Incretin Effect Due to Increased Secretion and Decreased Clearance of Insulin in Normal Humans

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To assess the contribution of changes in insulin secretion and clearance to the incretin effect (greater insulinemia after oral than after intravenous glucose), 10 healthy subjects were studied after oral glucose (1 g/kg body wt) and again when glucose was infused intravenously at rates to match arterialized plasma glucose concentrations after oral glucose. Although basal and integrated plasma glucose did not differ between oral and intravenous glucose, integrated responses of insulin (3.3 ± 0.5 vs. 1.8 ± 0.4 mU · ml^{-1} · 240 min^{-1}, $P < .001$), C-peptide (456.5 ± 58.5 vs. 327.9 ± 46.3 ng · ml^{-1} · 240 min^{-1}, $P = .002$), gastric inhibitory polypeptide, (16.8 ± 3.5 vs. −2.8 ± 1.0 μg · ml^{-1} · 240 min^{-1}, $P < .001$), and insulin secretion (6.6 ± 1.1 vs. 4.7 ± 0.7 U · 240 min^{-1}, $P = .003$) were greater with oral than intravenous glucose. However, insulin clearance, whether calculated as the molar ratio of integrated C-peptide to integrated insulin responses (6.9 ± 0.7 vs. 14.2 ± 3.8, $P = .005$) or from the formula insulin clearance equals insulin secretion divided by integrated insulin responses (1.1 ± 0.2 vs. 2.5 ± 0.7 L · min^{-1} · m^{-2}, respectively, $P = .002$), was less for oral than for intravenous glucose. Therefore, the incretin effect is mediated both by increased secretion and decreased clearance of insulin. *Diabetes* 37:200–203, 1988

It has been repeatedly demonstrated that peripheral venous insulin concentrations are greater after enteral (1–3) than after intravenous (4,5) administration of comparable glucose loads. The higher insulin concentration after oral glucose is called the incretin effect and has been ascribed to the stimulation of insulin secretion by gastrointestinal hormones. Among the latter, gastric inhibitory polypeptide (GIP) is believed to be the major incretin factor (6). It has generally been assumed that the incretin effect is due to enhanced insulin secretion (7). Recently, Gibby and Hales (8) have implicated decreased hepatic insulin clearance as a mechanism for the incretin effect. However, the method used to quantitate hepatic clearance of insulin has been seriously challenged (9,10).

This study was conducted to determine whether the incretin effect is mediated by increased insulin secretion and/or decreased insulin clearance. Insulin secretion was determined with a validated kinetic model (11), and insulin clearance was calculated by two complementary but independent methods. To avoid the limitations associated with venous glucose clamps, glucose was infused intravenously at rates to match arterialized plasma glucose concentrations generated from oral glucose ingestion.

MATERIALS AND METHODS

Five lean [body mass index (BMI) 24.8 ± 0.7 (mean ± SE)] healthy men (aged 28.8 ± 1.8 yr) and five lean (BMI 20.2 ± 1.0) healthy age-matched women (29.2 ± 2.3 yr) were studied on two separate occasions after an overnight fast. Leanness was defined as BMI <27 for men and <25 for women (12). The range of body weight for women was 49.2–61.1 kg and for men was 68.3–98.6 kg. Subjects were taking no medications and had no family history of diabetes. All studies were conducted at the General Clinical Research Center of the Mayo Foundation after written informed consent had been obtained.

On the first occasion, each subject ingested glucose (1 g/kg body wt) orally. Arterialized blood samples were obtained through an 18-gauge indwelling plastic cannula (Jelco, Rariton, NJ) inserted into a dorsal hand vein in a retrograde fashion; the hand was maintained at 50–60°C in a temperature-controlled enclosure (13). Blood was sampled from −30 to 240 min at 10-min intervals for the first 2 h and at 30-min intervals thereafter. On the second occasion, 50% glucose was infused through an 18-gauge indwelling can-

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nula inserted into a contralateral forearm vein with a Harvard pump (Harvard Apparatus, Millis, MA) at rates adjusted manually to achieve arterialized plasma glucose concentrations identical to or slightly above those obtained after oral glucose ingestion (14). Plasma glucose was measured by a glucose oxidase method (YSI model 23A, Yellow Springs, OH). Blood samples for insulin and C-peptide measurements were collected in tubes containing 0.1 M benzamidine. Blood samples for GIP measurements were collected in tubes containing Trasylol (500 KIU/ml; Sigma, St. Louis, MO) and EDTA. All samples for hormone determination were chilled, centrifuged at 4°C, and frozen until assayed. Insulin was measured by the method of Herbert et al. (15). C-peptide was measured by the method of Heding (16) with Novo antibody 1221. Plasma GIP was measured by radioimmunoassay with antiserum R4817 as previously described (17). Insulin secretion was determined by the kinetic model of Eaton et al. (11), which has been validated for this purpose (18–20). The C-peptide kinetic constants generated by Polonsky et al. (21) were used. Insulin clearance was determined by calculation of the molar ratios of the integrated [area under the curve (AUC)] responses of C-peptide and insulin after oral and intravenous glucose. The limitations of the C-peptide–to-insulin molar ratio at individual sampling points (9,10) do not apply when integrated responses are employed (22). Insulin clearance was also calculated with the formula insulin clearance (ml/min) equals insulin secretion (μU/min) divided by AUC of insulin concentrations (μU/ml) (23,24). Insulin clearance was then adjusted for surface area. AUC was calculated for the data points from basal to the resumption of basal concentrations with the trapezoidal rule. The formula

$$\frac{\text{AUC oral glucose} - \text{AUC intravenous glucose}}{\text{AUC oral glucose}} \times 100$$

was used to calculate the incretin effect (25). Statistical analysis was performed by analysis of variance on the ranks of the data.

RESULTS

All results are shown in Fig. 1.

Glucose. There were no significant differences between intravenous and oral glucose for basal (95 ± 1 vs. 95 ± 3 mg/dl, respectively) and integrated postprandial (5.5 ± 0.6 vs. 5.1 ± 1.1 g · dl^{-1} · 240 min^{-1}, $P = .155$) plasma glucose. Peak postprandial plasma glucose was higher after intravenous than after oral glucose (180 ± 4 vs. 174 ± 5 mg/dl, $P = .023$). Mean plasma glucose concentrations from intravenous glucose were 1.4 ± 0.9% higher than those after oral glucose ingestion.

GIP. Basal plasma GIP concentrations did not differ before oral and intravenous glucose administration (120.4 ± 20.2 vs. 96.1 ± 20.6 pg/ml). GIP increased to peak postprandial levels of 277.7 ± 23.8 pg/ml after oral glucose but were unchanged (92.7 ± 18.4 pg/ml) after intravenous glucose. Integrated postprandial GIP concentrations (16.8 ± 3.5 vs. −2.8 ± 1.0 μg · ml^{-1} · 240 min^{-1}, $P < .001$) were significantly greater after oral than after intravenous glucose administration.

Insulin. Basal plasma insulin concentrations did not differ before oral and intravenous glucose administration (6.3 ± 0.6 vs. 6.8 ± 1.3 μU/ml). Peak postprandial (47.1 ± 6.5 vs. 27.8 ± 4.2 μU/ml, $P = .011$) and integrated postprandial (3.3 ± 0.5 vs. 1.8 ± 0.4 mU · ml^{-1} · 240 min^{-1}, $P < .001$) insulin concentrations increased more after oral than after intravenous glucose administration.

C-Peptide. Basal plasma C-peptide concentrations did not differ before oral and intravenous glucose administration (1.48 ± 0.14 vs. 1.45 ± 0.15 ng/ml). Peak postprandial (5.73 ± 0.55 vs. 4.48 ± 0.40 ng/ml, $P < .001$) and integrated postprandial (456.5 ± 58.5 vs. 327.9 ± 46.3 ng · ml^{-1} · 240 min^{-1}, $P = .002$) C-peptide concentrations increased more after oral than after intravenous glucose administration.

Insulin secretion. Basal rates of insulin secretion did not differ before oral and intravenous glucose administration (20.6 ± 5.6 vs. 19.0 ± 4.0 mU/min). Insulin secretion increased more after oral than after intravenous glucose administration (6.6 ± 1.1 vs. 4.7 ± 0.7 U · 240 min^{-1}, $P = .003$). The incretin effect accounted for 19.6 ± 7.1% of the enhanced insulin secretion observed after oral glucose administration.

Insulin clearance. The molar ratio of integrated concentrations of C-peptide to integrated concentrations of insulin was significantly lower after oral than after intravenous glucose administration (6.9 ± 0.7 vs. 14.2 ± 3.8, $P = .005$), indicating decreased insulin clearance. The clearance rate of insulin also was significantly less after oral than after intravenous glucose when calculated directly (1.1 ± 0.2 vs. 2.5 ± 0.7 L · min^{-1} · m^{-2}, $P = .002$).

Effect of gender. With the exception of slightly lower basal ($P < .01$) and peak postprandial ($P < .01$) plasma glucose concentrations in women, no other parameter noted above was significantly influenced by gender.

DISCUSSION

These studies confirm the previous observation that peripheral venous insulin concentrations are greater after enteral than after intravenous administration of glucose, despite comparable plasma glucose concentrations. Our data indicate that oral glucose ingestion results in both increased insulin secretion and decreased hepatic insulin clearance. After completion of our studies, Nauck et al. (25) reported similar conclusions with a similar experimental design, with the exception that venous rather than arterial glucose concentrations were matched during oral and intravenous glucose administration. Matching venous glucose concentrations has been criticized because the arteriovenous difference of plasma glucose is larger after oral than after intravenous glucose administration (4,7). Matching venous glucose concentrations will result in higher arterial glucose concentrations during oral than during intravenous glucose administration; the greater arterial glucose concentration will increase insulin secretion, thereby potentially creating an artifactual incretin effect. Furthermore, hyperglycemia may decrease hepatic insulin clearance (26), which also could artifactually inflate the incretin effect.

Our studies are not limited by these potential disadvantages. Our results indicate that the increased insulin secretion and decreased insulin clearance after oral glucose cannot be accounted for by differences in arterial glucose concentrations. Although our results and those of Nauck et al. (25) agree qualitatively, our estimate of the contribution

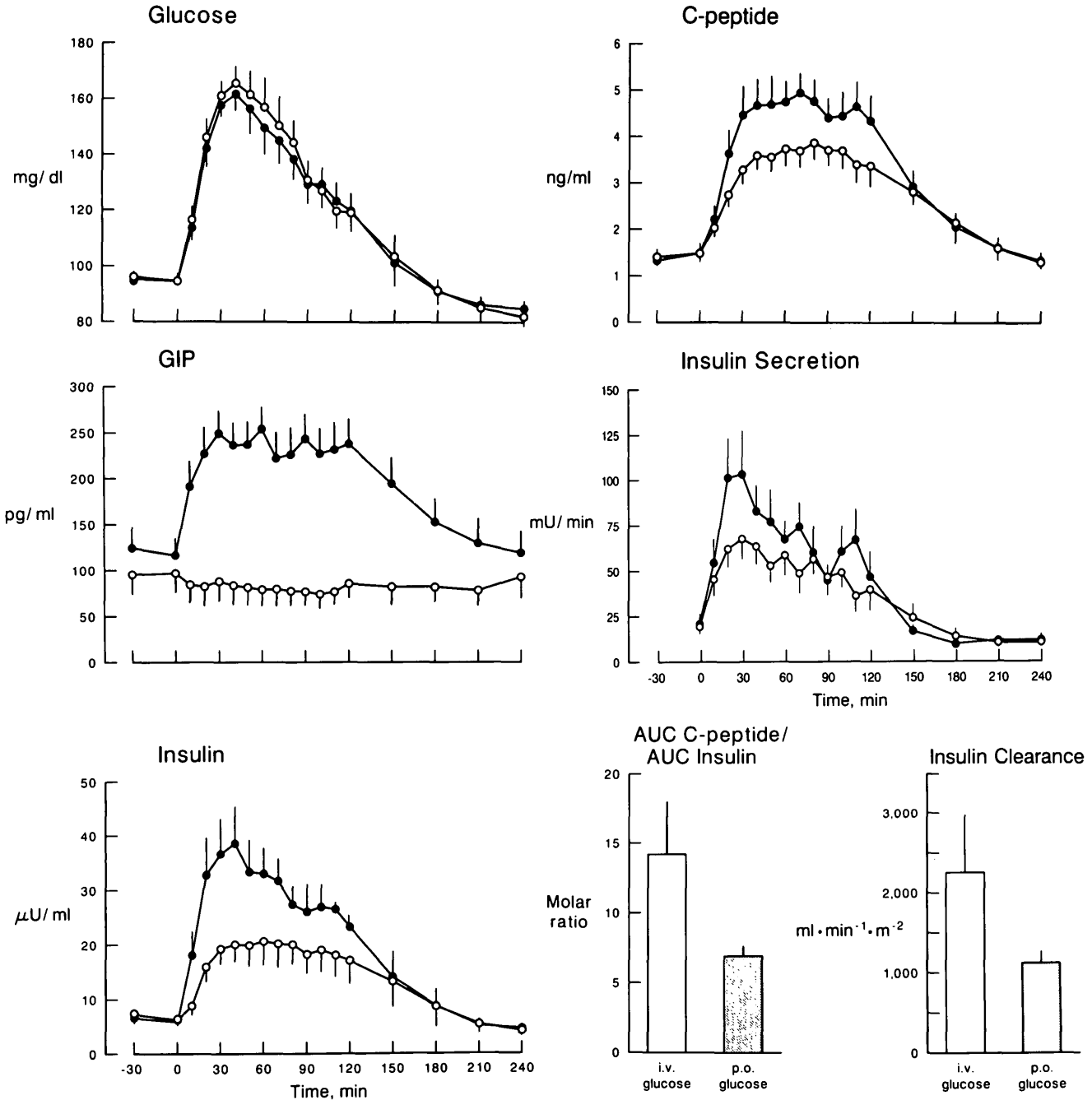

FIG. 1. Plasma glucose, insulin, C-peptide, GIP, insulin secretion, and insulin clearance levels and molar ratio of integrated C-peptide to integrated insulin response to oral glucose (1 g/kg) or to intravenous glucose administered at rates to match arterialized venous plasma glucose concentrations resulting from oral glucose. Data are expressed as means ± SE. AUC, area under curve; i.v., intravenous (○), p.o., oral (●).

of insulin secretion to the incretin effect is somewhat less (19.6 ± 7.1%) than theirs (26.2 ± 12 and 61.0 ± 4.1%) for glucose loads (50 and 100 g, respectively) comparable to those used in this study. The higher insulin secretion observed by these investigators was probably due to higher arterial glucose concentrations after oral glucose ingestion compared with intravenous glucose infusion. Of perhaps greater importance, despite the use of different approaches to the estimation of insulin clearance, both Nauck et al. (25) and we have documented a decrease in clearance of insulin after oral glucose.

The observation that the incretin effect is due to both increased insulin secretion and decreased insulin clearance has both physiological and pathophysiological implications. The mechanism by which gastrointestinal hormones decrease insulin clearance is not known. However, it may be more than coincidence that potential incretins and insulin are both secreted into the portal venous system, resulting in the liver being the initial organ exposed. Hanks et al. (27) have reported no effect of GIP on hepatic insulin extraction with an isolated perfused rat liver system. However, further studies are required to determine if the various incretin candidates, such as GIP, alter hepatocyte insulin binding and/or degradation. Although a role for GIP in the hyperinsulin-

emia of obesity has been postulated (28), experimental data conflict (29). This controversy may be partly due to the failure to distinguish between the effect of incretins such as GIP on insulin secretion and clearance. Additional experiments are required to determine if incretins also influence insulin secretion and clearance in disease states characterized by altered carbohydrate metabolism.

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