment after the 40-g i.v. dose was +9.2 mmol/L.

The concentration of GIP and glucose required to produce significant potentiation of the insulin response appears to be in the pharmacologic, rather than physiologic, range. DIABETES 33:389–393, April 1984.

The marked improvement in glucose tolerance observed after oral glucose, compared with glucose given by the intravenous (i.v.) route, is mainly due to the greatly augmented insulin response seen with the former.[1,2] This potentiation suggests the possibility of insulin-augmenting factors from the upper small intestine.[3] In 1969, Unger and Eisentraut used the term "enteroinsular axis" to describe the positive gut effects on the endocrine pancreas.[4] Much earlier, La Barre and colleagues demonstrated that an impure preparation of secretin improved glucose tolerance.[5,6] The term incretin was employed to describe the active component responsible for this improvement. Although, in the strict sense, these terms apply to the endocrine pancreas as a whole, they are most commonly used to describe effects on the beta cell only.

The identity of this gut messenger still remains unknown, but it is generally agreed that one or more of the polypeptide hormones of the upper small intestine are involved. These candidate incretins include gastrin, secretin, glucose-dependent insulinotropic polypeptide (GIP), vasoactive intestinal polypeptide, cholecystokinin-pancreozymin, and enteroglucagon.[7–10] Of these, however, only GIP is currently regarded as a serious contender.[11–15]

Previous reports on the effects of infused GIP in man[11–16] have demonstrated that hyperglycemia is a prerequisite for this hormone to exert its insulinotropic effect. However, the doses of GIP and glucose used have been outside the normal physiologic range. The present study was undertaken, first, to investigate the degree of glycemia required for GIP infused at physiologic concentrations to cause a significant augmentation of insulin release, and, second, to ascertain the levels of circulating GIP necessary to reproduce the magnitude of insulin release seen after oral glucose.

MATERIALS AND METHODS

Experimental subjects. Five healthy volunteers were investigated, two males and three females, with a mean age of 27.4 yr (range, 22–35 yr) and of normal weight (62.8 ± 5.0 kg, mean ± SEM). All subjects had normal glucose tolerance and no family history of diabetes mellitus. None of the volunteers were taking medication immediately before or during the period of study. Fully informed consent was obtained from each subject, and ethical permission for the infusion of GIP was obtained from the Ethical Committee of the Hammersmith Hospital. All investigations were carried out in the morning, after a fast of at least 14 h duration. Subjects were studied in the Metabolic Unit, in a recumbent position, after an initial period of bed rest.

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Preparation of GIP for infusion. Pure porcine GIP (Prof. J. C. Brown, University of British Columbia, Vancouver, Canada) was weighed directly on an electrostatic balance (Cahn-M10, Cahn Instruments, Paramount, California). A sterile solution was obtained by dissolving the GIP in 0.9% saline containing 1.5% propyl alcohol. This solution was then immediately lyophilized to 1.33 Pa and sealed in vacuo.

At the time of infusion, the GIP was redissolved in 0.15 M saline containing 200 μmol/L human serum albumin (HSA, Lister Blood Products Laboratory, Elstree, Herts, United Kingdom), again under sterile conditions.

Glucose and GIP infusions. Volunteers were investigated at 0730 h after an overnight fast. In random order, on separate occasions, they were given 10 g, 20 g, or 40 g of i.v. glucose as a 20% dextrose solution, over a 30-min period. These infusions were accompanied with either 0.9% saline infusion or an infusion of 0.5 pmol/kg min^{-1} GIP also over 30 min. A further infusion of 40 g glucose was also given, but this time accompanied by three times (1.5 pmol/kg min^{-1}) the dose of GIP. All infusions were given into an antecubital vein via a 19-gauge butterfly cannula using a syringe ram pump (Harvard compact infusion pump, Harvard Instruments, Millis, Massachusetts) through a short, sterile, single-use manometer line (Portex tubing, Hythe, Kent, United Kingdom).

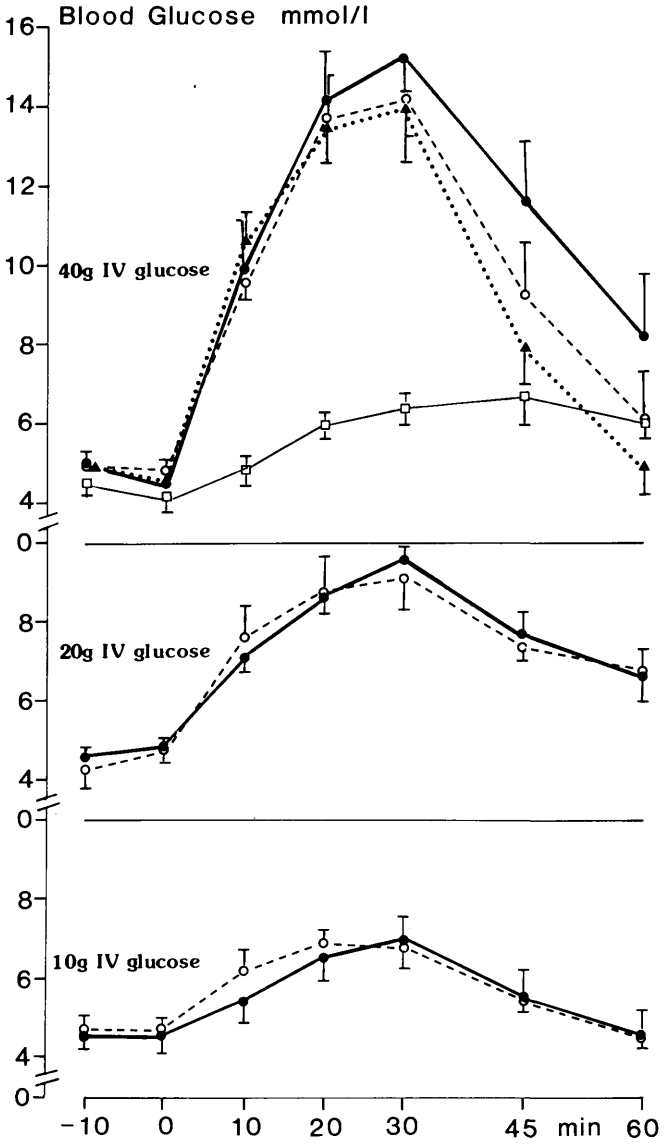

FIGURE 1. Glucose levels after 10 g, 20 g, and 40 g i.v. glucose with 0.0 (●), 0.5 (○), or 1.5 pmol/kg min^{-1} GIP infusion (▲), or 100 g oral glucose (□).

Our previous work has shown that infusion of GIP at a rate of 0.5 pmol/kg min^{-1} resulted in circulating concentrations of GIP similar to those seen after oral glucose or a mixed meal.[17]

To allow for losses due to adsorption of the peptide to the infusion line, the infusate was flushed through the tubing externally for 5 min before infusion.

To quantify the GIP, insulin, and blood glucose responses to oral glucose, each subject was also given a drink of 300 ml water containing 100 g dextrose.

Blood for estimation of GIP, insulin, and blood glucose was drawn from a catheter placed in the contralateral arm. This was kept patent by 0.9% saline and 3.8% sodium citrate solution.

Blood sampling. Eleven milliliters of whole blood was taken from each subject 10 min before, at the start of infusion (time 0), and at 10, 20, 30, 45, and 60 min afterward. One milliliter of this blood was placed in fluoride-oxalate tubes for measurement of blood glucose by a glucose-oxidase method modified for use on an autoanalyzer.[18] The remaining 10 ml was put into chilled lithium-heparin tubes containing 400 KIU/ml aprotinin (Trasylol, Bayer Company, FRG). The blood was immediately centrifuged for 5 min at 1600 × *g*, the plasma decanted and stored deep frozen (−20°C) until assay.

Hormone assays. Insulin was measured using commercially obtained reagents. The antiserum was raised against human insulin in guinea pigs (Wellcome Ltd., Beckenham, Kent, United Kingdom). Radiolabeled ^{125}I-insulin was used as tracer (IM 38, Radiochemical Centre, Amersham, Bucks, United Kingdom). After a 72-h incubation period, the free and bound fractions were separated by dextran-coated charcoal. The sensitivity of the assay (± 2 SD from the zero standard) was 6 pmol/L. Intra-assay variation was 8.2%.

GIP was estimated by a radioimmunoassay that has been fully described elsewhere.[19] The antibody raised in a rabbit, and used at a final dilution of 1:96,000, bound approximately 50% of the 1.5 fmol of ^{125}I-GIP added to each tube, in the absence of unlabeled hormone.

Labeled GIP was prepared by a modification of a lactoperoxidase method.[20] The specific activity of this preparation was 65 Bq/fmol at the time of assay. The sensitivity of the assay was 3 pmol/L and intra-assay variation for the physiologic range was 7.6%.

All samples were measured in single-hormone assays as duplicates, set up on one day to overcome possible losses due to thawing and refreezing.

Analysis of data. Individual datum points are expressed as mean ± SEM (df = n − 1). Hormone secretion was calculated as the integrated incremental response (IIR). This was determined by calculation of the area under the curve by the trapezoidal method; the component representing basal secretion was then subtracted. Significance of difference was obtained by application of Student's paired *t* test. Differences of $P < 0.05$ were considered to be significant.

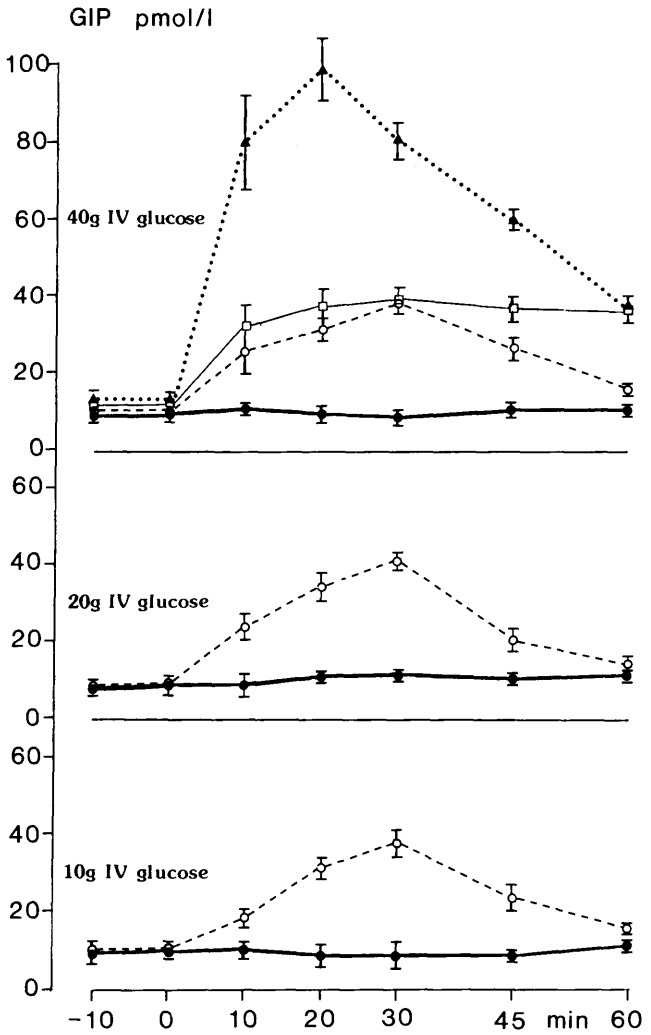

FIGURE 2. GIP response to 10 g, 20 g, and 40 g i.v. glucose with 0.0 (●), 0.5 (○), or 1.5 pmol/kg min^{-1} GIP infusion (▲), or 100 g oral glucose (□).

RESULTS

Blood glucose. Levels for each experiment are shown in Figure 1. Glucose concentrations over the experimental period (IIR) are shown in Figure 4A. After the 10-g i.v. glucose infusion with saline, a peak glucose level of 6.9 ± 0.7 mmol/L was achieved at 30 min from a mean basal value of 4.5 ± 0.4 mmol/L. After the 20-g dose, the 30-min peak was 9.6 ± 0.4 mmol/L from a mean basal value of 4.7 ± 0.2 mmol/L. The 40-g i.v. glucose gave a peak glucose at 30 min of 15.3 ± 1.4 mmol/L from a mean basal concentration of 4.7 ± 0.3 mmol/L. Simultaneous infusions of GIP did not significantly alter these responses. The peak glucose of 6.6 ± 0.6 mmol/L after oral loading occurred at 45 min, which approximated that of the 10-g i.v. glucose infusion.

GIP. Figure 2 shows the concentration of plasma GIP for each experiment. Figure 4B shows the areas under the GIP curve obtained during each infusion. Intravenous glucose alone produced no change in plasma concentrations of GIP. After infusion of low-dose GIP, a mean peak was achieved at 30 min (from all low-dose infusions) of 38.6 ± 3.3 pmol/L, from a mean basal concentration of 9.6 ± 1.3 pmol/L. This did not differ significantly from the peak mean GIP secretion seen after 100 g oral glucose (38 ± 3 pmol/L at 60 min). The peak mean plasma GIP concentration obtained after high-dose GIP infusion occurred slightly earlier, at 20 min, and was 99.2 ± 8.1 pmol/L. The 30-min value was 80.4 ± 5.2 pmol/L, with a basal GIP of 9.5 ± 0.8 pmol/L.

Insulin. The time course of the response to 10 g, 20 g, and 40 g i.v. glucose with and without GIP is shown in Figure 3. The integrated incremental response of insulin for each experiment is shown in Figure 4C. Simultaneous infusions of GIP and 10 g or 20 g glucose resulted in no significant increase of the insulin response compared with i.v. glucose alone. Peak insulin concentrations to i.v. glucose alone occurred at 20 min for both glucose doses and was 128 ± 16.4 pmol/L for 10 g and 191.2 ± 25.8 for 20 g. The basal insulin concentrations were 48.2 ± 10.3 and 44.4 ± 7.5 pmol/L, respectively. With the greater glycemia resulting from the 40 g glucose, the infusion of low-dose GIP caused an approximately twofold increase in the response of insulin (30-min increment, 602 ± 247 pmol/L; compared with 40 g i.v. glucose alone, 356 ± 100 pmol/L, $P < 0.05$). High-dose GIP caused a further potentiation of the insulin response (30-min increment, 972 ± 191 pmol/L; $P < 0.01$ versus glucose alone; $P < 0.02$ versus low-dose GIP). This was of similar

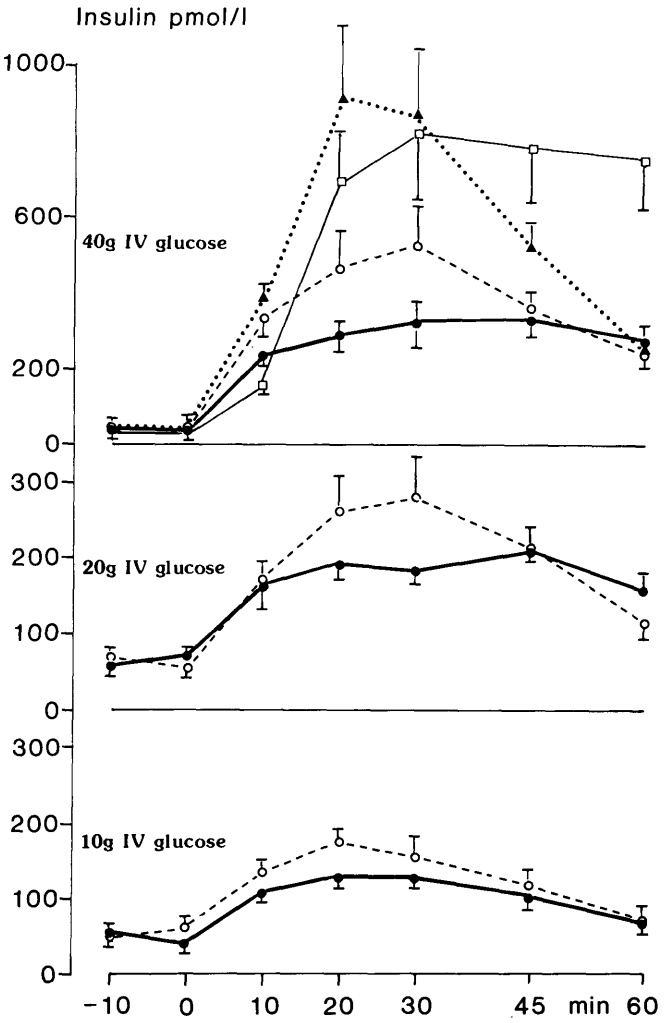

FIGURE 3. Insulin response to 10 g, 20 g, and 40 g i.v. glucose with 0.0 (●), 0.5 (○), or 1.5 pmol/kg min^{-1} GIP infusion (▲), or 100 g oral glucose (□).

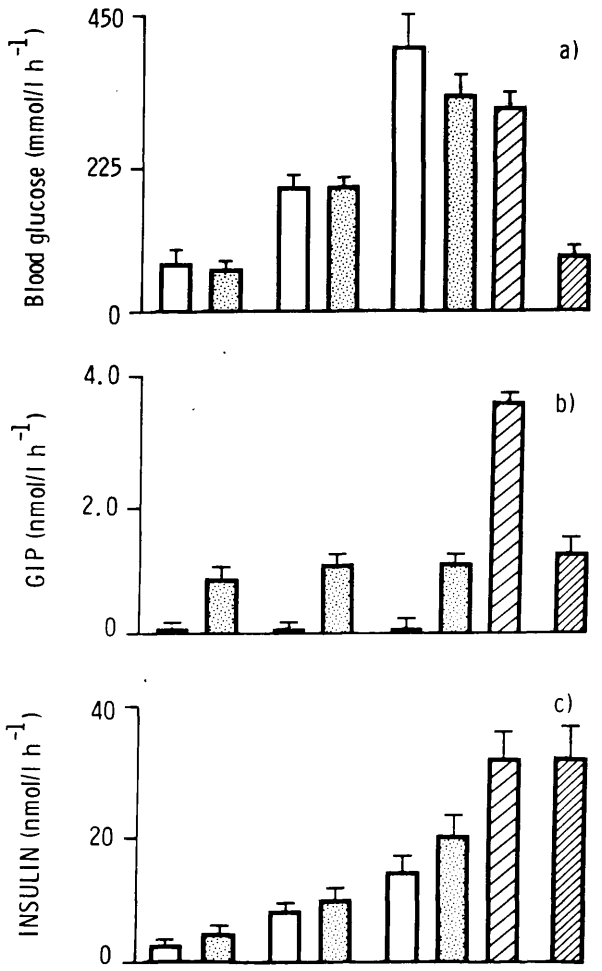

FIGURE 4. (a) Integrated incremental response of blood glucose (mmol/L h^{-1} ± SEM) after 10, 20, and 40 g i.v. glucose without (open bars) or with a GIP infusion of 0.5 pmol/kg min^{-1} (stippled bars) or 1.5 pmol/kg min^{-1} (open-hatch bar), and after 100 g oral glucose (close-hatch bar). (b) Integrated incremental responses of GIP (nmol/L h^{-1} ± SEM) after 10, 20, and 40 g i.v. glucose without (open bars) or with a GIP infusion of 0.5 pmol/kg min^{-1} (stippled bars) or 1.5 pmol/kg min^{-1} (open-hatch bar), and after 100 g oral glucose (close-hatch bar). (c) Integrated incremental responses of insulin (nmol/L h^{-1} ± SEM) after 10, 20, and 40 g i.v. glucose without (open bars) or with a GIP infusion of 0.5 pmol/kg min^{-1} (stippled bars) or 1.5 pmol/kg min^{-1} (open-hatch bar), and after 100 g oral glucose (close-hatch bar).

magnitude to the response observable after oral glucose (30-min increment, 828 ± 183 pmol/L).

DISCUSSION

The results of this study demonstrate that exogenous infusion of GIP, at a dose that reproduces the endogenous release of this hormone after oral glucose, does not produce a significant augmentation of the insulin response unless the accompanying blood glucose concentrations are clearly supranormal. In addition, the concentrations of exogenous GIP required to achieve insulin release comparable with that of postprandial insulinemia is some three times higher than that seen physiologically. Previous experiments using exogenous infusions of GIP have suggested that this hormone is potently insulinotropic. Dupre et al.[11] infused GIP at a rate of approximately 2.8 pmol/kg min^{-1} (to achieve circulating concentrations of 200 pmol/L) with a simultaneous glucose infusion, which, in the absence of infused GIP, produced an increment of 6.1 mmol/L in blood glucose. This resulted in an incremental rise of insulin of 510 pmol/L. Elahi et al.,[16] using a glucose-clamp technique, infused GIP at half the dose used by Dupre and colleagues, which was comparable with the high dose used in this study, yet they achieved circulating levels of over 500 pmol/L GIP. The ensuing insulin response, on a background of a glucose concentration of 7.9 mmol/L above the fasting level, was in excess of 4000 pmol/L. However, when the same experiment was performed at lower and more physiologic glucose concentrations (+3.0 mmol/L) the peak increment of insulin approximated 330 pmol/L, which was not much greater than the insulin response elicited by the i.v. glucose alone.

The discrepancies in the measured circulating GIP levels of the two studies described above can only be partially explained in terms of duration of infusion. Dupre and colleagues infused GIP for 30 min only, whereas Elahi et al. continued for 90 min. The assay system used for both studies[21] was the same, suggesting that differential peptide losses due to adsorption to infusion lines, or other causes, may be significant.[22,23] In the present study, care was taken to minimize peptide losses by allowing saturation of the infusion system, and all dose-rate calculations were made on the basis of the radioimmunoassayable GIP, both in the infusates and in the plasma.

While the present study cannot support the view that GIP is a physiologically important insulinotropic hormone, it does give results consistent with previous results, and again demonstrates the glucose dependency of GIP's insulin-releasing properties.[11,16,24] It is evident that the plasma glucose concentration required before GIP can enhance insulin secretion is far higher in both this study (+10.6 mmol/L) and in that of Elahi et al.(+7.9 mmol/L) than would be seen in the peripheral blood of healthy subjects or, indeed, at the beta cell, after an oral glucose load in man.

Anderson et al.[24] have shown that there is a threshold rise in glucose of +1.1 mmol/L before GIP can be insulinotropic. This was deduced from studies with oral glucose and a glycemic-clamp technique. Their use of oral glucose, unfortunately, does not preclude stimulation of insulin release by some gut factor other than GIP. It is also clear from infusion studies that significantly higher blood glucose concentrations are usually required before GIP potentiates any insulin secretion.

The results of the experiments described here also suggest that the degree of enhancement of insulin secretion is dependent on the concentration of plasma GIP when the same high concentration of circulating glucose is reached. The amount of GIP required to produce an insulin concentration of similar magnitude to that observed after oral glucose, however, is outside the physiologic range, at least as found by this laboratory,[17,19,25–27] especially when glucose is used as the only stimulant.

All the studies described here, and, indeed, all exogenous studies published to date have been carried out using porcine GIP, and it is possible that this is different from human GIP and, therefore, less potently insulinotropic in man. We have, however, shown previously that human and porcine GIP behave similarly on chromatographic analysis, and that

they have closely similar plasma half-lives in man.[17,28] We have also demonstrated that endogenous human GIP, stimulated by fat on a background hyperglycemia of +2.8 mmol/L above basal, failed to augment the insulin response beyond that stimulated by i.v. glucose alone.[29]

On the basis of these findings, we suggest that it is not proven that the role of GIP in normal, healthy man is primarily one of augmentating insulin release. It is possible that the human GIP-like molecule or a related peptide may be more potently insulinotropic, but, in the absence of any data on this point, it must remain a speculation. It also remains a possibility that GIP, together with other gut hormones or meal-stimulated neurotransmission, might contribute to enhanced insulin secretion. It is conceivable that under conditions of pathologic hyperglycemia, GIP may act as a "fail-safe" mechanism, and this would be consistent with the raised GIP found by some workers in type II diabetes mellitus.[30,31]

There is the possibility that a hormone such as GIP, which is secreted in response to both fat and glucose, is important in the maintenance of neonatal glucose homeostasis. Since breast milk is rich in fat and lactose, the release of GIP to switch on glucose-homeostatic and insulin-induced lipogenic mechanisms provides protection from glycemic stress and allows the laying down of adipose tissue for insulation and energy storage.[32,33]

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