Human insulin release processes measured by intraportal sampling

NIELS PØRKSEN,1 THORBJØRN GRØFTE,2 JACOB GREISEN,2 ANETTE MENGEL,1 CLAUS JUHL,1 JOHANNES D. VELDHUIS,3 OLE SCHMITZ,1 MARTIN RÖSSLLE,4 AND HENDRIK VILSTRUP2

Department of 1Endocrinology and Metabolism M, and 2Medicine V (Hepatology and Gastroenterology), Aarhus University Hospital, 8000 Aarhus C, Denmark; 3Department of Medicine and National Science Foundation Center for Biological Timing, University of Virginia, Charlottesville, Virginia 22908; and 4Department of Hepatology, Freiburg University Hospital, Freiburg, Germany

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Am J Physiol Endocrinol Metab 282: E695–E702, 2002; 10.1152/ajpendo.00516.2000.—Insulin is secreted as a series of punctuated secretory bursts superimposed on variable basal insulin release. The contribution of these secretory bursts to overall insulin secretion has been estimated on the basis of peripheral vein sampling in humans to encompass ≥75% of overall insulin release. A similar contribution of the pulsatile mode of release was inferred in a canine model by use of portal vein sampling. The primary regulation of insulin secretion is through perturbation of the mass and frequency of these secretory bursts. The mode of delivery of insulin into the circulation seems important for insulin action; therefore, physiological conditions that alter the pattern of insulin release may affect insulin action through this mechanism. Transepithelial intraportal shunt in humans may provide access to portal vein samples, thus potentially improving the sensitivity of detecting and quantitating the frequency, mass, and amplitude of secretory bursts along with basal release and the regularity of these variables. To establish the insulin-secretory mechanism in nondiabetic humans by the use of portal vein sampling, we here assessed the mass, frequency, amplitude, and overall contribution of pulsatile insulin secretion by deconvolution analysis of portal vein insulin profiles. We find that, in nondiabetic humans fasted overnight, the portal vein insulin concentration oscillates at a period of 4.1 ± 0.2 min/pulse and with secretory peak amplitudes averaging 660% of basal (interpulse) release. The frequency was confirmed by spectral and autocorrelation analyses. The punctuated insulin-secretory bursts partially overlap and are responsible for the majority (70 ± 4%) of insulin release. After ingestion of a mixed meal, the insulin release was increased through amplification of the secretory burst mass (507 ± 104 vs. 1,345 ± 211 pmol·l−1·min−1, P < 0.001), whereas frequency (4.4 ± 0.2 vs. 4.3 ± 0.2, P = 0.86) and basal secretion (62 ± 14 vs. 91 ± 22 pmol·l−1·min−1, P = 0.33) were unaffected. One subject with diabetes and cirrhosis had a similar insulin-secretory pattern, whereas a subject with insulin-dependent diabetes mellitus and minimal insulin release had preserved pulsatile release. A single subject was entrained to show agreement between entrained frequency and portal vein insulin oscillations. We conclude that insulin release in the human portal vein occurs at a mean periodicity of 4.4 ± 0.2 min with a high signal-to-noise ratio (pulse amplitude 660% of basal). The impact of noise on the detected high frequency cannot be excluded.

C-peptide; oscillations; cirrhosis; secretion; diabetes

INSULIN IS SECRETED in a pulsatile manner (4), resulting in high-frequency insulin concentration oscillations in the peripheral circulation. These high-frequency oscillations are apparently caused by interislet coordinate insulin-secretory bursts at a periodicity of 5–15 min/pulse (4, 7, 21). The contribution of these insulin-secretory bursts to overall insulin secretion has been quantified in a canine model by direct sampling across the pancreas (21) and in a human model by employing high-frequency sampling of peripheral venous blood, a highly specific insulin assay, and validated deconvolution analysis (20). In both species, the overall contribution of pulsatile insulin secretion to total insulin release is ≥70–75%. Furthermore, the mechanisms underlying changes in overall insulin secretion after stimulation (18, 19) and inhibition (16, 20) are exerted principally via modulations in the pulsatile component of insulin secretion, with changes in the mass and/or frequency of insulin-secretory bursts. It therefore appears that the pulsatile pattern is physiologically integrated to overall β-cell secretory performance, as underscored by impaired pulsatility in non-insulin-dependent diabetes mellitus (NIDDM) (6) and glucose-intolerant first-degree relatives of NIDDM patients (11) and by a defective release process in glucose-tolerant first-degree relatives of NIDDM patients (24). Conversely, more effective actions of insulin on muscle...
studied. The study was designed to allow multiple analyses of dance with the Helsinki Declaration. Ten volunteers were in animal models (18) and to some extent on portal vein sampling in animal models (18-20), in human patients with cirrhosis (27), and recently as reported in Ref. 25. Application of portal vein sampling in humans would offer insights into the pattern of insulin delivery to the liver in humans, allow estimates of true insulin release patterns under optimal sampling conditions, and permit comparisons with previously published literature on in vivo portal insulin secretion. One drawback would be noise from variable catheter placement and streaming, both of which may introduce false signals. Portal vein sampling through transjugular intrahepatic portosystemic shunt (TIPS) is possible in patients with cirrhosis or portal vein thrombosis. Because liver failure is associated with diabetes, in this context, the model may provide insight into the insulin-secretory role in this specific metabolic disease. In contrast, in isolated portal vein occlusion, pancreatically healthy subjects may be investigated.

The present studies were designed to accomplish sampling from the portal vein in humans and provide insulin concentration time series for detailed analysis of secretory patterns in nondiabetic individuals. The potential role of using TIPS for pancreatic hormonal release studies could thus be explored in these circumstances and in a single subject with type 1 diabetes but preserved (trivial) insulin release.

**METHODS**

**Subjects and Design**

The protocol was approved by the Ethics Committee of Freiburg University Hospital and was performed in accordance with the Helsinki Declaration. Ten volunteers were studied. The study was designed to allow multiple analyses of basal and mixed-meal-stimulated insulin release processes in a rather heterogeneous group of participants who needed transhepatic portal vein catheterization as part of TIPS treatment. The subjects did not receive any other medication than local anesthesia and their usual medicine on the day of the study. The treatments with steroids or β-blockers are shown in Table 1. In nine patients, we obtained portal vein concentration data before (F) and after (M) ingestion of a mixed meal to allow analysis of the mechanism by which insulin is stimulated. Furthermore, we analyzed portal and peripheral (P) insulin concentrations simultaneously in two subjects. In one subject, the ability to entrain portal and peripheral vein rapid pulsatile insulin secretion by a novel technique was used to assess glucose pulse-induced insulin release. Data on protocols completed by individual participants are shown in Table 1. The individual data on age, fasting glucose, and concomitant diseases are depicted in Table 1, but the 10 subjects all suffered from severe liver diseases of different causes. One subject (subject 4) suffered from type 1 diabetes. He had his last insulin injection 24 h before the study.

**Protocols**

After an overnight fast, the participants were brought to the Clinical Research Unit, University of Freiburg. The TIPS catheterization procedure has previously been described in detail (27). After puncture of the internal jugular vein and introduction of an 11-F sheath, a catheter was advanced into the right or middle hepatic vein. A puncture needle (modified Ross needle, Collx, Mönchengladbach, Germany) was inserted through the catheter, and the puncture of an intrahepatic branch of the portal system was performed under fluoroscopy and ultrasound guidance. After successful puncture, the catheter was placed in the mainstream of the extrahepatic portal vein to allow repeated blood sampling. In the patients with portal vein thrombosis, the catheter was placed at the confluence of the splenic and mesenteric veins. After blood sampling from the portal catheter and central venous sheath, the TIPS procedure was accomplished by implantation of a metallic stent into the parenchymal tract of the liver (27). In patients with portal vein thrombosis, TIPS and thrombolysis were performed the day before the study day. After catheter placement, 30 min were allowed as a resting period before sampling protocols were initiated. Sampling

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>BMI</th>
<th>Sex</th>
<th>Glucose (mg/dl)</th>
<th>Medical Treatment</th>
<th>Disease</th>
<th>Biochemistry Hb, 12–18 g/dl</th>
<th>Protocols</th>
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<tr>
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<td>55</td>
<td>24.4</td>
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<td>66</td>
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<td>F, M</td>
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<tr>
<td>2</td>
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<td>24.0</td>
<td>Male</td>
<td>145</td>
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<td>Hb 10.4, bilirubin 2.0</td>
<td>Type 2 DM, sclerosing cholangitis</td>
<td>F, M</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
<td>25.3</td>
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<td>100</td>
<td>+/−</td>
<td>Hb 17.0, bilirubin 2.4</td>
<td>Polycaemia vera, portal thrombosis</td>
<td>F, M</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>27.1</td>
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<td>130</td>
<td>−/−</td>
<td>Hb 12.1, bilirubin 0.8</td>
<td>IDDM, portal thrombosis</td>
<td>F, M</td>
</tr>
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<td>5</td>
<td>24</td>
<td>25.3</td>
<td>Male</td>
<td>80</td>
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<td>Hb 14.1, bilirubin 1.1</td>
<td>Hepatopulmonary disease</td>
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<td>22.9</td>
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<td>108</td>
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<tr>
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<td>23.0</td>
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<td>−/−</td>
<td>Hb 12.5, bilirubin 1.3</td>
<td>Portal thrombosis</td>
<td>F, M, P</td>
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<tr>
<td>9</td>
<td>57</td>
<td>22.5</td>
<td>Female</td>
<td>106</td>
<td>−/−</td>
<td>Hb 14.3, bilirubin 0.9</td>
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<td>F, M, P</td>
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<td>10</td>
<td>26</td>
<td>25.0</td>
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<td>88</td>
<td>−/−</td>
<td>Hb 14.1, bilirubin 1.1</td>
<td>Portal vein thrombosis</td>
<td>E</td>
</tr>
</tbody>
</table>

Summary of the 10 participants, showing characteristics [sex, age, body mass index (BMI)] and treatment with β-blockers or steroids that may impact on insulin secretion and basic biochemistry (glucose, Hb, and bilirubin). The primary disease and possible secondary cause of transhepatic intraportal shunt are depicted. DM, diabetes mellitus; IDDM, insulin-dependent DM. The protocols that they participated in are shown at the right. F, 40 min during short-term fasting; M, 40 min after mixed-meal ingestion; P, simultaneous sampling from portal vein and peripheral vein only at fasting state; E, entrainment by minimal glucose infusions every 10 min and with sampling from portal and peripheral vein.
was performed for 40 min from portal vein (F; n = 9) and peripheral vein catheters (P; n = 2) simultaneously in the basal state (protocol 1). After the basal period, the patients ingested a standardized mixed meal, and 40 min after meal ingestion, a second sampling period of 40 min (M; n = 9) was performed (protocol 2). One subject (subject 10) was studied during pulse induction, a recently introduced method whereby glucose was infused into a peripheral vein at a rate of 6 mg/kg for 1 min every 10 min. This method has been shown to allow the pulsatile insulin release to be controlled by predetermined intervals. Samples were collected simultaneously from the portal and peripheral circulation.

Samples were collected from the portal vein catheter and from a peripheral catheter placed in the femoral vein. Blood sampled at 1-min intervals was processed for later measurement of insulin concentrations; in addition, blood was collected at 10-min intervals for measurements of C-peptide and glucose concentrations. To ensure the patency of the portal vein sampling catheter, blood was collected by slow and continuous withdrawal. The femoral vein catheter was flushed with saline after each sample collection, and catheter dead space plus 0.8 ml was withdrawn before the next collection. All samples were stored at −20°C until analysis for concentration measurements.

**Assays**

**Glucose.** Plasma glucose concentrations were measured by the glucose oxidation method (Beckman Instruments, Palo Alto, CA).

**Insulin.** Serum insulin concentrations were measured in triplicate by a two-site immunospecific insulin ELISA, as previously described (1). In brief, the assay uses two monoclonal murine antibodies (Novo Nordisk, Bagsvaerd, Denmark) specific for insulin. The detection range of this insulin ELISA is 5–600 pM. At low (20 pM), medium (150 pM), medium-high (200 pM), and high (350 pM) plasma insulin concentrations, the interassay coefficients of variation (among triplicates) were 5.2, 3.7, 4.0, and 4.5%. Corresponding intra-assay variations were 3.0, 2.3, 2.1, and 2.0%. There is no cross-reactivity with C-peptide, insulin-like growth factor (IGF)-I, IGF-II, glucagon proinsulin, and split (32,33) and des(31,32)-proinsulin. The antibodies cross-react 30 and 63% with split (65,66)-proinsulin and des (64,65)-proinsulin, respectively (1).

**C-peptide.** C-peptide measurements were performed using a commercially available kit (K6218, DAKO Diagnostics, Cambridgeshire, UK). The assay is a two-site ELISA based on two monoclonal antibodies and employs the same principles referred to in Insulin. Each sample was assayed in duplicate, and the intra- and interassay (among triplicates) coefficients of variation were 2.2 and 3.3%, respectively. The detection limit was 35 pM.

**Data Analysis**

**Detection and quantification of pulsatile insulin secretion by deconvolution analysis.** The plasma insulin concentration time series were analyzed by deconvolution for purpose of detection and quantification of insulin-secretory bursts. Deconvolution of venous insulin concentration data was performed with a multiparameter technique (29), which requires the following assumptions. The venous plasma insulin concentrations measured in samples collected at 1-min intervals are 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), 3) a common half-duration (duration of and algebraically Gaussian secretory pulse at half-maximal amplitude), which is superimposed on 4) a basal time-invariant insulin-secretory rate, and 5) a biexponential insulin disappearance model. In the systemic circulation, disappearance consisted of assumed half-lives of 2.8 and 5.0 min and a slow component fraction of 0.28, as previously measured (21), whereas in the portal vein, disappearance consisted of assumed half-lives of 0.2 and 3.0 min and a fractional slow component of 0.065 based on repetitive analysis using a wide range of kinetic values and choosing the model with best agreement to the observed data (lowest sum of squared residuals). The kinetic values are in agreement with literature reports (21), although different values would be expected due to TIPS and liver diseases. Shunting of insulin, recirculation, and esophageal varices all contribute in varying degrees to the (already large) uncertainty in the estimated kinetic values. The assumed kinetic parameters would ideally be determined by bolus insulin injection upstream of the sampling catheter, but this would not be possible for ethical reasons. Therefore, the kinetic parameters are not measured directly and may be inaccurate, although a similar procedure for estimating insulin kinetics in a canine model with infusion and sampling catheters showed a good ability to quantitate parameters from fitting to endogenously released insulin. Assuming the foregoing nominal insulin disappearance values, we estimated the number, locations, amplitudes, and half-duration of insulin-secretory bursts, as well as a simultaneous zero or nonnegative basal insulin-secretory rate, for each data set by nonlinear least squares fitting of the multiparameter convolution integral to each insulin time series. A modified Gauss-Newton quadratically convergent iterative technique was employed with an inverse (sample variance) weighting function. Parameters were estimated until their values and the total fitted variance both varied by less than 1 part in 100,000. Asymmetric highly correlated variance spaces were calculated for each parameter by the Monte Carlo support plane procedure. Secretary rates are expressed as mass units of insulin (pmol) released per unit of distribution volume (liters) per unit of time (min). The mass of hormone secreted per burst (time integral of the calculated secretory burst) was thus computed as picomoles of insulin released per liter of corresponding distribution volume. Because the calculated values represent posthepatic insulin appearance for peripherally deconvoluted secretory rates, total insulin secretion was calculated using C-peptide concentrations (28). When the deconvolution analysis was performed, basal secretion was adjusted to allow accommodation of most interpulse troughs. Likewise, the secretory burst half-duration was adjusted to fit individual obvious secretory bursts, consisting of series of data points building up to a peak and down to a trough. All data analysis was performed in a blinded manner.

**Periodicity analysis.** The frequency of the insulin pulses may be estimated by spectral analysis or autocorrelation analysis, which evaluate the concentration time series for regular periodicities. Autocorrelation analysis examines the replicability of patterns by moving a template across the time series and estimates the correlation among data points at increasing time lags. Spectral analysis tests the comparability of the data with a sinusoidal variability and gives a spectral density peak as a measure of the signal at a given periodicity. Both methods are well established in mathematics and biology and have been used in the studies herein on insulin pulsatility.
Regularity Statistics

The pulsatile insulin release pattern may also be characterized in further detail to examine the reproducibility of the subordinate patterns in the data set. A validated mathematical approach is the application of approximate entropy (ApEn) (15, 24). ApEn is a recently introduced regularity statistics tool that measures the logarithmic likelihood that runs of patterns reproduce on the next incremental comparison. The method has proved useful in analysis of pulsatile insulin secretion (10, 24) for discriminating between pathophysiology and health. The method is robust to noise and to absolute differences in data.

Statistics

All data in the text and figures are given as means ± SE. Student’s two-tailed paired t-test was used to examine statistical significance.

RESULTS

Concentrations

The portal vein insulin concentrations showed large oscillations (~80–600%) both before and during meal ingestion (Fig. 1). In contrast, the peripheral vein concentrations oscillated by ~30%, thus revealing a 3- to 20-fold reduction in the insulin pulsatile signal (Fig. 2). Despite the reduced amplitude, the peripheral insulin pulses could still be detected as concentration changes (Fig. 2). From Fig. 1, it appears visually that the liver is exposed to large-amplitude insulin oscillations that occur at an interval of ~5 min/pulse and with mean changes of 100–600%. In subject 10, portal vein concentrations were controlled by the glucose infusion, indicating that the observed variability in portal vein insulin concentrations truly represents coordinate insulin release rather than noise (Fig. 2).

Secretion

Nondiabetic subjects: basal state. Insulin secretion was assessed (n = 8) by deconvolution analysis of serial portal vein serum insulin concentration measurements and revealed the presence of discrete, punctuated insulin-secretory bursts that were superimposed on

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Fig. 2. Patient 10. Simultaneously sampled portal vein (PV; top curve) and peripheral vein (PeV; bottom curve) concentrations (CONC) are shown (A) under overnight-fasted conditions. Minimal glucose infusion occurred at 0–1, 10–11, 20–21, and 30–31 min, resulting in presumed oscillations in glucose of ~5% but PV (top curve) and PeV oscillations in insulin. B: deconvoluted insulin-secretory (SECR) rates from peripheral insulin data, revealing large insulin-secretory bursts. There is some nonpulsatile basal release. C: deconvoluted insulin-secretory rates from PV data, again confirming large secretory bursts; but in this case, 2 pulses are detected at 12–15 min, possibly a false positive (nonentrained) pulse being included. D: PV-PeV difference (DIFF), confirming that, between pulses, very little insulin (if any) is secreted. The scales in the B and C are secretion expressed per volume of distribution, and for PV and PeV, these differ.
basal insulin secretion in all studies. For the nondiabetic subjects \((n = 7)\), analysis revealed, consistent with inspection of raw portal vein insulin concentration data, that the concentration changes arise from high frequency (i.e., low periodicity, \(4.4 \pm 0.2\) min/pulse), large amplitude (\(213 \pm 53\) pmol\(\cdot\)l\(^{-1}\)\cdot\)min\(^{-1}\)) insulin-secretory bursts, with little basal insulin secretion (\(65 \pm 14\) pmol\(\cdot\)l\(^{-1}\)\cdot\)min\(^{-1}\)). Consequently, the overall contribution of pulsatile insulin secretion was calculated as \(70 \pm 4\%\). On the basis of peripheral vein deconvolution in two subjects, a similar observation of frequency (\(5.0\) min/pulse) and relative pulsatile contribution to overall insulin release of \(70\%\) was made. The nature of the individual insulin-secretory burst in all cases was compatible with a Gaussian distribution and half-duration of \(2.3 \pm 0.2\) min. Analysis of simultaneously measured peripheral and portal vein insulin concentrations yielded good agreement between calculated secretory patterns at these two sampling sites. The changes in insulin secretion were reflected in C-peptide concentrations: peripheral vein \((1,019 \pm 277\) vs. \(1,682 \pm 750\) pM) and portal vein \((1,175 \pm 290\) vs. \(2,686 \pm 278\) pM) concentrations increased as expected after meal ingestion.

Postprandial insulin secretion. In the postprandial state, the insulin-secretory pattern was preserved \((n = 7\); Fig. 1). The mechanism of increasing the insulin secretion was by amplification of the insulin-secretory burst mass \((507 \pm 104\) vs. \(1,343 \pm 211\) pmol\(\cdot\)l\(^{-1}\)\cdot\)min\(^{-1}\), \(P < 0.001\)), whereas frequency \((4.4 \pm 0.2\) vs. \(4.3 \pm 0.2, P = 0.86\)) and basal secretion \((62 \pm 14\) vs. \(91 \pm 22\) pmol\(\cdot\)l\(^{-1}\)\cdot\)min\(^{-1}\), \(P = 0.33\)) were not significantly affected, although the latter might have been significant had a larger number of subjects been studied.

**Regularity Statistics**

The application of autocorrelation and spectral analyses resulted in significant periodicities in some, but not all, of the subjects. The results are shown in Table 2. The lack of significant periodicities is likely due to short sampling durations. The design was limited because of the underlying diseases of the participants and was originally chosen to allow for deconvolution, where sampling duration is of lesser importance. As is seen, spectral analysis showed significant peaks in seven of eight subjects in the non-glucose-induced studies, revealing a mean periodicity of \(4.50 \pm 0.63\) min/oscillation. Autocorrelation tended to show periodicities in five of eight subjects, with a mean periodicity of \(4.90 \pm 1.00\), although these were not significant in any subject. During glucose induction, there was a significant spectral density peak at 10-min intervals, as would be expected.

**Pulsatile Insulin Secretion in Type 1 Diabetes Mellitus?**

The subject with insulin-dependent diabetes mellitus (IDDM) had some variation in portal vein insulin concentrations. This variation was very modest; nonetheless, inspection of raw data indicated that the variation was not random (Fig. 3). Analysis by ApEn for data regularity could not detect any significant pattern compared with randomly shuffled data, and there was not a significant frequency by autocorrelation analysis of first-differenced data. However, for both statistical approaches, a \(P\) value of 0.1–0.15 indicated that the
lack of significance could potentially be secondary to the short data series (40 data points), which for both approaches is a small sample size for analysis. In contrast, deconvolution analysis, with use of 95% confidence intervals for all amplitudes considered jointly, suggested significant secretory burst activity. The C-peptide concentrations were, in almost all cases, below the detection limit (35 pM). The only detectable concentration occurred after meal ingestion and in the portal vein. By use of the concentrations read by the ELISA reader there was an increase in the portal vein vs. peripheral vein C-peptide concentrations (mean of 5 measurements, 13 vs. 7 pM) and a portal vein increase after meal ingestion (mean of 5 measurements, 13 vs. 32 pM), thus indicating the occurrence of minimal insulin release in response to meal ingestion. The absorbance detected by the ELISA reader showed the same changes.

**Insulin Secretion in Type 2 Diabetes and Cirrhosis**

One patient with diabetes and cirrhosis was studied. The subject did not receive antidiabetic drugs. Comparison of the deconvoluted secretory pattern in this subject with that of the nondiabetic subjects revealed an apparently similar pulsatile secretory pattern (Fig. 4). The burst periodicity and mass were (4.8 min/pulse and 563 pmol·1⁻¹·pulse⁻¹ before and 4.6 min/pulse and 2,305 pmol·1⁻¹·pulse⁻¹ after meal ingestion) similar to the mean.

**DISCUSSION**

We have employed transhepatic intraportal high-frequency blood sampling in humans to measure prehepatic insulin concentration time series in a highly sensitive and specific insulin ELISA. Data were subjected to deconvolution analysis to define secretory burst activity. These analyses reveal that the release of insulin consists of serial secretory burst-like events and is regulated by amplification of the secretory burst mass upon mixed-meal ingestion. In one subject with NIDDM and cirrhosis, a similar pattern was observed, whereas the insulin concentration data in one subject with type 1 diabetes suggested a minimally preserved release of low-amplitude secretory bursts.

Because the nature of insulin secretion is pulsatile (4) and the contribution by secretory bursts per se may be the major mechanism regulating release in fasting (20, 21), inhibited (16, 20), and stimulated (19, 22) states, we sought to characterize in vivo insulin secretion in humans by use of the portal vein sampling procedure combined with a deconvolution technique. Similar sampling protocols have previously been employed in humans (27) with the use of discrete pulse detection algorithms to identify significant oscillations in insulin concentrations and, recently (25), with the use of methods similar to those herein and reporting similar changes upon stimulation with hyperglycemia. Also, portal vein sampling has been used in one other species and combined with deconvolution techniques to allow quantitative measurements of insulin secretion (21). The techniques used confirm previous inferences on the importance of the pulsatile mode of release in overall insulin release. The present detailed analysis of the shape, frequency, and relative amplitude of insulin-secretory episodes in the human portal vein shows that the vast majority of insulin is secreted as high-frequency (~4.4 min/pulse), high-amplitude (~660% of basal secretion) pulses with a brief half-duration of ~2.3 min and with little interpulse secretion (referred to as basal secretion). Therefore, the insulin release patterns to which the liver is exposed are very dynamic and resemble an on/off delivery pattern. In a large number of in vivo (9, 12–14, 23) and in vitro (5) studies, this on/off signal pattern is important for insulin action. In addition to the enhanced actions on target tissues, the pulsatile pattern of insulin signaling via secretory bursts and secretory pauses may be important for optimal β-cell function, since this dynamic process may allow short-term “pancreatic rest/recovery” without the depolarization, increase of intracellular calcium, and ionic changes in the β-cells known to occur during a secretory burst (3, 8). Portal vein sampling has also been used recently (25) under basal and hyperglycemic conditions. The reported frequency of ~5 min/pulse is similar to the present periodicity, and similarly, hyperglycemia did not alter the frequency. The present study included a diabetic subject with cirrhosis as the likely cause and a subject in whom control of the pulsatile insulin release was attempted by punctuated glucose infusions, confirming that be-

![Graph](http://ajpendo.org)"
between-pulse secretory activity is minimal, if present at all. We acknowledge that noise may be dependent on catheter placement and that differences in frequency reported herein and previously (19) may depend on optimal sampling conditions. The problem with detection of false-positive pulses is clearly demonstrated in patient 10 (Fig. 2), in whom one false positive, nonentrained pulse was detected. However, this patient also demonstrates that portal vein concentrations are at times similar to peripheral vein concentrations, picked up as minimal basal secretion by portal vein deconvolution, whereas peripheral vein deconvolution detected some basal release.

The present study using portal vein catheterization revealed that overall insulin secretion after meal ingestion is modulated by changes in secretory burst amplitude (and, hence, mass), whereas no significant changes in frequency were observed in the subjects studied, which supports previous reports (2). The amplification of the secretory burst mass indicates that an increased amount of insulin is released per β-cell within each secretory event and/or that more β-cells are contributing to the secretory burst; i.e., that recruitment occurs. Our observations also imply that the hepatic insulin exposure patterns change with nutrient supply, which may be of importance for hepatic handling of nutrients.

Portal vein insulin concentration profiles and C-peptide concentrations in the IDDM patient suggested trivial insulin release but preserved pulsatile insulin secretion despite a very limited insulin-secretory capacity. Although these data did not reveal significant oscillatory patterns by autocorrelation analysis and ApEn, this could be an artifact resulting from the short sampling duration. Deconvolution analysis to detect any concerted secretory activity by use of defined detection criteria showed modest but significant insulin-secretory activity. If this observation is confirmed, it will indicate preserved interislet coordinating mechanisms in IDDM; that is, coordinating mechanism(s) do not arise from an insulin/target-organ/insulin feedback loop, because the very small changes in portal vein insulin concentrations are unlikely to have an impact on target organs. Therefore, this finding will support the hypothesis of an intrapancreatic neuronal pacemaker (17, 26).

The present study also included one subject with NIDDM and cirrhosis. In this subject, insulin release was not detectably different from that in the nondiabetic subjects. The cause of diabetes in this subject is unknown, but the presence of “normal” pulsatile insulin release suggests that the cause was cirrhosis rather than type 2 diabetes, because pulsatile insulin secretion is impaired in the latter. This would imply that the pulsatile release may be preserved in some secondary diabetes despite chronic hyperglycemia and the concomitant metabolic abnormalities.

The benefits of portal vein sampling in humans are, to some extent, vitiated by the primary diseases that make these subjects suitable for TIPS. Indeed, the patients studied herein had different primary diseases causing stress and, likely, variable insulin resistance. Nonetheless, the derived insulin-secretory profiles mimic both those reported in a healthy canine model with the use of portal vein sampling and in healthy humans evaluated via peripherally sampled blood, indicating that the model is not severely impaired by the primary diseases. To address specific questions, the portal vein sampling model may be the most accurate in affording detailed insights into insulin-secretory activity. Also, the present study shows that the model is very suitable for studies in liver disease, where prehepatic hormonal delivery is likely of great importance for hepatic performance. Further investigations using TIPS may prove useful to further elucidate metabolic derangement(s) in cirrhosis and other primary liver diseases. The obvious drawback is potential noise, causing detection of false pulses leading to a too-high estimate of frequency. The data herein suggest that this does play some role.

We conclude that human portal vein blood sampling corroborates previously reported insulin-secretory pattern(s) based on peripheral sampling and that the pulsatile mode is dominant (70 ± 4% of total insulin release) in the fasting and postprandial states. We suggest that the TIPS portal vein sampling model may be suitable for addressing specific questions on the dynamics of insulin release. Furthermore, data from one subject with IDDM suggest preserved insulin pulsatility despite a lack of insulin-to-glucose feedback mechanisms and very low secretory capacity. Finally, a cirrhotic-diabetic patient exhibited insulin secretion that evidently was not different from that in nondiabetic subjects.

The work for this study was conducted at Department of Medicine V (Hematology and Gastroenterology), Aarhus University Hospital.

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