Effects of Ingestion of Triglyceride or Galactose on Secretion of Gastric Inhibitory Polypeptide and on Responses to Intravenous Glucose in Normal and Diabetic Subjects

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SUMMARY

Responses of plasma immunoreactive gastric inhibitory polypeptide (IRGIP) to oral triglyceride or galactose were compared in normal and mildly diabetic (non-insulin-dependent) subjects. After triglyceride the responses of IRGIP were similar, but after galactose those of the diabetics were slightly exaggerated. Both stimuli evoked increments of plasma immunoreactive glucagon (IRG) in diabetics but not in normal subjects. Plasma immunoreactive insulin (IRI) did not change.

In normal subjects given oral triglyceride or galactose followed by intravenous (I.V.) glucose the early-phase response of plasma IRI was enhanced and glucose tolerance improved. In the diabetics, oral triglyceride did not affect insulin release or glucose tolerance after I.V. glucose; oral galactose elicited a slight increase of insulin release without improving glucose tolerance. In the diabetics the rise in plasma IRG after ingestion of triglyceride or galactose was maintained after I.V. glucose.

It is concluded that endogenous GIP is insulinotropic and that there is partial resistance to this action in diabetes. The results were compatible with feedback inhibition of GIP secretion by insulin and with the suggestion that the rise of plasma IRG associated with secretion of GIP in diabetics may be due to the glucagonotropic action of this peptide. DIABETES 27:327-33, March, 1978.

Gastric inhibitory polypeptide (GIP) administered by intravenous infusion augments glucose-stimulated insulin secretion in normal subjects,[1] may stimulate glucagon secretion in subjects with diabetes mellitus[2] or liver disease,[3] and can elicit insulin and glucagon release from rat pancreas in vitro.[2] Among the nutrients that evoke secretion of GIP during absorption from the gut,[2,4,5] galactose and triglyceride have little or no direct action on insulin or glucagon secretion. In the present study these nutrients were ingested by normal and diabetic subjects in order to determine whether endogenous GIP release is associated with responses of the endocrine pancreas corresponding to those attributed to exogenous porcine GIP.

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SUBJECTS, METHODS, AND PROCEDURES

Twenty-two normal (mean age 30±10 years, range 18-46 years) and 29 diabetic volunteers (mean age 37±12 years, range 19-54 years) took part in this study. None of the diabetics had been treated with insulin, but some were receiving oral hypoglycemic agents; these medications were interrupted for a minimum period of one week preceding a test. All subjects were within 10 per cent of ideal body weight, currently stable in weight, and fasted for 12 hours overnight before procedures.

Plasma glucose was determined on a Beckman glucose analyzer by a specific glucose oxidase technique. IRI was assayed by a modification of the radioimmunoassay procedure employing dextran-coated charcoal for separation of bound and free moieties of pep-

tide.[6] This assay was sensitive to IRI at a concentration ≥2.5 μU./ml. of plasma on addition of 0.1 ml. plasma. Aprotinin (Trasylol) was added (to a concentration of 500 K.I.U./ml.) to samples of plasma for IRG assays. IRG was assayed with iodine^{125} labeled by the chloramine-T method and purified on an anion-exchange column.[7] In studies with the antiserum employed (donated by Dr. J. Manns, University of Saskatchewan), total plasma IRG and changes in plasma IRG after ingestion of glucose were similar to those estimated with antibody 30-K in this laboratory, and the changes were shown by gel chromatography to be largely confined to the 3,500 component of plasma IRG. Changes in plasma IRG in the present study were therefore taken to represent pancreatic glucagon and are presented as differences from baseline. The assay detected IRG at concentrations ≥25 pg./ml. in plasma, with addition of 0.1 ml. of plasma. Plasma immunoreactive GIP was assayed with antibody donated by Dr. J. C. Brown, University of British Columbia; this assay detected GIP at concentrations ≥50 pg./ml. plasma, with addition of 0.1 ml. of plasma.[2]

Basal venous blood samples were taken before administration of test substances, and thereafter samples were drawn at intervals for estimation of glucose, IRI, and IRGIP. To test effects of ingestion of triglyceride or galactose, subjects took 100 ml. emulsified corn oil (Lipomul, 66 gm. triglyceride) or galactose (50 gm. in 100 ml. water) as drinks consumed within two minutes. To examine responses to these stimuli in the presence of elevated plasma glucose concentration, the effects of ingestion of triglyceride or galactose followed by intravenous injection of glucose were tested. Each subject underwent standard intravenous glucose tolerance tests (25-gm. glucose intravenous (I.V.) bolus injection) on two occasions separated by three to seven days. On one occasion (varied order) intravenous glucose was preceded (15 minutes) by oral triglyceride or galactose: the interval was selected after preliminary studies showed that plasma IRGIP attained potentially insulinotropic[1] levels by this time.

The statistical significance of differences between mean values in diabetic and normal groups was tested by Student's *t*-test. When paired studies were performed the significance of differences was tested by the paired *t*-test, which was also applied to the means of differences from 0 time values in order to determine whether changes of glucose, IRI, IRG, or IRGIP were significant within groups.

RESULTS

There were no systematic differences in the plasma concentrations of IRI and IRGIP of normal and dia-

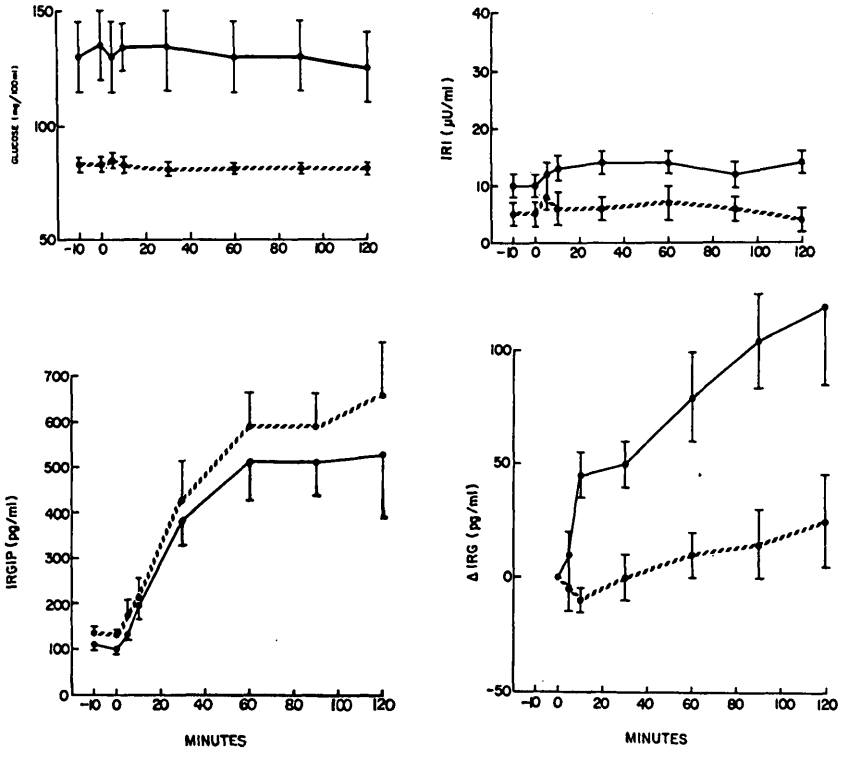

FIGURE 1

Effects of ingestion of triglyceride on plasma glucose, immunoreactive insulin, immunoreactive glucagon, and immunoreactive GIP in nine normal and 12 diabetic subjects. (Interrupted lines show normal subjects, vertical bars S.E.M.)

betic subjects in the basal state, but mean plasma glucose was significantly higher in the diabetics under all conditions, as shown in the figures. Basal plasma total IRG was not significantly different in normal (143±21 pg./ml. S.E.M.) and diabetic (164±21 pg./ml.) subjects, and changes of IRG from values at 0 time are shown in the figures.

Studies with Ingestion of Triglyceride Alone

The effects of ingestion of triglyceride on plasma glucose, IRI, IRGIP, and IRG in normal and diabetic subjects are shown in figure 1. While mean plasma IRGIP rose progressively in both groups, there was no change in mean plasma glucose or IRI. The rise of IRGIP was significant in terms of changes from baseline by 10 minutes in both groups ($p<0.05$) and continued through the two hours of the study; there was no significant difference between the responses of normal and diabetic subjects. Mean plasma IRG did not change significantly in the normal subjects; by contrast, in the diabetics it rose progressively, and this was significant by 10 minutes ($p<0.01$) with mean maximum increment of 146±31 pg./ml. ($p<0.005$).

Studies with Ingestion of Galactose Alone

The effects of ingestion of 50 gm. galactose on plasma glucose, IRI, IRGIP, and IRG in normal and diabetic subjects are shown in figure 2. Mean plasma glucose rose in both groups of subjects. The increments were statistically significant in the normal subjects only at 30 minutes (mean 22±1.6 mg./100 ml., $p<0.001$). In the diabetic subjects the rise in plasma glucose was also significant by 30 minutes (mean 23±4.4 mg./100 ml., $p<0.01$) and remained so through 90 minutes (mean 15±4.8, $p<0.05$). Mean plasma IRI rose slightly in the normal group; this increment was significant by 10 minutes (7.0±2.2, $p<0.05$ μU./ml.) and persisted through 60 minutes (16±6.1 μU./ml., $p<0.05$). The mean levels of IRI in the plasma rose less in the diabetics, and this change was not statistically significant. Mean plasma IRGIP rose promptly after ingestion of galactose in normal and diabetic subjects and reached a peak at 30 minutes in both groups. Mean plasma IRGIP was higher in the diabetic than normal subjects 10 minutes after ingestion of galactose ($p<0.02$), but the difference between the two groups at other times was not statistically significant. There was no significant change in mean plasma IRG in the normal subjects, whereas in the diabetics the modest peak mean increment of 36±12 pg./ml. was significant ($p<0.05$) at 30 minutes after the drink.

Effects of Ingestion of Triglyceride on Response to Intravenous Glucose

When glucose was given I.V. after ingestion of triglyceride there was enhancement of the initial (five minutes postinjection) response of plasma IRI (88±15

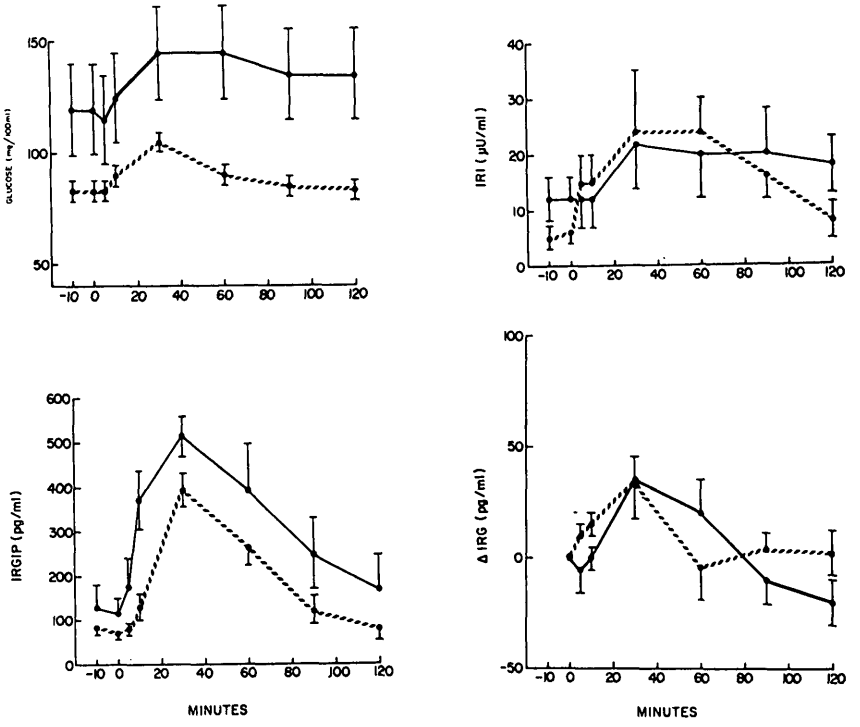

FIGURE 2

Effects of ingestion of galactose on plasma glucose, immunoreactive insulin, immunoreactive glucagon and immunoreactive GIP in seven normal and six diabetic subjects. (Interrupted lines show normal subjects, vertical bars S.E.M.)

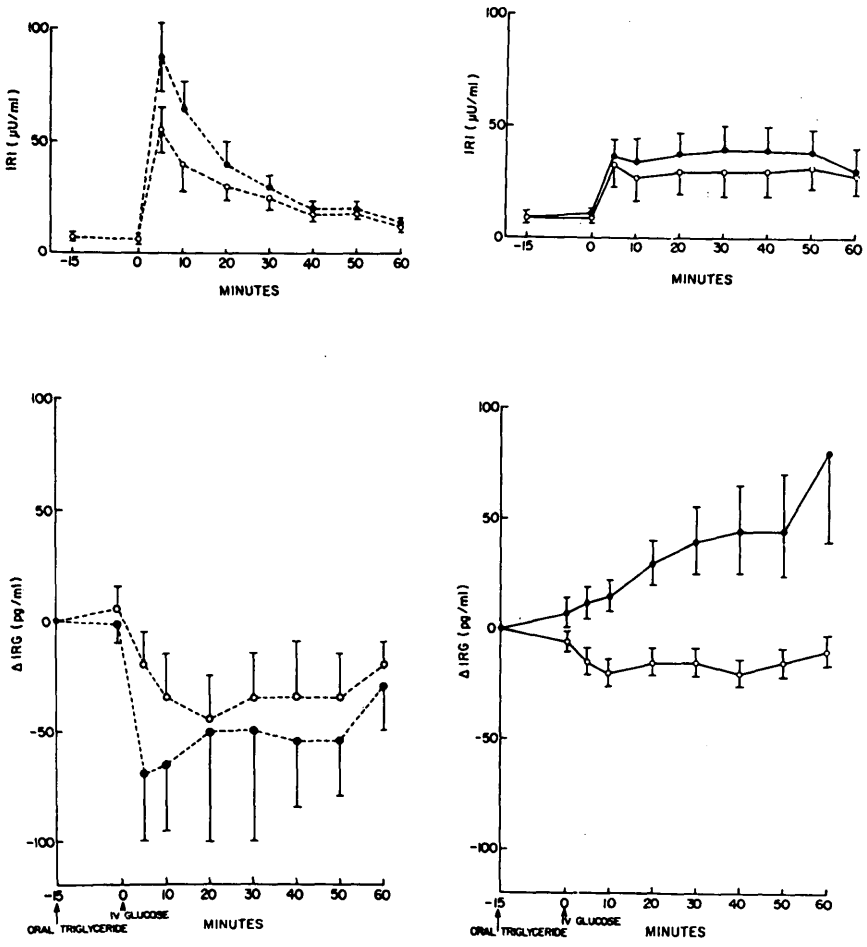

FIGURE 3

Effects of ingestion of triglyceride on responses to subsequent intravenous injection of glucose in six normal and seven diabetic subjects showing responses of plasma IRI and IRG. (Interrupted lines show normal subjects, solid symbols show test with oral triglyceride, vertical bars S.E.M.)

μU./ml., cf. 55±10 μU./ml., p <0.01, figure 3). The mean value for plasma IRI after I.V. glucose in the diabetics was slightly higher after oral triglyceride, but this difference was not significant at any time.

After oral triglyceride the rate constant for glucose disappearance was significantly increased in the normal subjects, but in the diabetics there was no difference between the rates of disappearance of glucose on the two occasions (table 1).

After injection of glucose with or without oral triglyceride, plasma IRG was suppressed in the normal subjects. The mean maximum suppression was 55±18 pg./ml. (p <0.05) after glucose alone and 88±33 pg./ml. (p <0.05) after glucose and oral triglyceride; the difference between the mean decrements was not statistically significant. In the diabetics, whereas mean plasma IRG fell after injection of glucose alone (mean maximum decrement 25±3.3 pg./ml., p <0.01), there was a large rise of plasma IRG when triglyceride was given, with a mean maximum increment of 66±21 pg./ml. (p <0.05).

After intravenous glucose alone, mean plasma IRGIP tended to fall in normal subjects, as noted in

TABLE 1

Fasting plasma glucose (FPG) and rate constants for glucose disappearance (K) after I.V. glucose with and without oral galactose or triglyceride in normal and diabetic subjects

Test	Normal subjects		Diabetic subjects	
	FPG mg./100 ml.	K %/min.	FPG mg./100 ml.	K %/min.
I.V. glucose alone	89±5	1.7±0.12	107±11	0.8±0.08
Oral galactose + I.V. glucose	87±3	3.3±0.30	101±9	0.7±0.05
I.V. glucose alone	86±3	1.8±0.15	108±8	0.7±0.10
Oral triglyceride + I.V. glucose	80±2	2.8±0.20	110±8	0.8±0.15

earlier studies,[4] but did not change significantly in either normal or diabetic subjects. The means of plasma IRGIP after ingestion of triglyceride with subsequent I.V. injection of glucose in normal and diabetic subjects are compared in figure 4. When I.V. glucose was given after oral triglyceride the response of plasma IRGIP in normal subjects differed from that observed when triglyceride was given alone (figure 1). Mean plasma IRGIP rose through the early period after the drink on both occasions, but after I.V. glucose there was a secondary fall in the interval 10 to 30 minutes in all normal subjects, and the mean of paired differences between individual values at 10 minutes and 30 minutes was statistically significant (mean 99±34 pg./ml., $p < 0.05$). There was no secondary fall of plasma IRGIP after I.V. glucose in the diabetics.

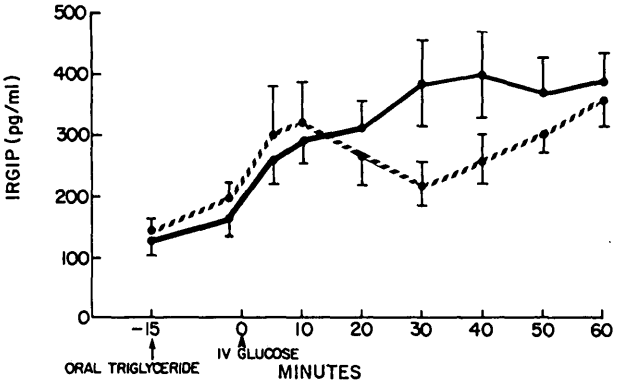

FIG. 4. Changes in plasma IRGIP after ingestion of triglyceride followed by intravenous injection of glucose in six normal and seven diabetic subjects. (Interrupted lines show normal subjects, vertical bars S.E.M.)

Effects of Ingestion of Galactose on Responses to Intravenous Glucose

The mean plasma IRI five minutes after I.V. glucose in normal subjects was significantly greater when oral galactose was given (139±40 μU./ml. vs. 66±11 μU./ml., mean paired difference $p < 0.01$, figure 5). In the diabetics, mean plasma IRI five minutes after I.V. glucose was also higher when oral galactose was given (34±6 μU./ml. vs. 21±4 μU./ml., mean paired difference $p < 0.02$). However, the mean proportionate enhancement of the early insulin response by oral galactose was smaller in the diabetics, and the mean peak plasma IRI attained after the combined stimulus was less than 25 per cent that of the normal subjects ($p < 0.001$).

In the normal subjects the mean rate of disappearance of glucose was increased after oral galactose, but this effect was not apparent in the diabetics (table 1).

In the normal subjects plasma IRG was suppressed after I.V. glucose on both occasions, and the mean maximum suppression was similar (54±6.3 and 64±5.2 pg./ml., both with $p < 0.01$). In the diabetics I.V. glucose alone again resulted in suppression of plasma IRG (mean maximum decrement of 41±7.8 pg./ml., $p < 0.02$) but when oral galactose was given before I.V. glucose there was a rise in plasma IRG (mean maximum increment of 77±30 pg./ml., $p < 0.05$).

The mean concentrations of IRGIP in plasma of both groups are shown in figure 6. As in the study with oral galactose alone, the early rise of plasma IRGIP was greater in the diabetic group, the mean levels being significantly different at the time of injection of glucose and five minutes afterwards ($p < 0.05$). Plasma IRGIP rose to a mean peak 35 minutes after ingestion of the galactose in normal subjects and 10 minutes later in the diabetics; the values at this time and subsequently were not significantly different.

DISCUSSION

The basal levels of IRI, IRGIP, and IRG in the plasma of the diabetic subjects were not significantly different from those of the normal subjects, as found earlier in similar equally mild maturity-onset diabetics.[2,8]

Effects of Ingestion of Triglyceride

After oral triglyceride, the secretion of IRGIP in the normal subjects was not associated with changes of plasma glucose, IRI, or IRG. This is in accordance with the lack of effects of intravenous infusion of physiologic doses of GIP on plasma IRI or IRG in fasting normal man.[1,2] The rise of plasma IRG in the diabetic subjects after ingestion of triglyceride may correspond to that observed in normal dogs, which has been attributed to humoral stimulation of pancreatic glucagon secretion.[9] Failure of suppression or paradoxical elevation of plasma IRG after ingestion of glucose in maturity-onset diabetes has also been reported.[8,10] The previously suggested increased sensitivity to the alphacytotrophic action of GIP in diabetes was offered as an explanation of abnormal glucagon responses after oral glucose, as supported by stimulation of plasma IRG by exogenous GIP in maturity-onset diabetes in man[2] and in experimental diabetes in rats.[11] Thus endogenous GIP may be responsible for the rise in plasma IRG in the diabetics in the present study with other stimuli to GIP secretion.

The responses of normal and diabetic subjects to

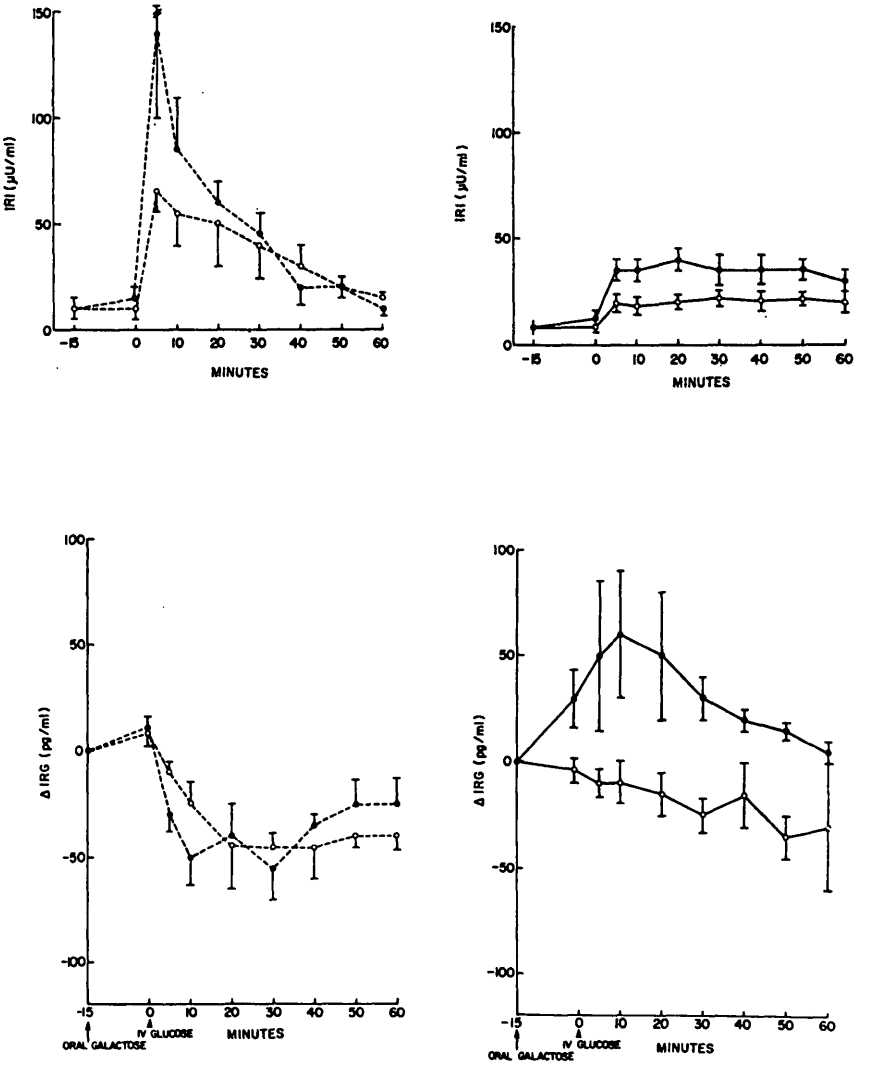

FIGURE 5

Effect of ingestion of galactose on responses to subsequent intravenous injection of glucose in five normal and six diabetic subjects, showing responses of plasma IRI and IRG. (Interrupted lines show normal subjects, solid symbols show test with oral galactose, vertical bars S.E.M.)

I.V. glucose in the presence of elevation of plasma IRGIP after oral triglyceride correspond to effects observed during intravenous administration of GIP.[1,2] The normal subjects showed enhancement of the insulin response to the I.V. bolus of glucose, associated with improved glucose tolerance. A similar but protracted enhancement of insulin secretion has been reported during prolonged I.V. infusion of glucose after oral triglyceride.[11] In the present study, the diabetics showed apparent resistance to the insulin-releasing effects of endogenous GIP and elevation of plasma IRG persisted in spite of I.V. administration of glucose.

The secondary fall of plasma IRGIP when I.V. glucose was administered after oral triglyceride in the normal subjects would be expected if endogenous insulin, like exogenous insulin,[2] inhibits release of GIP. The impaired insulin response to glucose in the pres-

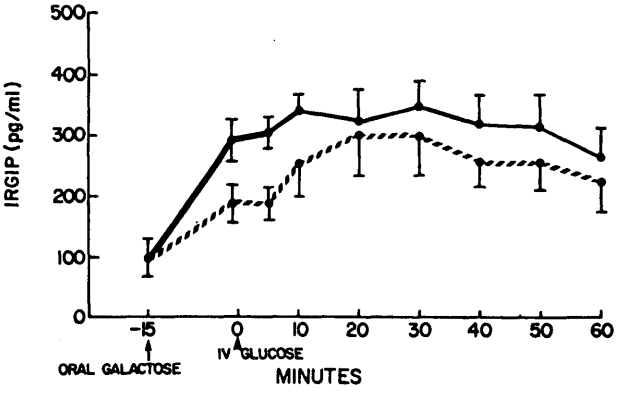

FIG. 6. Changes in plasma IRGIP after ingestion of galactose followed by intravenous injection of glucose in five normal and six diabetic subjects. (Interrupted lines show normal subjects, vertical bars S.E.M.)

ence of elevated plasma IRGIP in the diabetics is in accordance with observations with exogenous GIP in similar subjects,[2] and the failure of I.V. glucose to suppress IRGIP after oral triglyceride in the diabetics could be attributed to their obtunded insulin response.

Effects of Ingestion of Galactose

In general the effects of ingestion of galactose were compatible with similar interpretations in terms of the proposed actions of GIP. In the normal subjects, oral galactose alone showed modest hyperglycemic and insulin-releasing effects, which may be attributed to conversion of galactose to glucose, and to the action of glucose together with GIP on the beta cell. In the light of the postulated inhibition of GIP secretion by insulin, the finding that the mean plasma IRGIP response after oral galactose was smaller in the normal subjects than in the diabetics would be expected. In the study of effects of oral galactose on the response to I.V. glucose in normal subjects, the enhancement of plasma IRI and improvement of glucose tolerance were of similar degree to the corresponding effect after oral triglyceride and were recorded in the presence of similar levels of plasma IRGIP. In the diabetics, oral galactose, unlike oral triglyceride, resulted in enhancement of the early rise of plasma IRI after I.V. glucose, but this was not sufficient to affect glucose tolerance. This difference between the effects of oral triglyceride and oral galactose on the response to I.V. glucose in diabetic subjects may be due to the fact that mean plasma IRGIP was higher at the time of injection of glucose in the diabetic group after oral galactose than after oral triglyceride. The failure of plasma IRGIP to show a significant secondary fall when I.V. glucose was given after oral galactose in normal subjects is not in conflict with the suggestion that endogenous insulin suppresses GIP secretion; such an effect might not be demonstrable under these conditions because of the insulinotropic action of oral galactose, which was apparent before the intravenous glucose was given.

Taken together, the findings in normal subjects support the suggestions that endogenous GIP is insulinotropic in the presence of a raised plasma glucose concentration and that insulin exerts feedback inhibition of GIP secretion. The results also suggest that, although there is partial resistance to the insulinotropic action of GIP in diabetes, this hormone can participate in the enteroinsular augmentation of insulin release demonstrated in maturity-onset diabetes.[12] The rise in plasma IRG after oral triglyceride or galactose in the diabetics points out the probable importance of intestinal factors in abnormalities of glucagon secretion after ingestion of nutrients that lack direct effects on the alpha cell and is compatible with the suggestion that GIP is the agent concerned. However, further characterization of the biologic significance of this response of plasma IRG is necessary. In conclusion, the present findings support the suggestion that effects on insulin and glucagon secretion that have been attributed to intestinal humoral factors may be mediated by gastric inhibitory polypeptide in diabetic as well as in normal subjects.

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