Reduction of Insulin Clearance During Hyperglycemic Clamp

Dose-Response Study in Normal Humans

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Insulin secretion and clearance were studied in eight normal subjects who underwent hyperglycemic clamp studies at plasma glucose levels of 120, 225, and 300 mg/dl on three occasions. Insulin secretion rates were calculated during a 1-h baseline period and during 3 h of glucose clamping from a two-compartmental analysis of peripheral C-peptide concentrations with individual kinetic parameters derived after intravenous bolus injections of biosynthetic human C-peptide. At the 300-mg/dl clamp level, the insulin secretion rate increased to a value 9.9 ± 0.7 times that of basal at the end of the clamp (mean ± SE), whereas over the same period, the peripheral insulin concentrations increased to a greater extent, reaching a value 15.4 ± 1.2 times that of basal (P = .002). This greater relative increase in the insulin concentration in comparison with the corresponding insulin secretion rate suggests a reduction in the clearance of endogenous insulin. A similar trend was seen at the 225-mg/dl clamp level, but the relative increase in the insulin concentration (9.9 ± 1.5 times that of basal) was not significantly higher than the relative increase in the insulin secretion rate (8.1 ± 0.5 times that of basal, P = .17). At the 120-mg/dl clamp level, the relative increases in the insulin secretion rate (2.7 ± 0.2 times that of basal) and the insulin concentration (2.4 ± 0.2 times that of basal) were similar (P = .26), indicating no reduction in endogenous insulin clearance during moderate stimulation of insulin secretion. In conclusion, a reduction in endogenous insulin clearance occurs during greater stimulation of insulin secretion at higher glucose-clamp levels. These data suggest that endogenous insulin clearance is nonlinear and shows evidence of saturation at high physiologic insulin concentrations. *Diabetes* 37: 1351–57, 1988

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The liver is the major site of insulin removal from the circulation, and in the fasting state this organ extracts ~50% of newly secreted insulin during its first pass before reaching the peripheral circulation (1–5). The regulation of hepatic insulin extraction during stimulation of insulin secretion remains the subject of considerable controversy. Studies with oral glucose administration showed a reduction (6–8), no change (9,10), or an increase in fractional hepatic insulin extraction (11–13). Widely differing experimental techniques, as well as species differences, may be responsible for these discrepancies. Few studies have investigated hepatic insulin extraction during intravenous glucose administration (13). Ishida et al. (13) observed unchanged hepatic insulin extraction after intraportal and peripheral intravenous glucose administration in dogs.

Quantitation of hepatic insulin extraction in humans has been complicated by the difficulty in measuring insulin in portal venous blood. With the availability of biosynthetic human C-peptide (BHCP) for experimental use (14,15), we have demonstrated that pancreatic insulin secretion rates can be accurately derived under non-steady-state conditions from peripheral C-peptide concentrations with an open two-compartment model of C-peptide kinetics as proposed by Eaton et al. (8,16). In this study we used this approach to investigate the relationship between the insulin secretion rate and the peripheral insulin concentration during hyperglycemic clamp studies at three glucose levels in normal subjects.

MATERIALS AND METHODS

SUBJECTS

Studies were performed on eight healthy nonobese volunteers (4 men, 4 women; mean ± SE age 26.1 ± 1.9 yr; weight

67.9 ± 4.7 kg; body mass index 22.4 ± 0.7 kg/m^2; ideal body weight 102.9 ± 4.4%). No subject had a personal or family history of diabetes mellitus. Liver-function tests were normal. All studies were carried out in the clinical research center of the University of Chicago after written informed consent had been obtained. The experimental protocol was approved by the institutional review board.

EXPERIMENTAL PROTOCOLS

All studies were performed after a 10-h overnight fast. During each experiment, an intravenous sampling catheter was inserted into the dorsum of the hand, and 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 occasions within a 4-wk period. The individual studies were performed as follows.

Intravenous bolus injection of BHCP. After inhibition of endogenous C-peptide and insulin secretion by a primed constant intravenous infusion of somatostatin (500 μg/h, Bachem, Torrance, CA), each subject received an intravenous bolus injection of 150 μg BHCP (Lilly, Indianapolis, IN). The details of this protocol have been described (15). Analysis of the resulting C-peptide decay curves allowed the kinetic parameters of C-peptide for an open two-compartment model of C-peptide distribution to be individually derived in each subject (17).

Hyperglycemic clamp studies. To compare the effects of increasing glucose-clamp levels on insulin secretion and clearance, each subject was studied on three occasions. After a 60-min basal period during which glucose, insulin, and C-peptide were measured at 15-min intervals, glucose was infused intravenously to raise the plasma glucose concentration acutely to 120, 225, or 300 mg/dl. The plasma glucose concentration was held constant at the respective glucose level for 180 min by adjusting the glucose infusion rate every 5 min based on the peripheral plasma glucose concentration. The order of the experiments was randomized. In each study, the peripheral insulin and C-peptide concentrations were measured at the following times after starting the glucose infusion: 1, 3, 5, 7, 9, 12, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, and 180 min.

SAMPLE COLLECTION AND 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 aprotinin (Trasylol, Bayer, FBA Pharmaceuticals, New York) and 1.2 mg/ml EDTA. Plasma was separated and stored frozen at −20°C until assayed. Blood for measurement of glucose was centrifuged in tubes containing NaF to inhibit enzymatic glycolysis, and plasma glucose was measured immediately with a glucose analyzer (YSI model 23A, Yellow Springs, OH) by the glucose oxidase method. Serum insulin was assayed by a double-antibody technique (18). Human C-peptide immunoreactivity in plasma was measured with the M 1230 antibody as previously described (19). BHCP and ^{125}I-labeled Tyr-BHCP were used as assay standard and tracer, respectively (20).

CALCULATIONS

Insulin secretion rates. Each C-peptide decay curve was resolved into the sum of two exponential functions by nonlinear least-squares regression analysis, allowing the kinetics of C-peptide to be individually defined (15,17). Pancreatic insulin secretion rates were derived by application of an open two-compartment model of C-peptide distribution and metabolism as proposed by Eaton et al. (8,16). We previously demonstrated in our laboratory that this model allows accurate estimates of insulin secretion rates to be derived under non-steady-state conditions (14,15).

Relative changes in insulin secretion rate and peripheral insulin concentration. To define the relationship between the pancreatic insulin secretion rate and the simultaneously measured peripheral insulin concentration at each clamp level, the insulin secretion rates and the peripheral insulin concentrations were expressed as percentages of their respective mean basal values, which were taken as 100%. Because insulin has a high metabolic clearance rate, a similar increase in the insulin secretion rate and the peripheral insulin concentration in response to the glucose infusion was taken as evidence that no change in the clearance of endogenously secreted insulin had occurred (21). A greater relative increase in the peripheral insulin concentration than the insulin secretion rate was assumed to indicate a reduction in the clearance of endogenously secreted insulin.

As an alternative approach, we also calculated the ratio of the total area under the insulin secretion rate curve to the total area under the peripheral serum insulin concentration curve for both the basal period and the poststimulatory period at each glucose-clamp level. This ratio is an indicator of insulin clearance in that it compares the amount of insulin secreted with the amount of insulin in the peripheral circulation (21). This approach gives an integrated view of changes in insulin clearance but does not allow transient changes to be detected.

STATISTICAL ANALYSIS

Results are expressed as means ± SE. Areas under the concentration and secretion-rate curves were calculated by the trapezoidal rule. Nonlinear least-squares regression analysis of the C-peptide decay curves was performed with the BMDP 3R program (BMDP Statistical Software, Los Angeles, CA). Means were compared by the paired two-tailed *t* test or by two-way analysis of variance (ANOVA) with subsequent Bonferroni's (Dunn's) *t* test where indicated. *P* values $<.05$ were considered statistically significant. Data analysis was performed with the Statistical Analysis System (version 6 edition for personal computers, SAS, Cary, NC).

RESULTS

Decay curves of C-peptide. After 60 min of somatostatin infusion, the plasma C-peptide concentration was suppressed to 0.08 ± 0.01 pmol/ml. Two minutes after the C-peptide bolus injection, the plasma C-peptide was 10.8 ± 0.3 pmol/ml. The subsequent fall in plasma C-peptide concentration demonstrated a fast and slow component with half-disappearance times of 5.3 ± 0.5 and 31.8 ± 1.9 min, respectively. The metabolic clearance rate of the injected human C-peptide was 4.01 ± 0.17 ml · min^{-1} · kg^{-1}

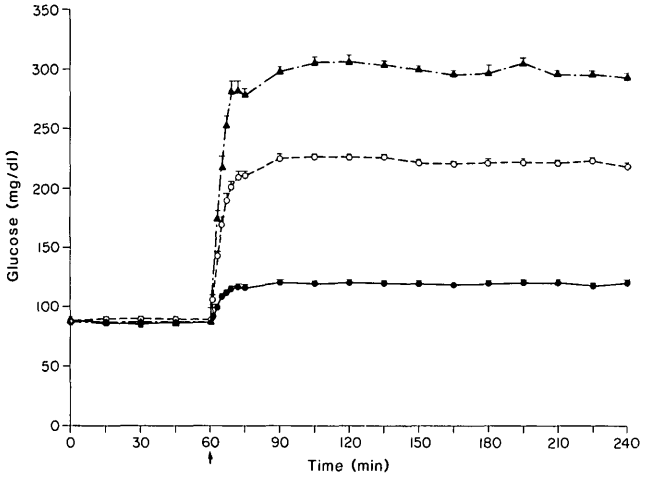

FIG. 1. Peripheral concentrations (mean ± SE) of arterialized plasma glucose in basal period and during hyperglycemic clamp studies at glucose levels of 120, 225, and 300 mg/dl. *Arrow* indicates initiation of glucose infusion.

(149.1 ± 4.8 ml · min^{-1} · m^{-2}). The fractional kinetic rate constants for C-peptide derived by two-compartmental analysis of the C-peptide decay curves were as follows: k_1 = 0.0482 ± 0.0048 min^{-1}, k_2 = 0.0513 ± 0.0045 min^{-1}, k_3 = 0.0601 ± 0.0035 min^{-1}; distribution volume of the central accessible compartment 68.2 ± 4.7 ml/kg (2536.2 ± 162.8 ml/m^2). The parameters are similar to those previously reported by our laboratory (15) and other investigators (22,23).

Peripheral concentrations of glucose, insulin, and C-peptide. The mean concentrations of glucose, insulin, and C-peptide in the basal state (0–60 min) and during the three hyperglycemic clamp studies (60–240 min) at plasma glucose levels of 120, 225, and 300 mg/dl are illustrated in Figs. 1 and 2. The mean basal concentrations of glucose, insulin, and C-peptide were similar on the 3 study days (overall mean for plasma glucose 87.8 ± 1.3 mg/dl, insulin 6.1 ± 0.8 μU/ml, and C-peptide 0.34 ± 0.03 pmol/ml). On average, 39.8 ± 3.6, 132.9 ± 9.5, and 203.3 ± 9.1 g glucose were infused over the 3 h of the three glucose-clamp studies. Plasma glucose concentrations of 119.7 ± 0.2, 222.2 ± 0.9, and 297.8 ± 1.4 mg/dl, respectively, were achieved during the period 75–240 min. The mean coefficient of variation of plasma glucose during this period was 2.4 ± 0.3% at the 120-mg/dl, 3.8 ± 0.3% at the 225-mg/dl, and 4.5 ± 0.5% at the 300-mg/dl clamp levels.

The peripheral insulin concentrations showed a biphasic pattern in response to hyperglycemic glucose clamping at each of the three glucose levels. In contrast to the insulin concentrations, the first phase of insulin secretion was not well reflected in the peripheral C-peptide concentrations.

Insulin secretion rates. The insulin secretion rates during the basal period and during the three hyperglycemic clamp studies are shown in Fig. 2. The basal insulin secretion rate was not significantly different among the 3 study days (overall mean 81.2 ± 7.7 pmol/min). The insulin secretory response to each of the hyperglycemic clamps demonstrated a clear biphasic pattern. At the 120-mg/dl clamp level, the insulin secretion rate was constant during the second phase,

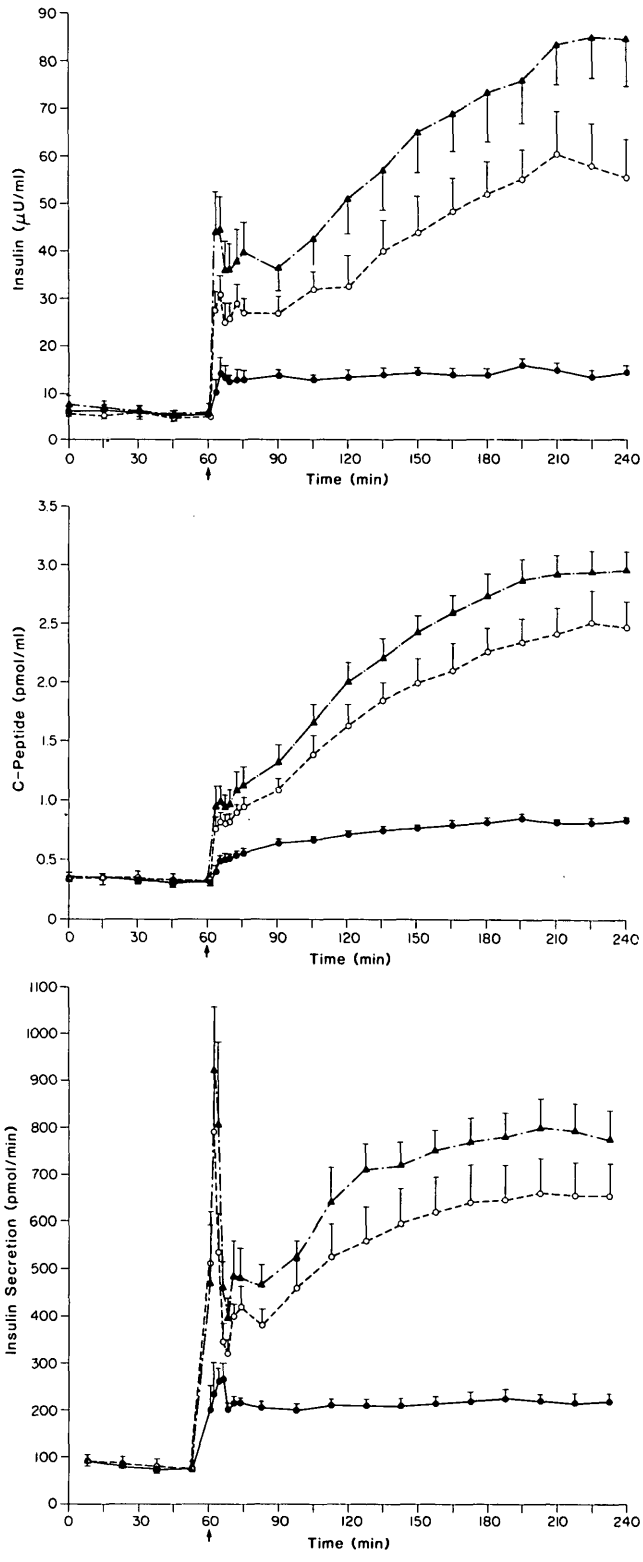

FIG. 2. Peripheral concentrations (means ± SE) of insulin (*top*) and C-peptide (*middle*) and insulin secretion rates (*bottom*) in basal period and during hyperglycemic clamp studies at plasma glucose levels of 120 (●), 225 (○), and 300 (▲) mg/dl. *Arrow* indicates initiation of glucose infusion.

similar to the peripheral insulin concentration at this level. At the 225- and 300-mg/dl clamp levels, the second-phase insulin secretory response leveled off with increasing duration of glucose clamping, but the peripheral insulin concentration still continued to increase. Thus, in the final 2 h of the glucose infusion at the 300-mg/dl clamp level, the insulin secretion rate increased by ~21%, from 641 ± 75 to 777 ± 58 pmol/min at the completion of the glucose infusion. During the same period, the peripheral insulin concentration increased by ~65%, from 51 ± 8 to 84 ± 10 μU/ml.

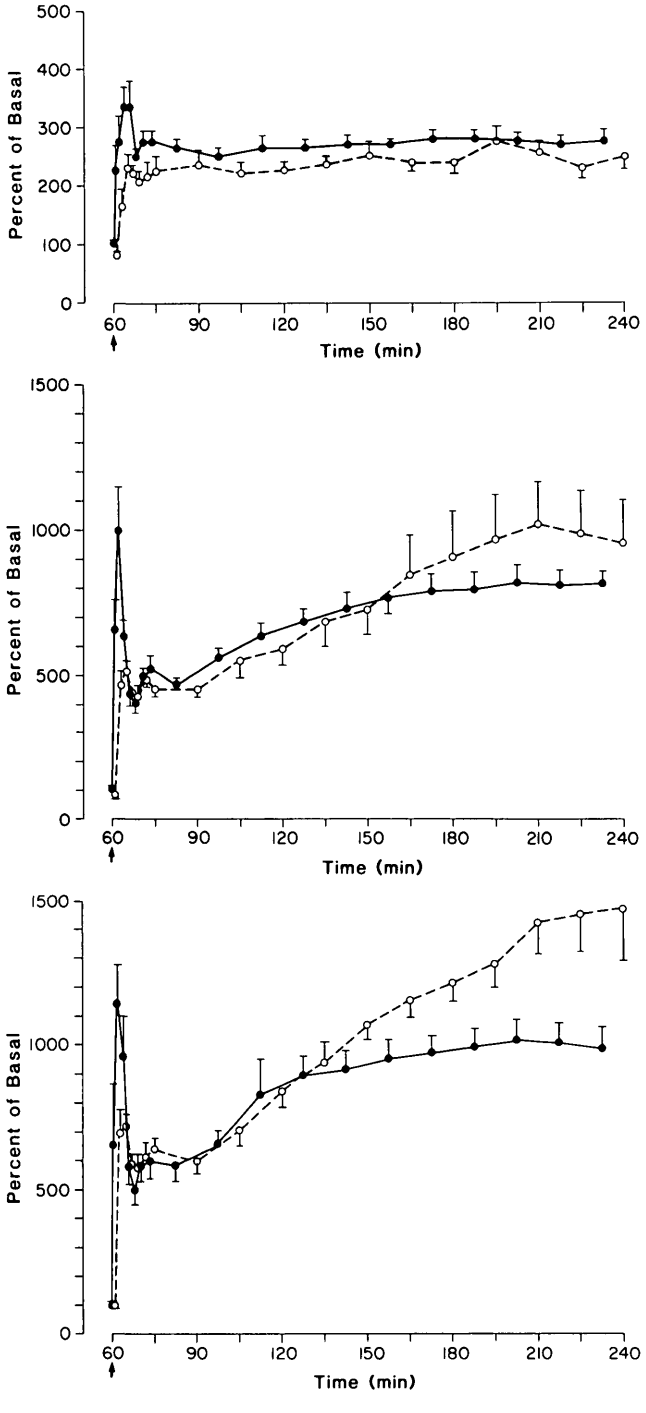

FIG. 3. Insulin secretion rates (●) and peripheral insulin concentrations (○), expressed as percentages of their respective mean basal values, in response to hyperglycemic glucose infusion at clamp levels of 120 (*top*), 225 (*middle*), and 300 (*bottom*) mg/dl (mean ± SE). Note that scale of vertical axis in *top panel* is double scale for other panels. *Arrow* indicates initiation of glucose infusion.

Relationship between insulin secretion rate and peripheral insulin concentration. To explore the relationship between the increases in the insulin secretion rate and the simultaneously measured peripheral insulin concentration at each clamp level, the insulin secretion rates and peripheral insulin concentrations (at each sampling time point) were expressed as the percentage of their respective mean basal values, which were taken as 100%. The data are shown in Fig. 3. Statistical comparisons between the relative increases in the insulin secretion rate and insulin concentration were made during the last 30 min of the clamps (Fig. 4).

During the first 75 min of the 300-mg/dl clamp (Fig. 3, *bottom*), the relative changes in the insulin concentration closely followed the relative changes in the insulin secretion rate. Then, at an average absolute insulin concentration of 57 ± 8 μU/ml (Fig. 2), the relative increase in the insulin concentration began to exceed the relative increase in the insulin secretion rate. Subsequently, with continuing stimulation of insulin secretion, this difference between the relative increases in insulin concentration and secretion rate became more pronounced. Thus, whereas the insulin secretion rate increased to 993.8 ± 72.6% of basal between 210 and 240 min, the peripheral insulin concentration increased to a significantly greater extent, reaching 1539.6 ± 124.4% of basal during the same period (P = .002). This greater increase in the peripheral insulin concentration compared with the corresponding insulin secretion rate when both are viewed in relation to their basal values can only be explained by a decrease in the clearance of endogenously secreted insulin. The relative increase in insulin concentration was considerably greater than that of insulin secretion in six subjects and slightly lower in two subjects (Fig. 4).

During the 225-mg/dl clamp (Fig. 3, *middle*), the relative increase in the peripheral insulin concentration (988.4 ± 146.1% of basal between 210 and 240 min) tended to be higher than the relative increase in the insulin secretion rate during the same interval (812.2 ± 47.6% of basal), but the difference was not statistically significant (P = .17). The relative increase in insulin concentration was higher than that in insulin secretion in seven subjects, although only slightly so in two of them (Fig. 4). In one subject the relative increase in insulin secretion rate was greater than the relative increase in peripheral insulin concentration.

At the 120-mg/dl clamp level (Fig. 3, *top*), the insulin secretion rate increased to 273.1 ± 16.6% of basal between 210 and 240 min, whereas the peripheral insulin concentration increased to 244.3 ± 21.0% of basal during the same time period (P = .26). In six of the eight subjects the relative increase in the insulin secretion rate was greater than that in insulin concentration (Fig. 4), whereas in the remaining two subjects the relative increase in the insulin concentration was greater than that in insulin secretion.

The above analysis suggested a reduction in the clearance of endogenous insulin at increasing glucose-clamp levels. As an alternative approach, we calculated the ratio of the total area under the insulin secretion rate curve to the total area under the peripheral insulin concentration curve

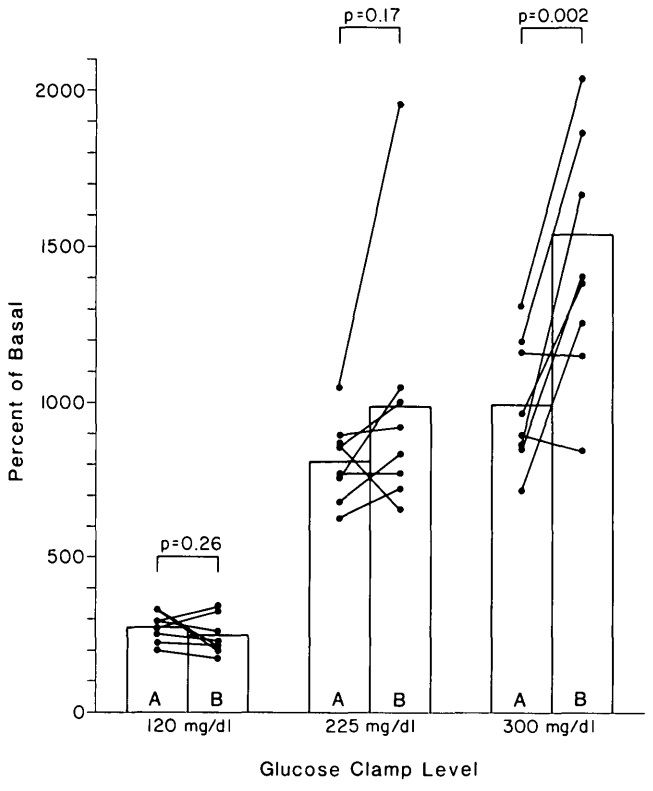

FIG. 4. Comparison between increase in insulin secretion rate (relative to basal) and peripheral insulin concentration (relative to basal) during last 30 min of hyperglycemic clamp at plasma glucose levels of 120, 225, and 300 mg/dl, respectively. *A*, insulin secretion rate; *B*, peripheral insulin concentration.

during the 60-min basal period and for the 180-min clamp period. The ratio during the basal period was not significantly different on the 3 study days and averaged 2215.9 ± 255.4 ml/min. The ratio of 1717.7 ± 135.9 ml/min during the clamp at 300 mg/dl was significantly ($P < .05$) lower than the ratio of 2373.0 ± 263.8 ml/min during the 120-mg/dl clamp. This result suggests a reduction in the overall insulin clearance at the 300-mg/dl clamp level in comparison with the 120-mg/dl clamp level. The ratio of 1976.8 ± 123.1 ml/min during the 225-mg/dl clamp was intermediate, although not significantly different from the values at the other two clamp levels.

Because the reduction in insulin clearance occurred with increasing duration of glucose clamping (Fig. 3), we also calculated this ratio separately for the second 90 min of the clamp studies. The ratio during the last 90 min at the 300-mg/dl clamp level (1543.6 ± 140.4 ml/min) was significantly ($P < 0.05$) lower than the corresponding ratio of 2305.5 ± 267.3 ml/min at the 120-mg/dl clamp level and the ratio during the basal period (2215.9 ± 255.4 ml/min). The ratio of 1801.0 ± 104.5 ml/min at the 225-mg/dl clamp level was intermediate and not significantly different from the two other clamp levels or the basal period. These results suggest that marked stimulation of insulin secretion during the second 90 min of the 300-mg/dl clamp is characterized by a significant reduction in insulin clearance in comparison with the basal fasting state and to the situation after moderate stimulation of insulin secretion (during the second 90 min of the 120-mg/dl clamp).

DISCUSSION

Several studies have shown that when insulin is infused into animals or humans, the rate of clearance of the administered insulin falls as its concentration increases (2,4,24–27). These data have been interpreted to indicate that insulin is removed by a saturable receptor-mediated mechanism in vivo, and the same conclusion has been reached with different in vitro systems (1,28). Thus, there is general agreement that insulin clearance is a saturable process, although the level at which saturation occurs is controversial (24, 25,27). Although most studies have concluded that the rate of insulin clearance is constant over the physiologic range, with saturation occurring only at supraphysiologic levels, researchers have suggested that saturation of the insulin clearance mechanism may play a role in the regulation of insulin concentrations within the physiologic range.

With the in vivo studies described above, we have examined the clearance of exogenous insulin infused into the peripheral circulation. Study of the clearance of endogenously secreted insulin had not been possible with previous methods. This study was therefore undertaken to examine changes in the clearance of endogenous insulin in response to intravenous glucose infused to three levels of plasma glucose. We have demonstrated that C-peptide clearance remains constant over a wide concentration range up to levels as high as 8 pmol/ml (15). Furthermore, the clearance of this peptide is unaffected by increases in the plasma glucose level to values as high as 300 mg/dl (29). Constancy of C-peptide clearance under these circumstances enabled us to derive accurate estimates of insulin secretion by mathematical deconvolution of peripheral C-peptide concentrations with individually derived C-peptide kinetics and a previously validated two-compartment model. This method allowed the relative changes in insulin secretion and simultaneously measured peripheral insulin concentrations to be examined in detail. At the lowest glucose concentration (120 mg/dl), insulin concentrations increased in parallel with the increase in insulin secretion, indicating that insulin clearance rates did not change during the study. At the highest glucose level, the insulin concentration increased to a greater extent than the increase in insulin secretion, and this discrepancy was most clearly evident during the final 60 min of the glucose infusion (Fig. 3). At that glucose level, the insulin secretion rate increased to a value 9.9 ± 0.7 times that of basal by the end of the clamp, but over the same period, the corresponding peripheral insulin concentrations increased 15.4 ± 1.2-fold. Peak insulin concentrations achieved during the high-dose glucose infusion were <90 μU/ml, i.e., concentrations that are still within the physiologic range. Although there was a tendency for insulin clearance rates to fall at the 225 mg/dl glucose level, the differences were not statistically significant. In only two subjects did the insulin concentrations fail to increase to a greater extent than the corresponding insulin secretion rate. The reason that insulin clearance did not fall in all subjects at the 300-mg/dl glucose level is uncertain but probably relates to variability in the secretory response to the intravenous glucose stimulus, as well as variability in the plasma concentration at which saturation of clearance mechanisms occurs.

As an alternative approach to the analysis of the data, we calculated the ratio between the total areas under the insulin

secretion rate curve and the peripheral insulin concentration curve, both for the baseline period and for each clamp level. This ratio was significantly lower at the 300-mg/dl clamp level (1718 ± 136 ml/min) than at the 120-mg/dl clamp level (2373 ± 264 ml/min), as well as the ratio measured during the basal period (2216 ± 255), suggesting a reduction in endogenous insulin clearance at the higher clamp level. The ratio was intermediate at the 225-mg/dl clamp level (1977 ± 123).

Although this study was not designed to determine whether the observed fall in insulin clearance was due to the increase in glucose concentration per se, this possibility is highly unlikely in view of previous studies that indicate that the prevailing glucose concentration does not affect the rate of clearance of insulin (13,30). A more likely explanation is that the reduction in insulin clearance is related to the greater increase in insulin secretion seen at the higher glucose levels. Because the liver plays the major role in insulin clearance, this fall in clearance may be due to saturation of the hepatic insulin-uptake process resulting from increased portal vein insulin concentrations. This postulate is compatible with the in vitro observations that hepatic insulin clearance occurs by a saturable receptor-mediated mechanism (1,28). An alternative explanation is that a time-dependent, dose-dependent saturation of low-affinity insulin receptors occurred, leading to a recirculation of insulin during the latter half of the glucose infusion. Nestler et al. (31) have observed a time-dependent effect of hyperinsulinemia on insulin clearance and postulated such a reservoir mechanism. This mechanism could explain the observation that the fall in clearance was observed only after the glucose had been infused for ~120 min. It could also explain the reason that Sherwin et al. (32) failed to observe a decrease in insulin clearance during a shorter (80-min) exogenous insulin infusion, although insulin concentrations as high as 200 μU/ml were reached. A further explanation relates to a recent report that indicates that after receptor-mediated endocytosis, a proportion of the internalized insulin undergoes rapid exocytosis without degradation (33). This phenomenon has been termed *retroendocytosis*. An increase in the rate of this process at higher insulin concentrations could result in a reduction of insulin clearance.

In summary, in this study we quantitated insulin secretion rates in normal humans during hyperglycemic glucose-clamp studies with individually derived kinetic parameters for C-peptide. We demonstrated that during greater stimulation of insulin secretion at higher glucose-clamp levels, a reduction in endogenous insulin clearance occurs. Thus, tests of β-cell function, which involve intravenous administration of glucose in high doses, should not assume that the resulting increases in peripheral insulin concentration are solely the result of increases in insulin secretion. Because endogenous insulin clearance may show evidence of saturation at physiologic insulin concentrations, the observed hyperinsulinemia may result from a combination of both increased insulin secretion and decreased insulin clearance.

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