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Pulsatile Insulin Secretion Dictates Systemic Insulin Delivery by Regulating Hepatic Insulin Extraction In Humans

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In health, insulin is secreted in discrete pulses into the portal vein, and the regulation of the rate of insulin secretion is accomplished by modulation of insulin pulse mass. Several lines of evidence suggest that the pattern of insulin delivery by the pancreas determines hepatic insulin clearance. In previous large animal studies, the amplitude of insulin pulses was related to the extent of insulin clearance. In humans (and in large animals), the amplitude of insulin oscillations is ~100-fold higher in the portal vein than in the systemic circulation, despite only a fivefold dilution, implying preferential hepatic extraction of insulin pulses. In the present study, by direct hepatic vein sampling in healthy humans, we sought to establish the extent of first-pass hepatic insulin extraction and to determine whether the pattern of insulin secretion (insulin pulse mass and amplitude) dictates the hepatic insulin clearance and thereby delivery of insulin to extrahepatic insulin-responsive tissues. Five nondiabetic subjects (two men and three women, mean age 32 years [range 25–39], BMI 24.9 kg/m^2 [21.2–27.1]) participated. Insulin and C-peptide delivery from the splanchnic bed was measured in basal overnight-fasted state and during a glucose infusion of 2 mg · kg^{-1} · min^{-1} by simultaneous sampling from the hepatic vein and an arterialized vein along with direct estimation of splanchnic blood flow. Fractional insulin extraction was calculated from the difference between the C-peptide and insulin delivery rates from the liver. The time patterns of insulin concentrations and hepatic insulin clearance were analyzed by deconvolution and Cluster analysis, respectively. Cross-correlation analysis was used to relate C-peptide secretion and insulin clearance. Glucose infusion increased peripheral glucose concentrations from 5.4 ± 0.1 to 6.4 ± 0.4 mmol/l ($P < 0.05$). Likewise, insulin and C-peptide concentrations increased during glucose infusion ($P < 0.05$). Hepatic insulin clearance increased with glucose infusion (1.06 ± 0.18 vs. 2.55 ± 0.38 pmol · kg^{-1} · min^{-1}; $P < 0.01$), but fractional hepatic insulin clearance was stable (78.2 ± 4.4 vs. 84 0. ± 3.9%, respectively; $P = 0.18$). Insulin secretory–burst mass rose during glucose infusion ($P < 0.05$), whereas the interburst interval remained unchanged (4.4 ± 0.2 vs. 4.5 ± 0.3 min; $P = 0.36$). Cluster analysis identified an oscillatory pattern in insulin clearance, with peaks occurring approximately every 5 min. Cross-correlation analysis between prehepatic C-peptide secretion and hepatic insulin clearance demonstrated a significant positive association without detectable (<1 min) time lag. Insulin secretory–burst mass strongly predicted insulin clearance ($r = 0.81$, $P = 0.0043$). In conclusion, in humans, ~80% of insulin is extracted during the first liver passage. The liver rapidly responds to fluctuations in insulin secretion, preferentially extracting insulin delivered in pulses. The mass (and therefore amplitude) of insulin pulses traversing the liver is the predominant determinant of hepatic insulin clearance. Therefore, through this means, the pulse mass of insulin release dictates both hepatic (directly) as well as extra-hepatic (indirectly) insulin delivery. These findings emphasize the dual role of the liver and pancreas and their relationship mediated through magnitude of insulin pulse mass in regulating the quantity and pattern of systemic insulin delivery. *Diabetes* 54:1649–1656, 2005

Following insulin secretion, the first organ of impact is the liver, where a proportion of secreted insulin is cleared through a receptor-mediated process (1–3). The proportion of insulin cleared has been estimated as ~50% in dogs (2,4) and ~40–80% in humans (5–8). Insulin is secreted in discrete secretory bursts occurring approximately every 4 min (9,10). Some studies have suggested that the pulsatile pattern of insulin delivery to the liver is important in insulin action (11,12). Regulation of insulin secretion is predominantly accomplished by modulation of burst mass, which is increased in response to glucose ingestion or infusion, sulfonylurea ingestion, and GLP-1 infusion (13–15) and decreased in response to somatostatin or IGF-1 infusion (16) and induction of anesthesia (17). One consequence of the intermittent release of insulin into the portal vein is that the insulin concentration profile presented to the liver exhibits striking oscillations (amplitude ~1,000–5,000 pmol/l) (18). The amplitude of insulin pulses in the systemic circulation is only ~1% of that in the portal vein (18). Although such damping is presumably at least in part due to the fivefold dilution of portal-vein blood by ancillary venous return, the extent of amplitude quenching suggests that the liver may preferentially clear insulin pulses.

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Several lines of evidence support a hypothesis of pulse amplitude–dependent insulin extraction. For example, low-dose somatostatin infusion suppressed insulin pulse size by ~50% in the portal vein of the dog but did not change systemic insulin concentrations because insulin clearance also decreased by 50% (16). Moreover, insulin clearance was directly related to the amplitude of prehepatic insulin pulses (16). Insulin clearance similarly fell in pigs when β-cell mass was reduced with alloxan, leading to a decrease in insulin pulse mass and a proportionate reduction in insulin clearance (19). Patients with type 2 diabetes have attenuated insulin pulses (20–23) and impaired hepatic extraction of endogenous insulin (24,25). Taken together, these data support the notion that hepatic insulin clearance might be pulsatile, with the liver preferentially removing insulin pulses, putatively via a receptor-mediated process (3). A complementary implication is that pulsatile insulin delivery may determine the hepatic actions of insulin, e.g., in suppressing hepatic glucose release. Both issues are important to establish, given the low amplitude of insulin pulses delivered to the liver in diabetes and the absence of insulin pulses engendered with conventional therapeutic insulin. To address the foregoing issues, we simultaneously measured minute-by-minute insulin secretion and clearance in healthy nondiabetic humans by direct hepatic vein catheterization during the overnight-fasted basal state and subsequent glucose infusion.

By these means, we addressed the following questions. *1*) What is the extent of hepatic insulin clearance in the fasting state and during intravenous glucose infusion? *2*) Is insulin cleared in a pulsatile fashion? *3*) Does insulin clearance oscillate concordantly with insulin pulses? *4*) Is there a direct proportionality between the amplitude (or mass) of insulin secretory bursts and hepatic clearance of endogenous insulin?

RESEARCH DESIGN AND METHODS

The study was approved by the Mayo Clinic Institutional Review Board. Written informed consent was obtained from all study participants. Five healthy subjects (two men and three women) mean age 32 years (range 25–39), with a mean BMI of 24.9 kg/m^2 (21.2–27.1) participated. All subjects were nondiabetic based on fasting plasma glucose concentration (mean fasting plasma glucose 5.4 mmol/l [5.1–5.8]) and HbA_{1c} values (mean 4.8% [4.5–5.0]). Insulin and C-peptide delivery from the splanchnic bed was measured in the basal overnight-fasted state and then during a constant glucose infusion of 2 mg · kg^{-1} · min^{-1} by concurrent sampling from the hepatic vein and an arterialized hand vein catheter. The constant glucose infusion rather than a hyperglycemic clamp was selected to avoid induction of spurious insulin pulses arising from fluctuations in glucose infusion rate (26,27). Splanchnic blood flow was measured by indocyanine green infusion. Fractional insulin extraction by the liver was calculated from the difference between the C-peptide and insulin delivery rates from the liver.

Subjects were admitted to the General Clinical Research Center at the Mayo Clinic, Rochester, Minnesota, the day before study. Participants ate a standard evening meal and fasted overnight. On the morning of the study, a hepatic-vein sampling catheter was placed percutaneously via a femoral vein under local anesthesia using fluoroscopic guidance. A second sampling catheter was placed retrogradely in a dorsal hand vein, and the hand was heated to 55°C in a thermoregulated Perspex hand warmer to achieve arterialization. A third catheter was placed in the contralateral antecubital vein for the infusion of glucose and indocyanine green (Cardio-Green; Becton Dickinson Microbiology Systems, Cockeysville, MD).

After placement of the catheters, following a 45-min rest period (t = −45 to 0 min), blood was sampled concurrently at 1-min intervals from the hepatic vein and arterialized dorsal hand vein for 40 min (t = 0–40 min). After this sampling period, a glucose infusion (2 mg · kg^{-1} · min^{-1}) was started and continued for 70 min (t = 40–110 min). Blood samples were again obtained concurrently at 1-min intervals from the hepatic vein and arterialized dorsal hand vein catheters for a further 40 min (t = 70–110 min). All blood samples for measurement of insulin were immediately separated at the bedside in chilled microcentrifuges, and the plasma was stored at −20°C until insulin was measured. Plasma glucose concentrations were measured immediately at the bed side. Plasma for measurement of indocynanine green concentrations was maintained on ice until the end of the study and then measured within 1 h.

Assays. Plasma glucose concentrations were measured by the glucose oxidase method with a Glucose Analyzer 2 (Beckman Coulter, Palo Alto, CA). Plasma insulin concentration was measured in triplicate by a two-site immunoenzymatic assay performed on the Access automated immunoassay system (Beckman Instruments, Chaska, MN). Intra-assay coefficients of variation (CVs) were 2% at 6.75 mU/l and 2.6% at 116 mU/l, and interassay CVs were 3.9% at 12.7 mU/l, 3.9% at 48.8 mU/l, and 4.6% at 121 mU/l. Plasma C-peptide was measured using a direct, double-antibody, sequential radioimmunoassay (Linco Research, St. Charles, MO) with interassay CVs of 4.9, 4.3, and 8.0% at 0.43, 1.75, and 4.36 nmol/l, respectively. Indocyanine green concentrations were measured as previously described (28). The hematocrit was measured by a hematocritogram to allow computation of plasma flow (below).

Calculations. Results are presented as the mean ± SEM. Splanchnic blood flow was measured by the constant-infusion technique using indocyanine green dye as previously described (28). Due to blood volume limitations, splanchnic blood flow was measured at 10-min intervals and the intervening 1-min data obtained by interpolating in a linear manner between the measured flow rates. Splanchnic plasma flow was calculated by multiplying the splanchnic blood flow by (1.0 − fractional hematocrit). Insulin and C-peptide delivery from the splanchnic bed was calculated from the plasma concentration difference in the hepatic vein versus the arterialized vein multiplied by the splanchnic plasma flow. Fractional hepatic extraction of insulin was calculated from the difference between the C-peptide and the insulin delivery rates as a fraction of the endogenous C-peptide secretion rate. This approach requires the following assumptions. *1*) There is negligible C-peptide clearance by the liver, *2*) splanchnic insulin clearance is due principally to hepatic insulin clearance, *3*) there is minimal (<1 min) delay between the hepatic vein and arterial sampling sites, and *4*) portal blood flow is relatively stable from minute to minute.

For intra-individual comparisons of hepatic insulin clearance during basal conditions and during glucose infusion, the mean values of each period were computed. Thus, each data point was derived from 40 individual time points from each sampling site.

Pulsatile insulin secretion was quantified by deconvolution of 1-min arterialized plasma insulin concentrations as previously validated and described (29). This deconvolution approach is a multiparameter technique that requires the following assumptions. The plasma insulin concentrations measured in samples collected at 1-min intervals were assumed to result from five determinable and correlated parameters: *1*) a finite number of discrete insulin secretory bursts occurring at specific times and having *2*) individual amplitudes (maximal rate of secretion attained within a burst) and *3*) a common half-duration (duration of an algebraically Gaussian secretory pulse at half-maximal amplitude or 2.354 times the SD of the Gaussian waveform), which are superimposed upon a *4*) basal time-invariant insulin secretory rate and *5*) a biexponential insulin disappearance model in the systemic circulation consisting of estimated half-lives of 2.8 and 5.0 min and a fractional slow compartment of 28%, as previously measured (29). In this model, the mass secreted per burst is the algebraic product of the amplitude, SD of the Gaussian and $\sqrt{(2\pi)}$.

To address the question of whether hepatic insulin clearance is pulsatile, we examined the calculated minute-to-minute hepatic insulin clearance rates. Cluster analysis was used as a model-free method to detect time-varying excursions in minute-to-minute clearance values. The t statistics used for evaluating significant upstrokes or downstrokes in the insulin time series were taken as 2.0 with corresponding estimated cluster sizes of 2 and 1 for nadirs and peaks, respectively. This technique estimates the frequency and amplitude of oscillations in a data series without any assumptions about or knowledge of half-lives in the system.

In addition, the rhythmicity of the insulin clearance time series was quantitated by cosine regression analysis as described previously (30). This procedure entails unweighted regression of a cosine function of variable periodicity on the observed clearance time series. Ninety-five percent statistical confidence intervals were determined for the fitted amplitude (50% of the nadir-peak difference), period (min), and mesor (cosine mean).

To assess whether the dynamics of insulin delivery influence hepatic insulin clearance, cross-correlation analysis was applied to relate C-peptide secretion to insulin clearance on a sample-by-sample basis. This procedure consists of linear correlations carried out repeatedly at various time lags between the paired values. Thus, each C-peptide secretion value is compared with a time-delayed measure (e.g., lag time minus 2 min) of hepatic clearance.

At a zero time lag, simultaneous values are correlated. Since this analysis considers each time point individually, these calculations were based on a total of 400 values.

Linear regression analyses were carried out between insulin burst mass, burst amplitude, and the nonpulsatile component of insulin secretion and corresponding hepatic insulin clearance using GraphPad Prism, version 3.0 (San Diego, CA). In addition, two-way ANCOVA was carried out using insulin clearance during the glucose infusion as the dependent variable, insulin pulse mass as the independent variable, basal insulin clearance as the covariate, and the individual subjects as the random factors. Parameters obtained from a total of 100 (peripheral vein) and 90 (hepatic vein) pulses were included in this analysis. Two-way ANOVA and repeated-measures ANOVA were carried out using Statistica, version 6 (Statsoft, Tulsa, OK).

RESULTS

Appropriate location of the hepatic-vein catheter was verified by a higher glucose concentration in the hepatic vein than in the arterialized hand vein in each subject (5.8 ± 0.2 vs. 5.4 ± 0.1 mmol/l, $P < 0.01$). Glucose infusion increased arterialized glucose concentrations to 6.4 ± 0.4 mmol/l ($P < 0.05$) (Fig. 1). As expected, plasma insulin and C-peptide concentrations increased during glucose infusion ($P < 0.05$), and concentrations of both insulin and C-peptide were higher at all time points in the hepatic vein than in the arterialized vein ($P < 0.05$) (Fig. 1). Splanchnic plasma flow decreased slightly during the glucose infusion compared with the basal period (0.54 ± 0.07 vs. 0.60 ± 0.05 l/min; $P < 0.05$).

The computed endogenous C-peptide secretion rate increased approximately threefold during the glucose infusion (1.3 ± 0.2 vs. 3.0 ± 0.4 pmol · kg^{-1} · min^{-1} ; basal versus glucose infusion, $P < 0.01$) (Fig. 2). Delivery of insulin from the splanchnic bed increased ~1.5-fold during glucose infusion (0.27 ± 0.06 vs. 0.42 ± 0.09 pmol · kg^{-1} · min^{-1}; $P < 0.05$) (Fig. 2). Therefore, as expected, hepatic insulin clearance increased by ~2.5-fold during glucose infusion (1.06 ± 0.18 vs. 2.55 ± 0.38 pmol · kg^{-1} · min^{-1}; $P < 0.01$). However, fractional hepatic clearance of endogenously secreted insulin did not increase during glucose infusion (84.0 ± 3.9 vs. 78.2 ± 4.4%; $P = 0.18$) (Fig. 3).

Inspection of 1-min arterialized insulin concentration data revealed ~4-min oscillations, with larger amplitude oscillations evident in the hepatic vein insulin concentration time series (Fig. 4). As expected, the amplitude of the insulin oscillations increased markedly during glucose infusion. Deconvolution analysis affirmed the presence of pulsatile insulin secretion. While the insulin pulse mass increased by 1.3-fold ($P < 0.05$), insulin pulse interval remained unchanged (4.4 ± 0.2 vs. 4.5 ± 0.3 min; $P = 0.36$).

Inspection of minute-by-minute hepatic insulin clearance rates (Fig. 4) in each subject also revealed oscillations, confirmed by Cluster analysis with an interval of 5.3 ± 0.6 min in the basal state and 4.9 ± 0.6 min during glucose infusion ($P = 0.56$). Periodicity in the insulin clearance time series was confirmed by cosine regression. The mean period was 7.7 ± 0.6 min in the basal state and 7.4 ± 0.6 min during glucose infusion ($P = 0.68$). The mean amplitude (one-half of the difference between the peak and the nadir) was 16 ± 3 and 27 ± 7 ml/min, respectively ($P = 0.11$), and the mesor (computed mean about which the clearance time series oscillates) was 72 ± 15 and 173 ± 28 ml/min ($P = 0.0029$).

Cross-correlation analysis between C-peptide secretion and hepatic insulin clearance demonstrated a significant relationship at a lag period of <1 min (Fig. 5), indicating that prehepatic insulin secretion determines nearly simultaneous insulin clearance by the liver.

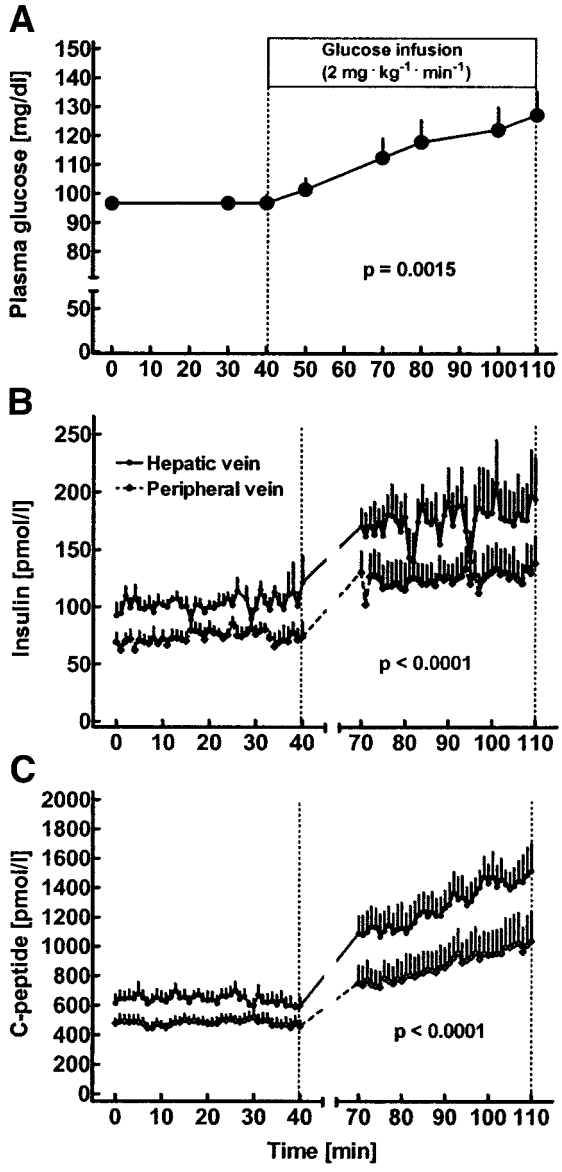

FIG. 1. Plasma concentrations of glucose (*A*), insulin (*B*), and C-peptide (*C*) in five healthy subjects studied in the fasting state and during an intravenous glucose infusion (2 mg · kg^{-1} · min^{-1}). Plasma glucose levels were obtained from blood samples from an arterialized vein. Insulin and C-peptide concentrations were derived from blood samples obtained from an arterialized peripheral vein (dotted lines) and from the hepatic vein (continued lines). Values are presented as means ± SEM ($n = 5$). *P* values were calculated by repeated-measures ANOVA.

Linear regression analysis established a strongly positive association between insulin clearance and the mean insulin secretory-burst mass and amplitude ($r = 0.81$, $P = 0.0043$) (Fig. 6). As a rule, these associations were stronger in the samples from the hepatic vein than in those from the arterialized peripheral vein. The association between insulin burst mass and hepatic insulin clearance was confirmed by ANCOVA including fasting insulin clearance as a covariate and glucose infusion as a fixed factor ($r^2 = 0.823$, $P < 0.0001$). In contrast, the basal, nonpulsatile

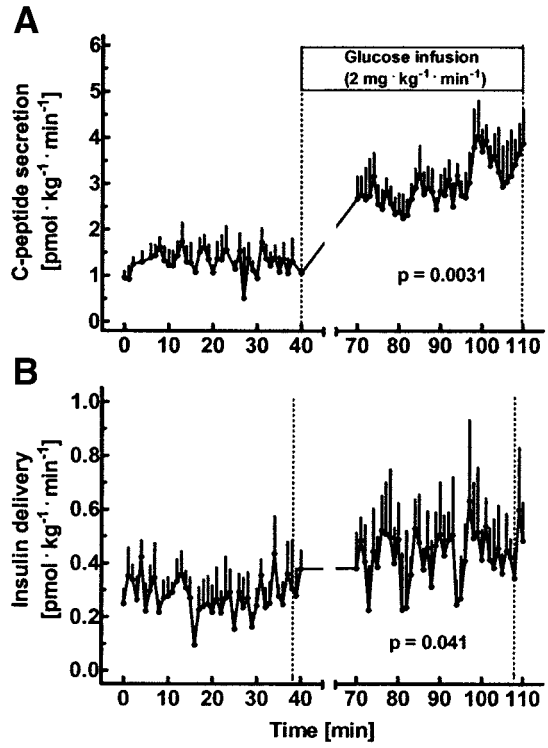

FIG. 2. Calculated C-peptide secretion rates (*A*) and insulin delivery rates from the liver (*B*) in five healthy subjects studied in the fasting state and during an intravenous glucose infusion (2 mg · kg^{-1} · min^{-1}). Values are presented as means ± SEM (n = 5). *P* values were calculated by repeated-measures ANOVA.

component of insulin secretion was unrelated to insulin clearance (Fig. 6).

DISCUSSION

The current data indicate that ~80% of endogenous insulin secretion is extracted during the first liver passage and that insulin clearance is pulsatile, wherein the rate of prehepatic insulin secretion primarily dictates time-varying clearance of endogenously secreted insulin. Moreover, the pattern of insulin delivery by the pancreas (pulse mass and therefore pulse amplitude) is the predominant determinant of momentary hepatic insulin clearance. By implication, regulation of insulin secretory-burst mass directly dictates the insulin concentration wavefront presented to hepatocytes and indirectly dictates (by modulating hepatic insulin clearance) insulin delivery to extrahepatic insulin-sensitive tissues.

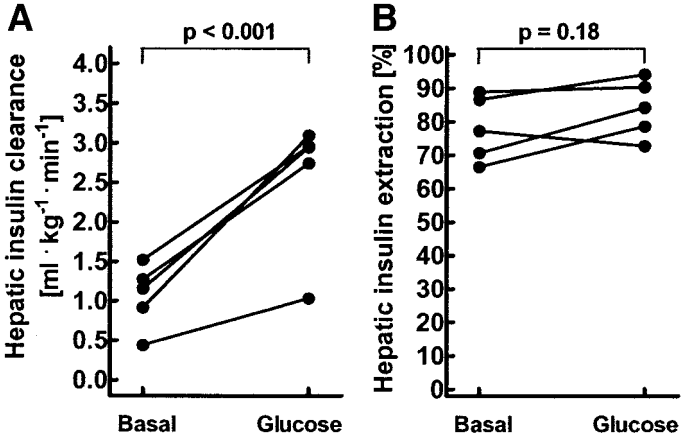

FIG. 3. Absolute hepatic clearance rates (*A*) and fractional insulin extraction (*B*) in five healthy subjects studied in the fasting state and during an intravenous glucose infusion (2 mg · kg^{-1} · min^{-1}). Data represent means of 40 individual values in each subject during the respective experimental periods. *P* values were calculated by paired ANOVA.

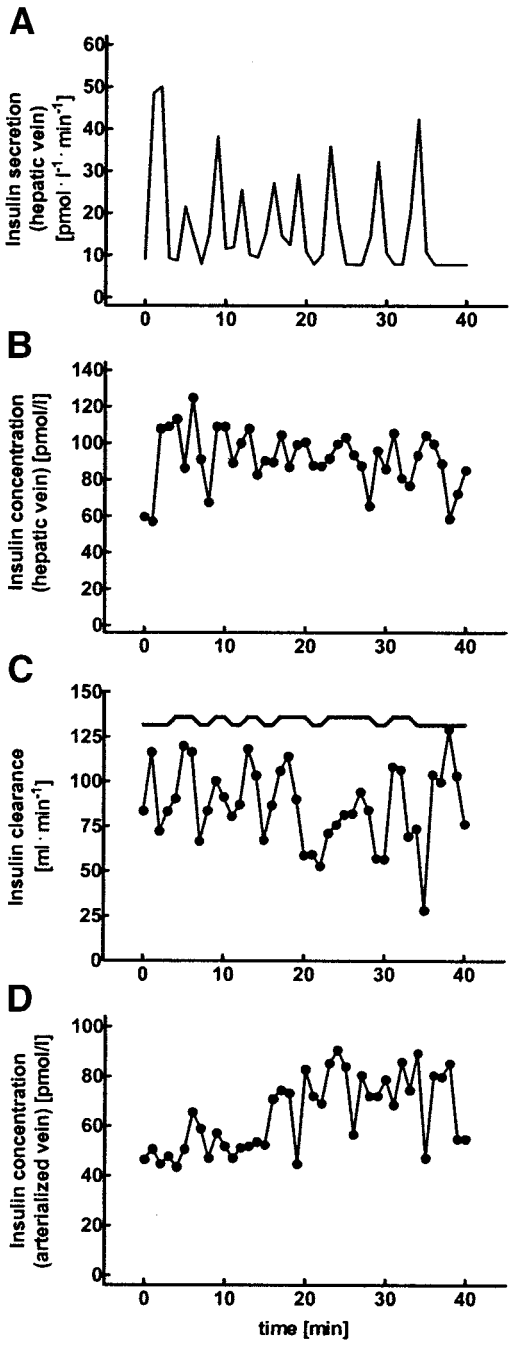

FIG. 4. Insulin secretion rates (*A*) and insulin plasma concentrations (*B*) from the hepatic vein in a representative nondiabetic individual studied over 40 min in the fasting state. *C*: Corresponding serial insulin clearance data from the same individual. Fluctuations in the solid line at the top of the panel show the result of the Cluster analysis. Each peak of this line represents a significant upstroke in the time series of insulin clearance. *D*: Corresponding plasma insulin concentration from the arterialized peripheral vein in the same individual.

A major insight from the accompanying analysis is that the liver responds minute-by-minute to changes in insulin

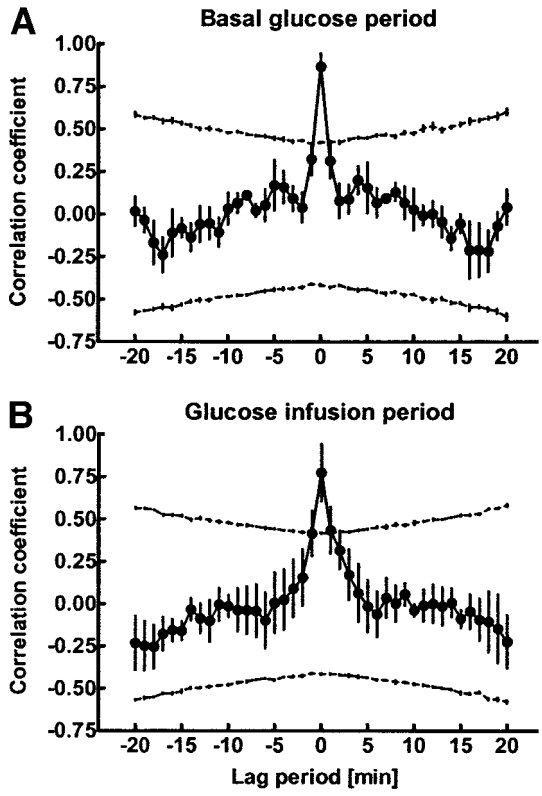

FIG. 5. Cross-correlation analysis of C-peptide secretion rates and hepatic insulin clearance in five healthy subjects studied over 40 min in the fasting state (*A*) and during an intravenous glucose infusion (2 mg · kg^{-1} · min^{-1}) (*B*). These data are presented as means ± SEM and illustrate that changes in C-peptide secretion occur almost simultaneously with changes in insulin clearance ($P < 0.05$).

concentration by adaptively changing insulin clearance. This minute-to-minute adaptation has important implications in delivery of insulin to the systemic circulation. In health, insulin is secreted in distinct secretory bursts every ~4 min (10). The minute-to-minute adaptive changes in insulin clearance by the liver reported here in vivo reflect greater extraction of the insulin within than between insulin secretory bursts. The fact that absolute insulin clearance increases with insulin concentration (6,31) agrees with the present observations that the liver clears larger insulin secretory bursts to a greater extent than smaller insulin pulses, given that at least 70% of insulin secretion is pulsatile. In addition, analyses based on direct portal-vein catheterization established an ~100-fold higher amplitude of insulin concentration oscillations in the portal vein than in the peripheral circulation in humans (18).

Conversely, partial attenuation of insulin pulse mass (and amplitude), for example by low-dose somatostatin infusion, diminishes hepatic insulin clearance commensurately, so that systemic insulin concentrations are unchanged (16). The relationship between the size of insulin secretory bursts presented to the liver and concurrent insulin clearance implies that the liver provides important secondary adaptive regulation of insulin access to the systemic circulation. Thus, when insulin secretion is relatively low, and insulin pulses are small, an adaptive decrease in the rate of transhepatic insulin clearance would tend to sustain basal insulin levels in the systemic circulation. As a consequence of the liver's capacity to rapidly modify insulin clearance, the secretion and the hepatic extraction of insulin are closely linked (6,31). In fact, the present data demonstrate that changes in insulin clearance accompany the changes in prehepatic insulin secretion with a lag period of <1 min. Thus, the rate of prehepatic insulin secretion dictates the actual amount of insulin extraction by the liver.

The markedly greater extraction of insulin within insulin secretory bursts is predicted by insulin receptor–binding kinetics. Thus, maximal insulin binding by hepatocytes coincides with the insulin concentration range present in the insulin concentration wavefront within a pulse presented to the liver in the portal vein, ~1,000–4,000 pmol/l. In contrast, hepatocyte insulin binding is much less avid at insulin concentrations typically present in the trough of an insulin pulse train, which represents a basal nonpulsatile component of release (32–34). High binding in insulin pulses would be expected to result in endocytosis of a high proportion of insulin delivered to the hepatic sinusoids. Interestingly, insulin receptors are recycled to the hepatocyte membrane after prior internalization of the receptor/insulin ligand complex in ~4 min (3,35,36), thus restoring potentially unoccupied receptors for successive insulin secretory bursts. Consistent with this concept, in vitro studies reveal that insulin-receptor internalization and reinsertion into the plasma membrane in hepatocytes can be entrained to insulin pulses delivered at a physiological frequency (36). While a major proportion of insulin is completely removed by the liver after internalization of the insulin-receptor complex, some amounts of insulin can be released back into the circulation after hepatocyte receptor binding or internalization (37). Therefore, it is possible that some insulin bound to the hepatocytes during an insulin wavefront reenters the circulation during the subsequent period of low insulin secretion. However, the quantitative impact of this mechanism compared with the amplitude of prehepatic insulin secretory pulses appears negligible.

The present observations allow the hypothesis that hepatocyte insulin-receptor turnover is entrained to pulsatile insulin clearance as well as local insulin action. In patients with type 2 diabetes, insulin secretion is impaired due to decreased insulin pulse mass (19,22,23). It is therefore of interest that in type 2 diabetes, hepatic insulin extraction is diminished (24,25) and that as a consequence, systemic insulin concentrations are relatively preserved (38). Indeed, the latter has often obscured the severity of the defect in insulin secretion in type 2 diabetes. In principle, the dual adaptive role of the pancreas and liver immodulating systemic insulin concentrations could protect patients with type 2 diabetes from more serious effects of diminished systemic insulin concentrations, for example ketoacidosis (39).

Another clinically relevant aspect of pulsatile insulin delivery that has been proposed is the possibility that pulsatile insulin delivery may avoid downregulation of hepatocyte insulin receptors compared with a comparable constant delivery of insulin (40). Indeed, in vitro studies have affirmed this notion (36). The minute-to-minute responsiveness of the liver to insulin pulses reported here reinforces the likely importance of puslatile insulin deliv-

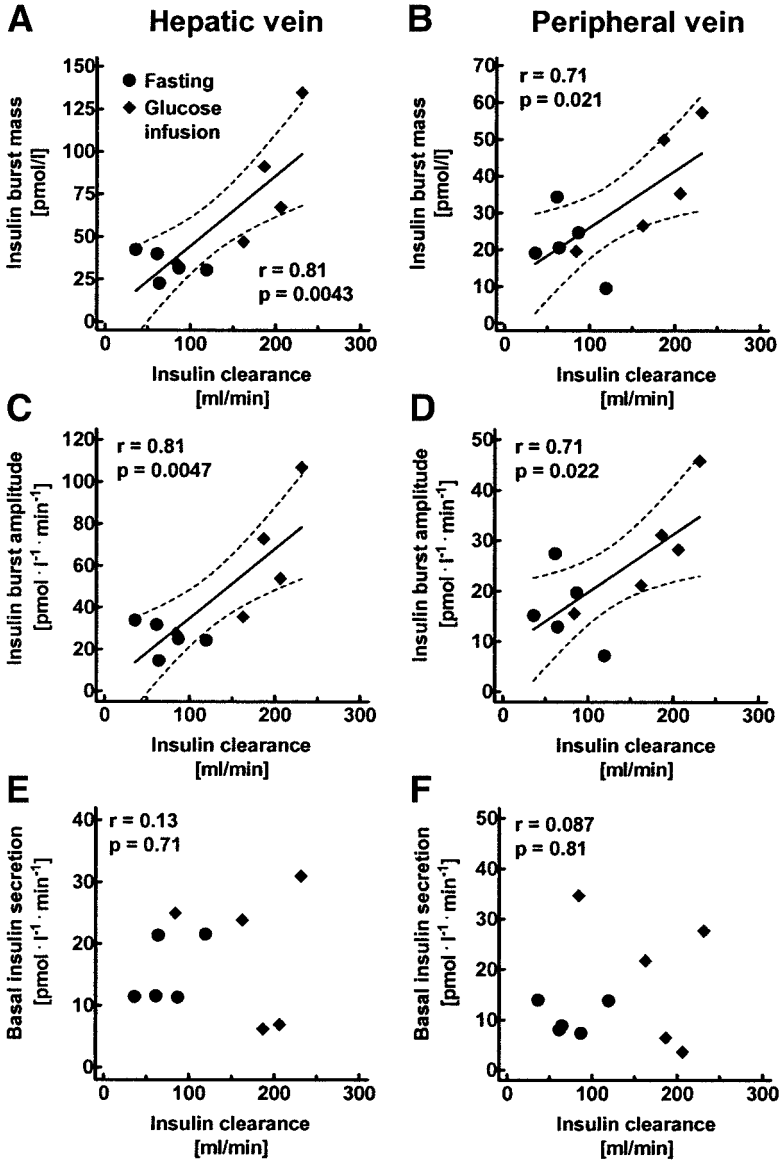

FIG. 6. Linear regression analysis between insulin clearance and respective insulin burst mass (*A* and *B*), insulin burst amplitude (*C* and *D*), and the basal, nonpulsatile insulin secretion (*E* and *F*) determined in the overnight-fasting state (●) and during an intravenous glucose infusion (♦). Left panels indicate the associations determined from the hepatic vein, and right panels associations with samples collected in arterialized peripheral blood.

ery to the liver. However, it is not technically feasible to reproduce oscillations of insulin concentration in the human portal vein that resemble those in vivo (~5,000 pmol/l over ~2 min every 4 min) by an infusion of insulin into the systemic circulation. Thus, the precise importance of pulsatile insulin secretion to insulin action in vivo has yet to be determined. In relation to this, it is also of interest that in vitro studies have suggested that insulin binding to the hepatocyte is most efficient at insulin concentrations present in the high-amplitude oscillations arising from insulin secretory bursts (41–43). Such values far exceed those in the systemic circulation or between insulin pulses in the portal vein. The in vitro observation is consistent with our finding that insulin pulse mass (and amplitude) but not basal (nonpulsatile) insulin secretion predicts hepatic insulin clearance.

The calculated fractional hepatic insulin extraction reported in the present study is higher than the estimated ~50% in the dog (2,4) but consistent with values reported in most human studies (5–7,44). In particular, based on a similar hepatic-vein sampling technique, hepatic first-pass extraction of insulin has been estimated as 70–85% by different groups (7,45,46). This relatively high hepatic insulin extraction in humans serves to emphasize both the importance of the liver as an insulin-responsive and insulin-regulating organ, wherein extraction acts as a gating mechanism to regulate the access of this hormone to other insulin-sensitive tissues. The potential importance of the liver in this regard is that a small decrease in hepatic insulin extraction would be expected to have a much greater impact on peripheral insulin delivery than a comparable increase in prehepatic insulin secretion. On the other hand, as reported in the present study, the islet apparently dictates the extent of hepatic insulin extraction largely through the mechanism of insulin pulse mass, an elegant mechanism empowering the islet with the opportunity to abruptly increase hepatic insulin delivery (for example after meal ingestion), while only minimally increasing peripheral insulin delivery. Also, under conditions of relative insulin deficiency (for example decreased β-cell mass in type 1 or type 2 diabetes) the diminished insulin pulse mass would be expected to allow a relatively high proportion of insulin to escape first-pass hepatic extraction, being made available for peripheral insulin

delivery. Thus, while the present studies serve to emphasize the importance of pulsatile insulin secretion for the regulation of hepatic insulin clearance in healthy lean subjects, it remains unknown whether this relationship is comparable in people who are obese and/or have type 2 diabetes.

A limitation of these along with many other intensive metabolic studies is that conclusions are drawn from studies of a relatively small number of individuals from the population. In the case of the present studies, the relatively invasive nature of hepatic vein catheterization also limits the number of study subjects. Despite this limitation, two factors contribute to our confidence that our primary conclusion "in humans pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction" is valid. First is the density of data that contributed to this analysis in each subject. Blood samples were collected at 1-min intervals at two different sampling sites (hepatic vein and arterialized vein) during both experimental periods (basal and glucose stimulation). Thus, 800 C-peptide and 800 insulin concentrations (each the mean of a triplicate measure) were analyzed to examine the relationship between hepatic insulin clearance and C-peptide secretion rates, so that the relationship was examined in 190 insulin pulses. Second, the relationship revealing that pulsatile insulin secretion drives hepatic insulin clearance was significantly present in each sampling period (both basal and glucose stimulated) in each individual study subject, i.e., in all 10 40-min sampling intervals.

In conclusion, in humans ~80% of endogenously secreted insulin is extracted during the first liver passage. The liver is rapidly responsive to fluctuations in delivered insulin concentration, preferentially extracting insulin released in pulses. As a consequence, the liver quenches the delivery of very large oscillations in insulin concentrations from the portal vein into the systemic circulation. These clinical studies emphasize the significance of pulsatile portal-vein insulin secretion not only by way of presenting the insulin concentration wavefront to the liver, but also by regulating the delivery of insulin into the systemic circulation by dictating the fractional hepatic extraction.

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