Insulin Secretion and Clearance

Comparison After Oral and Intravenous Glucose

E. TIMOTHY SHAPIRO, HARTMUT TILLIL, M. ANNETTE MILLER, BRUCE H. FRANK, JOHN A. GALLOWAY, ARTHUR H. RUBENSTEIN, AND KENNETH S. POLONSKY

SUMMARY

Insulin secretion and clearance in response to the administration of oral and intravenous glucose was investigated in nine normal men. C-peptide metabolic kinetics were calculated by analysis of individual C-peptide decay curves after the bolus injection of biosynthetic human C-peptide. Glucose was administered to the subjects on three occasions: as a 75-g oral dose, a 75-g i.v. infusion, and an intravenous glucose infusion at a variable rate adjusted to mimic the peripheral glucose levels obtained after the oral glucose load (matching experiment). Glucose, insulin, and C-peptide concentrations were measured for the subsequent 5 h. The glucose level after the oral glucose load (115.9 ± 2.6 mg/dl, mean ± SE) closely approximated that after the matching experiment (120.5 ± 2.5 mg/dl) but was significantly lower than after 75 g i.v. glucose (127.7 ± 3.4 mg/dl, $P < .05$). Analysis of the areas under the peripheral concentration curves (60–360 min) showed that the responses of both insulin (52.7 ± 5.6 and 46.5 ± 4.5 pmol · ml^{-1} · 300 min^{-1}) and C-peptide (252.7 ± 27.5 and 267.0 ± 21.6 pmol · ml^{-1} · 300 min^{-1}) were not significantly different after the oral and 75-g i.v. glucose studies, respectively, whereas in the matching experiment, both the insulin (26.1 ± 3.9 pmol · ml^{-1} · 300 min^{-1}) and C-peptide (178.0 ± 18.9 pmol · ml^{-1} · 300 min^{-1}) responses were lower ($P < .05$) than in the other two studies. Insulin secretory rates were derived from peripheral C-peptide concentrations with an open two-compartment model and individually derived model parameters. The basal insulin secretion rate was 86.8 ± 2.9 pmol/min. The insulin secretory response over the 300 min was 66.2 ± 4.8 nmol after oral glucose. This was similar to that after 75 g i.v. glucose (72.4 ± 4.1 nmol), whereas that secreted in response to the matching experiment was lower (47.6 ± 4.1 nmol, $P < .05$). As a measure of the clearance of endogenous insulin, the ratio between the area under the insulin secretory curve and the area under the peripheral insulin concentration curve was calculated. This ratio was similar (1906 ± 149 ml/min) during the baseline period and the matching glucose infusion (2042 ± 245 ml/min) but was significantly lower after oral glucose (1330 ± 112 ml/min, $P < .05$). The incretin effect calculated based on the insulin secretion rate (25 ± 9.2%) appeared to be less than if the calculations were based on peripheral insulin levels. These data demonstrate that equivalent doses of glucose administered orally and intravenously elicit an equivalent insulin secretory response. However, when the arterialized plasma glucose curve after 75 g oral glucose is matched by an intravenous glucose infusion, only 35.6 ± 2.9 g glucose was infused, and the intravenous glucose resulted in a lower secretory response. Furthermore, after oral administration of 75 g glucose a significant reduction in insulin clearance resulted. These data provide evidence that the hyperinsulinemia seen after oral glucose is due both to enhanced insulin secretion and diminished insulin clearance. *Diabetes* 36:1365–71, 1987

From the Department of Medicine, University of Chicago, Pritzker School of Medicine, Chicago, Illinois, and Eli Lilly Research Laboratories, Indianapolis, Indiana.

Address correspondence and reprint requests to Kenneth S. Polonsky, University of Chicago, Department of Medicine, Box 435, 5841 S. Maryland Avenue, Chicago, IL 60637.

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The liver is the major site of insulin metabolism, and in the fasting state, ~50% of insulin is extracted by that organ on the first pass (1–5). Whether hepatic insulin extraction may be altered by glucose administration has been the subject of considerable controversy. Faber et al. (6), Gibby and Hales (7), and Eaton et al. (8) reported a decrease in hepatic insulin clearance after oral glucose. Kaplan and Madison (9) found that hepatic insulin extraction fell after intravenous glucose. Waldhausl et al. (10) reported that the hepatic extraction of insulin was not altered

by oral glucose ingestion, and in dogs, Ishida et al. (11), Kaden et al. (2), and Jaspan and Polonsky (4) demonstrated increased insulin extraction after glucose ingestion. The reason for the variability among these studies is uncertain.

With the availability of biosynthetic human C-peptide for experimental use (12), we have demonstrated that insulin secretion rates can be accurately derived from peripheral C-peptide concentrations with a two-compartment kinetic model for C-peptide as proposed by Eaton et al. (13). In this study, we have used this approach to explore the relationship between insulin secretion rates and peripheral insulin concentrations after different routes of glucose administration.

MATERIALS AND METHODS

SUBJECTS

Experiments were performed in nine normal male volunteers aged 20–26 yr (mean ± SE 22.6 ± 0.6 yr). Mean (±SE) body mass index, body surface area, and percent ideal body weight were 23.3 ± 0.7 kg/m^2, 1.88 ± 0.07 m^2, and 103.0 ± 3.4%, respectively. No subject had a personal or family history of diabetes mellitus. All studies were carried out in the Clinical Research Center of the University of Chicago. The subjects gave written informed consent, and the protocols were approved by the Institutional Review Board.

EXPERIMENTAL PROTOCOL

All studies were performed after an overnight fast. During each experiment, an intravenous sampling catheter was inserted into the dorsum of the hand, and where necessary, an infusion catheter was inserted into a vein on the opposite hand. The hand with the sampling catheter was maintained in a heating blanket to ensure arterialization of the venous sample. Each subject was studied on four separate occasions within a 4-wk period and was kept on a weight-maintenance mixed diet throughout the duration of the study.

Bolus injection of biosynthetic human C-peptide. Endogenous insulin secretion was inhibited with an intravenous somatostatin infusion (500 μg/h; Bachem, Torrance, CA). To document its effectiveness, insulin and C-peptide concentrations were measured 60 min after the initiation of somatostatin. As a result of the somatostatin infusion, insulin concentration fell to 0.02 ± 0.01 pmol/ml and the C-peptide to 0.09 ± 0.02 pmol/ml. Each subject then received a bolus intravenous injection of 150 μg biosynthetic human C-peptide. Plasma C-peptide levels were measured at the following time intervals after the bolus injection: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 17, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 110, 120, 140, 160, and 180 min (Fig. 1).

Analysis of these decay curves allowed the kinetic parameters for C-peptide used in the application of the two-compartment model to be defined for each subject. Two minutes after administration of the C-peptide bolus, plasma concentration of C-peptide was 10.5 ± 0.8 pmol/ml. The subsequent fall in C-peptide plasma concentration demonstrated a fast and slow component, which had disappearance half-times of 5.20 ± 0.27 and 33.40 ± 0.98 min, respectively. The metabolic clearance rate of C-peptide was 152.97 ± 7.89 ml · m^{-2} · min^{-1}. The average kinetic rate constants for C-peptide derived by compartmental analysis of individual C-peptide decay curves were K_1 = 0.0481 ± 0.0049 min^{-1}, K_2 = 0.0426 ± 0.0015 min^{-1}, and K_3 = 0.0663 ± 0.0017 min^{-1}, and distribution volume was 2329.50 ± 153.06 ml/m^2.

Glucose administration. To compare the effects of different methods of glucose administration, each subject was studied according to three separate protocols. Each of these studies began with a 60-min basal period during which glucose, insulin, and C-peptide were measured at 15-min intervals. Next, subjects underwent the following interventions: *1*) ingestion of 75 g glucose (Dextol, Sherwood Medical, St. Louis, MO) diluted to 350 ml with water and ingested within 5 min; *2*) infusion of 75 g i.v. glucose over a 3-h period (a standard protocol was used in this study, with 9 g infused over the first 30 min, 36 and 24 g over the next 2 h, respectively, and 6 g over the final 30 min); and *3*) intravenous infusion of glucose at a varying rate to mimic the plasma glucose concentrations after oral glucose inges-

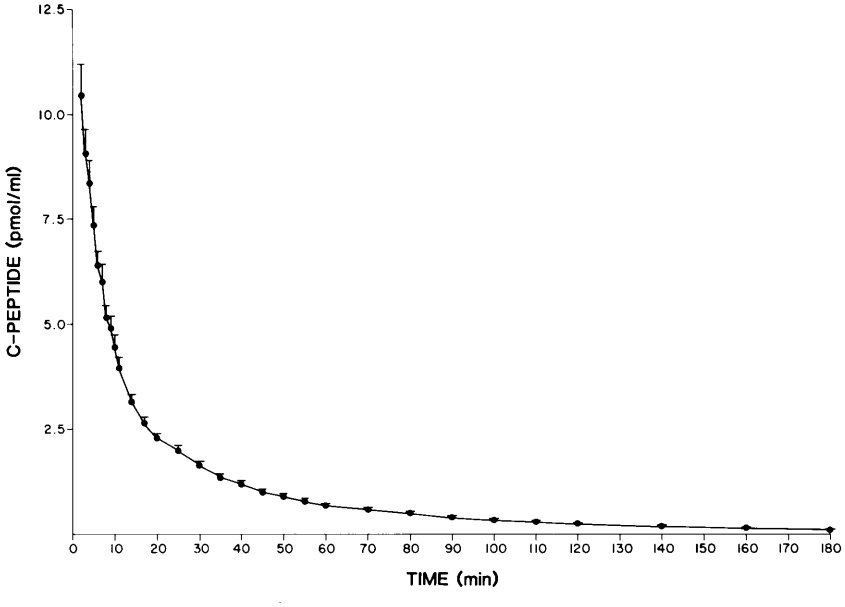

FIG. 1. Mean plasma C-peptide concentrations after intravenous bolus injection of biosynthetic human C-peptide in 9 normal subjects.

tion. This intervention is referred to as the *matching experiment*. In each of these studies, subsequent to the initial 1-h baseline period, peripheral glucose, insulin, and C-peptide levels were measured at the following times after glucose administration: 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 140, 160, 180, 210, 240, 270, and 300 min.

CALCULATIONS

Insulin secretion rates. The C-peptide decay curves were resolved into the sum of two exponentials by nonlinear least-squares regression analysis, and the metabolic clearance rates and fractional rate constants for C-peptide were derived by application of a two-compartment mathematical model as proposed by Eaton et al. (13) and Metzler (14). This model assumes that C-peptide distributes into a central compartment, from which sampling occurs, and a peripheral extravascular compartment. The central compartment consists of the plasma space and tissues in rapid equilibration with plasma. Fractional rate constants K_1 and K_2 describe the rate at which C-peptide passes from the central to the peripheral compartment (K_1) and back again (K_2). According to this model, C-peptide is irreversibly metabolized from the central compartment, K_3 being the rate constant that describes this process. These rate constants for each individual were then applied to the endogenous plasma C-peptide concentrations measured during the glucose administration studies, and individual insulin secretion rates were derived. In these studies a robust, locally weighted regression procedure described by Cleveland (15) was used to obtain a smooth representation of plasma C-peptide concentrations.

Endogenous insulin clearance. Standard approaches to the measurement of the clearance of exogenously administered insulin were adapted to enable the clearance of endogenously secreted insulin to be quantitated. In most experimental situations where insulin clearance is measured, the endogenous secretion rate of the peptide is not known, and the metabolic clearance rate is derived as the ratio of the exogenous infusion rate of the peptide and its steady-state plasma concentration. In our experiments, however, secretion rates of insulin were derived from peripheral C-peptide concentrations as described above. This enabled the clearance of endogenously secreted insulin to be calculated under basal steady-state conditions as the ratio of the basal insulin secretion rate and serum insulin concentration. The mathematical approach used in this analysis is identical to that proposed by Tait (16) but is adapted to a situation in which endogenous insulin production rates can be substituted for an exogenous insulin infusion.

The metabolic clearance rate of injected peptides can also be measured under non-steady-state conditions after bolus intravenous injections (17,18). Metabolic clearance rate is calculated as the ratio of the quantity of peptide in the bolus to the integrated area under the plasma concentration curve. An analogous approach was adopted for the estimation of endogenous insulin clearance after administration of the secretory stimuli. Clearance was calculated as the ratio of the respective areas under the secretion rate and peripheral serum concentration curves.

Incretin effect. The matching experiment enabled us to calculate the incretin effect (i.e., quantity of β-cell secretory response evoked by factors other than the plasma glucose concentration itself; 19) separately for peripheral insulin and C-peptide concentrations and insulin secretion rates. With the total areas under the respective curves in the 5 h after glucose, the incretin effect was calculated with the formula

$$\text{incretin effect} = \frac{(\text{area}_{\text{oral}} - \text{area}_{\text{match}})}{\text{area}_{\text{oral}}} \times 100\%$$

ANALYTICAL METHODS

Blood samples for insulin were allowed to clot at room temperature, and the serum was stored at −20°C until assayed. C-peptide samples were drawn into tubes at 4°C containing 500 KIU/ml Trasylol and 1.2 mg/ml EDTA. Plasma was separated and stored frozen until assayed. Serum insulin was assayed by a double-antibody technique (20). Human C-peptide immunoreactivity in plasma was measured as previously described (21). Plasma glucose was measured with a glucose analyzer (YSI, Yellow Springs, OH).

DATA ANALYSIS

The glucose, insulin, and C-peptide concentrations and insulin secretion rate responses to the three glucose challenges were compared by two-way analysis of variance. The significance of differences between means in each of the studies was evaluated at the 5% level by a two-tailed paired *t* test corrected for multiple comparisons by the Bonferroni-Dunn adjustment. Results are expressed as means ± SE. Data analysis was performed with the Statistical Analysis System (SAS Institute, Cary, NC), and nonlinear regression analysis was performed with the BMDP 3R program (BMDP Statistical Software, Los Angeles, CA). Areas under the concentration curves were calculated by the trapezoidal rule.

RESULTS

Peripheral concentrations of glucose, insulin, and C-peptide. Glucose, insulin, and C-peptide concentrations from the three protocols and the areas under the concentration curves from 60–360 min are shown in Fig. 2 and Table 1, respectively. On average, 35.6 ± 2.9 g glucose was infused intravenously to match the plasma glucose levels after oral glucose. The mean glucose level during the matching experiment (120.5 ± 2.5 mg/dl) closely approximated that achieved after oral glucose ingestion (115.9 ± 2.6 mg/dl), although the rapid fall in glucose after oral glucose could not be matched in some subjects. The mean glucose concentration during the infusion of 75 g i.v. glucose (127.7 ± 3.4 mg/dl) was significantly higher than that after oral glucose ($P < .05$), and the time course of the curve was different, having a delayed and higher peak.

Analysis of the areas under the peripheral concentration curves showed that the responses of both insulin (52.7 ± 5.6 and 46.5 ± 4.5 pmol · ml^{-1} · 300 min^{-1}) and C-peptide (252.7 ± 27.5 and 267.0 ± 21.6 pmol · ml^{-1} · 300 min^{-1}) were not significantly different after the oral and 75 g i.v. glucose studies, but in the matching experiments, both insulin (26.1 ± 3.9 pmol · ml^{-1} · 300 min^{-1}) and C-peptide (178.0 ± 18.9 pmol · ml^{-1} · 300 min^{-1}) responses were significantly lower ($P < .05$) than in the other two studies.

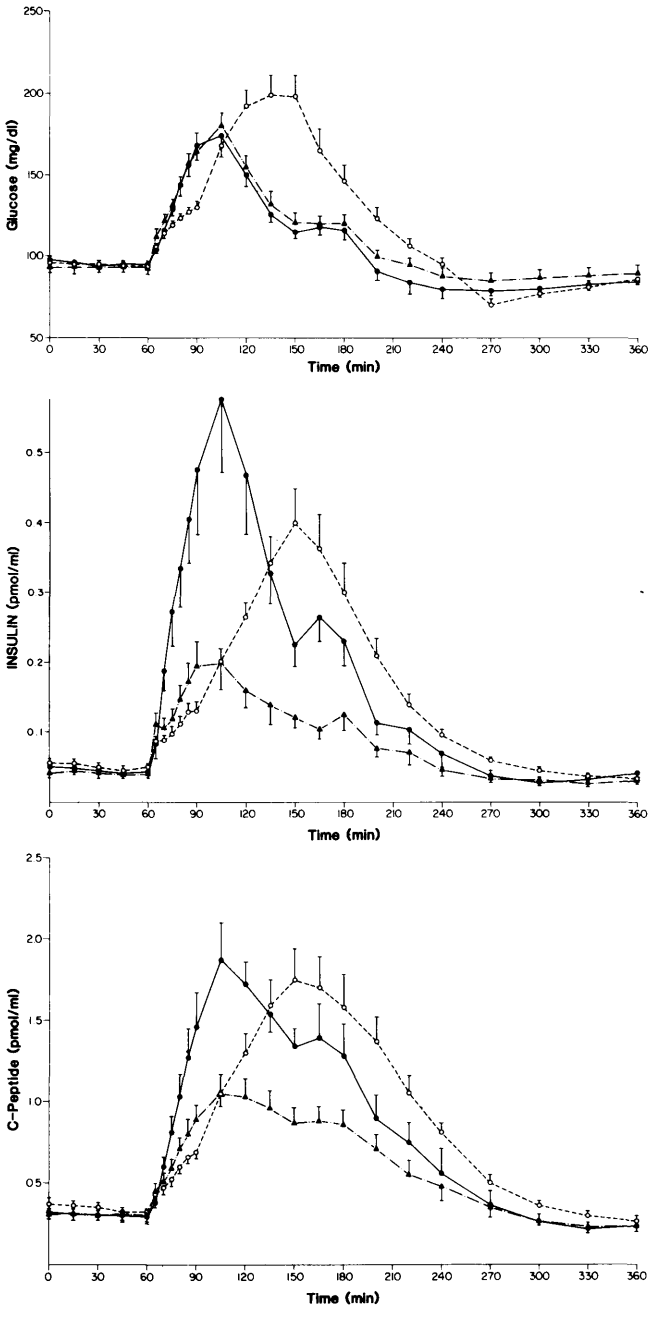

FIG. 2. Peripheral concentrations of arterialized glucose (*upper panel*), insulin (*middle panel*), and C-peptide (*lower panel*) in response to 75 g oral glucose (●), 75 g i.v. glucose (○), or matched intravenous glucose (▲).

Insulin secretion rates. The basal insulin secretion rate was 86.8 ± 2.9 pmol/min and did not differ significantly before the three studies. Secretion rates measured during each protocol are shown in Fig. 3. The insulin secretory response to oral glucose was similar to the response to 75 g i.v. glucose but was significantly greater ($P < .05$) than during the matching intravenous study, whether the total amount of insulin produced or the incremental secretory response over time was considered (Table 1). In the 5 h after oral glucose, 66.2 ± 4.8 nmol insulin was secreted; corresponding values of 72.4 ± 4.1 and 47.6 ± 4.1 nmol were secreted after 75 g i.v. glucose and the matching experiment, respectively. The incremental insulin secretion above baseline over 5 h was 41.3 ± 4.1, 44.1 ± 4.8, and 21.4 ± 3.4 nmol in each of these studies, respectively.

Effects on insulin clearance. As a measure of insulin clearance the relationship between insulin secretion and peripheral insulin concentration was compared under basal conditions and in the three experimental protocols. It can be seen from the data in Table 1 that the insulin secretory response to oral glucose was 146.9 ± 12% of the secretory response in the matching experiment. However, the integrated response in peripheral insulin concentrations after oral glucose was 219.9 ± 23.2% of the response during the matching experiment, suggesting diminished insulin clearance after oral glucose. This impression was confirmed by the analysis seen in Fig. 4, which depicts the ratio of the area under the insulin secretory rate curves and the corresponding peripheral insulin concentration curves. Because integrated secretion rate is expressed in picomoles and integrated insulin concentration as picomoles per milliliter per minute, the units of this ratio are milliliters per minute. As described in detail in MATERIALS AND METHODS, this ratio represents a measure of endogenous insulin clearance. Under basal conditions, the ratio of 1906 ± 149 ml/min did not differ significantly from the ratio during the matched intravenous experiment (2042 ± 245 ml/min). The mean ratio after oral glucose (1330 ± 112 ml/min) decreased from basal in all nine individuals and was significantly lower than the basal ratio. Although the individual ratios during the 75-g i.v. study were lower than those calculated for the basal period in most individuals (7 of 9), this decrease did not reach statistical significance with the Bonferroni adjustment for multiple comparisons (1628 ± 128 ml/min).

Incretin effect. The mean incretin effect based on the insulin secretion rate over the 300 min after the glucose stimuli was 25.5 ± 9.2% (66.5 ± 5.0 and 47.4 ± 4.0 nmol for the oral and matching experiments, respectively). Consistent with the evidence that insulin clearance is lower after oral than after intravenous glucose, the incretin effect based on peripheral insulin levels (50.3 ± 5.2%) was significantly higher than for insulin secretion (25.5 ± 9.2%). When the incremental areas were used to perform the same calculation, the mean incretin effect for insulin levels was 66.0 ± 6.4% compared to 43.9 ± 12.5% for insulin secretion rates.

DISCUSSION

Methodology for studying hepatic insulin extraction is either invasive (10), involving direct sampling of portal and hepatic circulation, or indirect, involving a number of unvalidated assumptions concerning C-peptide and insulin kinetics (6,7,22–26). In particular, differences in the C-peptide–to-insulin molar ratio, a commonly used measure of hepatic insulin extraction, may be difficult to interpret under non-steady-state conditions in view of the differences in the half-disappearance times of C-peptide and insulin (27). As a result of these methodological pitfalls, there is considerable confusion concerning the factors that regulate hepatic insulin extraction under physiologic circumstances (3,4,10,28–32).

We derived insulin secretion rates with a two-compartment mathematical model of C-peptide metabolism that we have

TABLE 1
Mean glucose levels and mean areas (60–360 min) under the peripheral insulin and C-peptide concentration curves for 3 experimental protocols with total and incremental insulin secretion over time

	Oral 75 g	Matching intravenous	75 g i.v.
Plasma concentrations			
Mean glucose level (mg/dl)	115.9 ± 2.6*	120.5 ± 2.5	127.7 ± 3.4*
Insulin (pmol · ml^{-1} · 300 min^{-1})	52.7 ± 5.6*	26.1 ± 3.9*†	46.5 ± 4.5†
C-peptide (pmol · ml^{-1} · 300 min^{-1})	252.7 ± 27.5*	178.0 ± 18.9*†	267.0 ± 21.6†
Secretion rates			
Insulin secretion (nmol)	66.2 ± 4.8*	47.6 ± 4.1*†	72.4 ± 4.1†
Incremental insulin secretion (nmol)	41.6 ± 4.3*	21.6 ± 3.5*†	44.4 ± 4.7†

Statistically significant differences ($P < .05$) between values in the same row but different columns are designated by the same symbol. To convert nanomoles to units of insulin, multiply by 0.145.

previously validated in animal and human experiments (12,33,34). Model parameters were derived in individual subjects by analysis of the C-peptide–decay curve.

Analysis of the insulin secretion rates revealed that when the arterialized plasma glucose concentration curve obtained after 75 g oral glucose was matched by an intravenous glucose infusion, the amount of insulin secreted in the 5 h after administration of the stimulus was considerably lower for intravenous than oral glucose (47.6 ± 4.1 vs. 66.2 ± 4.8 nmol; $P < .05$). Because in the former experiment an intravenous infusion of only 35.6 ± 2.9 g glucose was required to match the oral glucose concentration curves, an additional study was performed in which subjects received 75 g glucose intravenously. In this study, the amount of insulin secreted (72.4 ± 4.1 nmol) did not differ significantly from the amount secreted after oral glucose, although the plasma glucose concentrations were higher and the time course of the curve was different. However, if the degree of hyperglycemia is kept constant by matching arterialized plasma glucose concentrations after oral glucose with an intravenous glucose infusion, considerably less insulin is secreted after intravenous glucose. The difference in secretion in these two protocols has been termed the incretin effect (19), which in this study represented ~18.6 nmol insulin in the 5 h after the administration of the glucose stimulus or ~26% of the total quantity of insulin secreted after oral glucose. These conclusions regarding the difference between the effects of oral and intravenous glucose on insulin secretion are in agreement with previous reports in which indirect calculations were employed (6,32), but this is the first study in which the actual amount of insulin secreted after these various glucose stimuli could be accurately measured. We do not have a definite explanation for the greater hyperglycemia after intravenous glucose. It has been suggested that hepatic glucose uptake may be less after intravenous than oral glucose (35,36), although this has been disputed (37,38).

The availability of insulin secretion rates allowed the clearance of endogenously secreted insulin to be calculated as the ratio of the areas under the secretion and peripheral concentration curves for the three glucose studies and the baseline period. This approach, which is discussed in detail in MATERIALS AND METHODS, enabled us to compare the total amount of insulin reaching the peripheral circulation in relation to the amount of insulin secreted by the pancreas over the same time period in each experimental condition. Also note that, although we were unable to specifically determine whether changes in insulin clearance occurred at hepatic or peripheral sites or both, the liver is the most likely site at which these processes are regulated (2,3,5,11).

Under basal conditions the ratio of the integrated insulin secretion rate to the integrated insulin concentration was 1906 ± 149 ml/min, a value that was not significantly different in the matching glucose-infusion experiment (2042 ± 245 ml/min). After oral glucose ingestion, however, the ratio fell in all nine subjects to a mean value of 1330 ± 112 ml/min. This analysis indicates that in relation to insulin secretion rates, peripheral insulin concentrations are relatively higher after oral than after intravenous glucose, suggesting a significant fall in insulin clearance after oral glucose. The mag-

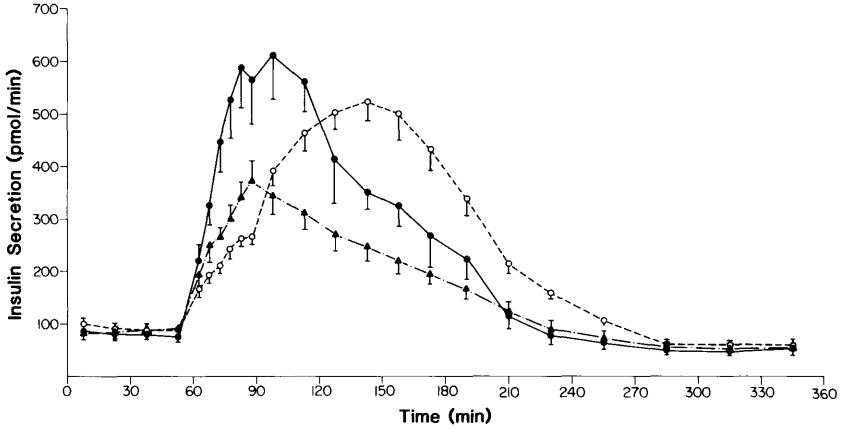

FIG. 3. Insulin secretion rates in response to 75 g oral glucose (●), 75 g i.v. glucose (○), or matched intravenous glucose (▲) calculated according to 2-compartment mathematical model.

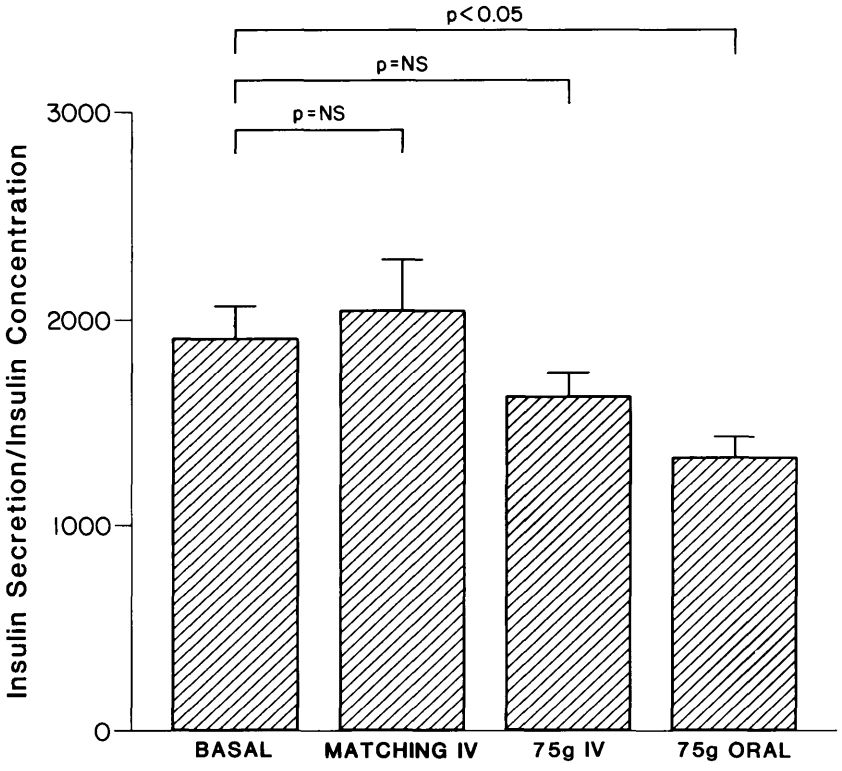

FIG. 4. Ratio between area under calculated insulin secretory rate curve and area under peripheral insulin concentration curve for basal period and in response to each of glucose stimuli. Ratio is measure of endogenous insulin clearance and is in units of milliliters per minute (i.e., pmol · pmol^{-1} · ml^{-1} · min^{-1}).

nitude of the reduction in this ratio suggests that there is an ~30% fall in insulin clearance after oral glucose compared to a low-dose matching intravenous glucose infusion. After the 75-g i.v. glucose infusion, the ratio was intermediate between the values obtained after oral glucose and the matching intravenous glucose-infusion study. However, this difference did not reach statistical significance, largely due to the correction factor used for multiple comparisons. Nevertheless, there appears to be a clear trend indicating that although a low-dose intravenous glucose infusion does not lower insulin clearance from its baseline value, a larger intravenous infusion may result in a reduction in insulin clearance. We are pursuing further experiments to resolve this issue.

The measure of endogenous insulin clearance derived herein is equivalent to the clearance of intraportally administered exogenous insulin. We have previously demonstrated in the dog that the clearance of intraportally administered insulin is approximately double the clearance of peripherally administered insulin, consistent with the hepatic extraction of ~50%, which has been repeatedly demonstrated in animal studies (30,39,40). We have had the opportunity to measure the clearance of peripherally administered insulin in the subjects who participated in the study, and the value was 464.9 ± 36.3 ml · m^{-2} · min^{-1}, compared to 1009.3 ± 67.5 ml · m^{-2} · min^{-1} for the clearance of endogenously secreted insulin. Because basal fractional hepatic insulin extraction can be calculated as 1 − peripheral insulin clearance/portal insulin clearance (17,18), the calculated basal hepatic insulin extraction was 52.1 ± 5.1%. These results are therefore consistent with previous data supporting the validity of the endogenous insulin-clearance measurements.

Studies have reached different conclusions concerning the effects of glucose administration on hepatic insulin extraction in human subjects (6–8,10,30–32). Whereas Waldhausl et al. (10) reported that hepatic insulin clearance was not altered by oral glucose, Faber et al. (6), Gibby and Hales (7), and Eaton et al. (8) reported decreased hepatic insulin clearance after oral glucose. Studies in the dog, including one from our own laboratory, have suggested that hepatic insulin extraction increases after oral glucose ingestion (2,4,11). The reason for these discrepancies is uncertain, although there were considerable differences in the experimental techniques. However, note that the conclusion of this study is in agreement with the only other study in which endogenous insulin secretion was accurately calculated in human subjects (34).

The mechanism whereby insulin clearance falls after 75 g oral glucose in humans is not known. One possibility is that it is a manifestation of saturation of hepatic insulin-uptake mechanisms due to the increase in secretion. Because receptor binding is the first step in initiating insulin degradation (41), saturation would most likely relate to saturation of binding to the insulin receptor. The insulin concentration at which saturation of clearance mechanisms occurs is controversial. Sonksen et al. (42) and Morishima et al. (43) reported a progressive fall in insulin clearance even within the physiologic range. However, other workers have found that insulin clearance only began to show evidence of saturation at concentrations above the physiologic range (1,44). Our data, therefore, support the idea that the clearance of insulin secreted endogenously into the portal vein may show evidence of saturation at physiologic concentrations.

In summary, this study has quantitated insulin secretory rates after oral and intravenous glucose with individual kinetic parameters for C-peptide. The data demonstrate that

in healthy subjects, ingestion of 75 g glucose is associated with a significant reduction in insulin clearance, presumably occurring at the level of the liver. Therefore, the hyperinsulinemia that occurs after oral glucose, in comparison to an isoglycemic intravenous glucose stimulus, results from a combination of increased insulin secretion and diminished insulin clearance.

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