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Direct Measurement of Pulsatile Insulin Secretion from the Portal Vein in Human Subjects*

SOON H. SONG, SUSAN S. McINTYRE, HASNAIN SHAH, JOHANNES D. VELDHUIS, PETER C. HAYES, AND PETER C. BUTLER

Liver Research Unit, Royal Infirmary of Edinburgh, University of Edinburgh (S.H.S., S.S.M., H.S., P.C.H.), EH3 9YW Edinburgh, Scotland; Department of Medicine, General Clinical Research Center, and National Science Foundation Center for Biological Timing, University of Virginia Health Sciences Center (J.D.V.), Charlottesville, Virginia 22908; and Division of Endocrinology, Keck School of Medicine (S.H.S., P.C.B.), Los Angeles, California 90033

ABSTRACT

Insulin is secreted in a high frequency pulsatile manner. These pulses are delivered directly into the portal vein and then undergo extraction and dilution before delivery into the systemic circulation. The reported frequency of these insulin pulses estimated in peripheral blood varies from an interpulse interval of 4–20 min. We postulated that this discrepancy is due to the attenuation of the pulse signal in the systemic circulation *vs.* the portal circulation. In the present study we measured pulsatile insulin release directly in the portal circulation of human subjects who had indwelling transjugular intrahepatic portasystemic stent shunts (TIPSS) to decompress portal hypertension. We quantitated pulsatile insulin secretion in both the overnight fasted state (fasting) and during a hyperglycemic clamp (8 mmol/L). Direct portal vein sampling established that pulsatile insulin secretion in humans has an interval (periodicity) of approximately 5 min. The amplitude (and mass) of the insulin concentration oscillations observed in the portal vein was approximately 5-fold greater than that observed in the arterialized vein and was similar to that observed in the dog. Increased insulin release during hyperglycemia was achieved through amplification of the insulin pulse mass. In conclusion, direct portal vein sampling in humans revealed that the interpulse interval of insulin pulses in humans is about 5 min, and this frequency is also observed when sampling from the systemic circulation using a highly specific insulin assay and 1-min sampling, but is about 4-fold greater than the frequency observed at this site using single site RIAs. We confirm that enhanced insulin release in response to hyperglycemia is achieved by amplification of these high frequency pulses. (*J Clin Endocrinol Metab* **85:** 4491–4499, 2000)

INSULIN IS SECRETED in high frequency pulses (1). This pattern of insulin secretion has been shown to be abnormal in subjects with type 2 diabetes (2) and their first degree relatives (3) and in patients at risk of developing type 1 diabetes (4). However, quantification of pulsatile insulin secretion is complex. Insulin pulses are secreted into the portal circulation and undergo significant hepatic extraction and waveform damping before entering the systemic circulation (5). We previously developed a canine model of direct portal vein catheterization to overcome this problem (6) and reported that sampling from the systemic circulation resulted in an underestimate of both the frequency of insulin pulses as well as the calculated proportion of insulin released in the pulsatile mode (5). Inevitably, studies of pulsatile secretion in humans have been confined to the systemic circulation (2–4, 7–12). Initially such studies reported a pulse frequency of 15–20 min (2–4, 7, 8), but more recently a pulse frequency of about 6 min has been suggested (11, 12). We have speculated that these differences may reflect the greater sensitivity of novel insulin assays applied in the latter studies, and that the true frequency of insulin pulses delivered into the portal circulation in humans may be 6–8 min. The latter would be comparable to the frequency observed in the isolated perfused pancreas (13) as well as the perifused islets (14, 15).

In the current study we applied a validated deconvolution technique for quantifying pulsatile insulin secretion to plasma insulin concentration profiles obtained simultaneously from the portal vein and the systemic circulation in human subjects with stable compensated hepatic cirrhosis and an *in situ* transjugular intrahepatic portasystemic stent shunt (TIPSS) (16). The TIPSS catheter allowed relatively noninvasive high frequency sampling of blood from the portal vein in conscious human subjects. Pulsatile insulin secretion was quantified in the fasting basal state as well as during a hyperglycemic clamp study.

Using this protocol we sought to address the following questions. First, is the frequency of pulsatile insulin secretion in humans (observed by direct sampling from the portal circulation) comparable to the frequency reported previously from studies in isolated islets, the isolated pancreas, and *in vivo* by sampling from the portal vein in dogs (interpulse interval, ~6–8 min or ~15–20 min as reported in some studies using the systemic sampling site in humans). Second, does hyperglycemia in the human (as in the dog) enhance insulin secretion through the specific mechanism of augmenting the mass of insulin bursts? Finally, we examined whether the amplitude of the insulin concentration wavefront to which the liver is exposed in humans approaches that observed in the portal vein of dogs (5, 6).

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Address all correspondence and requests for reprints to: Dr. Peter C. Butler, Division of Endocrinology and Diabetes, Keck School of Medicine, University of Southern California, 1333 San Pablo Street, BMT-B11, Los Angeles, California 90033. E-mail: pbutler@hsc.usc.edu.

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Subjects and Methods

Study subjects and design (Table 1)

This study was approved by the Lothian ethics committee (institutional review board equivalent), and all volunteers provided informed written consent. Inclusion criteria were patients with a patent TIPSS and known stable compensated liver disease. Patients were deliberately selected to represent those in which the predominant liver-related problem was cirrhosis and portal vein hypertension treated with a TIPSS, rather than progressive liver failure or active hepatic inflammation. Exclusion criteria include decompensated liver dysfunction, diabetes mellitus, a thrombosed stent, and/or a prolonged prothrombin time. All patients were studied 1–2 weeks before the TIPSS study and had a 75-g oral glucose tolerance test performed to exclude diabetes mellitus. Of six subjects screened, one was excluded from the study because of diabetes.

Five patients with *in situ* TIPSS were studied immediately after a routine follow-up assessment of the patency of their TIPSS. For this purpose patients were admitted overnight into the Royal Infirmary of Edinburgh. After an overnight fast a catheter was placed in the right jugular vein, and under ultrasound guidance it was passed through the TIPSS into the portal circulation. The purpose of the clinical assessment was to ensure that the TIPSS remained patent. Once this had been confirmed, a catheter was inserted via the right internal jugular introducer sheath into the portal vein to allow the present study to be completed.

Study protocol

All study subjects were admitted to the University of Edinburgh Wellcome Trust Clinical Research Facility at the Royal Infirmary of Edinburgh the night before the study and remained fasting overnight. On the morning of the study, each subject underwent TIPSS portogram performed by a radiologist as part of the routine follow-up assessment of the patency of the *in situ* TIPSS, and the sampling catheter was placed in the portal vein at the end of this procedure.

A peripheral indwelling sampling catheter was also inserted into a dorsal hand vein and the hand was warmed to 40 C by an electric blanket to permit subsequent sampling of arterialized blood. An iv catheter was also placed in an antecubital vein in the contralateral arm and infused with saline at 30 mL/h. Once all of the catheters were in place there was a 45-min rest period before commencement of the protocol at approximately 1000 h. At protocol time 0–40 min, simultaneous intensive 1-min sampling of arterialized blood and portal vein blood was performed to obtain the minutely insulin concentration profile at each site in the fasting state. At protocol time 40 min, a hyperglycemic clamp was commenced with the object of raising the arterialized plasma glucose concentration to 8–9 mmol/L. This was achieved by infusion of a variable rate glucose infusion (50% dextrose) administered by a programmable infusion pump (Harvard Infusion Pumps, Ayer, MA) on-line to a personal computer. Arterialized blood was sampled at 5-min intervals, and plasma glucose was measured within 2 min. Steady state hyperglycemia was achieved by protocol time 80 min (40 min after the clamp was begun), and then the second sampling period began. From protocol time 80–120 min, blood was sampled at 1-min intervals from the arterialized peripheral vein catheter and the portal vein catheter to determine the insulin concentration profile during the hyperglycemic clamp. Portal vein sampling was completed in all five study subjects in both the basal and hyperglycemic sampling periods. Arterialized sampling was completed in four of the five subjects; the peripheral sampling catheter was unreliable in the remaining subject. In two subjects portal vein blood flow was measured during the study by use of Doppler scanning.

TABLE 1. Patient characteristics

Case no.	Age (yr)	Sex	Wt (kg)	BMI (kg/m^2)	FPG (mmol/L)
1	58	F	60	23	4.4
2	61	M	71	22	5
3	43	M	71	24	4
4	61	M	100	33	5.8
5	54	M	117	36	5.4

FPG, Fasting plasma glucose; M, male; F, female; BMI, body mass index.

Assays

Plasma glucose concentrations were measured by the glucose oxidase method using a glucose analyzer (Beckman Coulter, Inc., Palo Alto, CA).

Plasma insulin concentrations were measured in duplicate by two-site immunospecific insulin enzyme-linked immunosorbent assay (ELISA), as previously described (11, 17). In brief, the assay uses two monoclonal murine antibodies (Novo Nordisk, Bagsvaerd, Denmark) specific for human insulin. The detection range of this insulin ELISA was 5–600 pmol/L. At medium (150 pmol/L), medium-high (200 pmol/L), and high (350 pmol/L) plasma insulin concentrations, the interassay coefficients of variation were 3.7%, 4.0%, and 4.5%, and the corresponding intraassay variations were 2.3%, 2.1%, and 2.0%. There was no cross-reactivity with proinsulin or split 32,33 and des-31,32 proinsulins, respectively.

The plasma C peptide concentration was assayed with a commercially available kit (K6218, DAKO Corp., Cambridgeshire, UK). The assay is a two-site ELISA based on two monoclonal antibodies, using the principles referred to above. Each sample was assayed in duplicate; intra- and interassay coefficients of variation were 2.2% and 3.3%, respectively.

Data analysis: detection and quantification of pulsatile insulin secretion by deconvolution analysis

The plasma insulin concentration-time series were analyzed by deconvolution as previously validated (18) to detect and quantify insulin secretory bursts (6). Deconvolution of plasma insulin concentration data

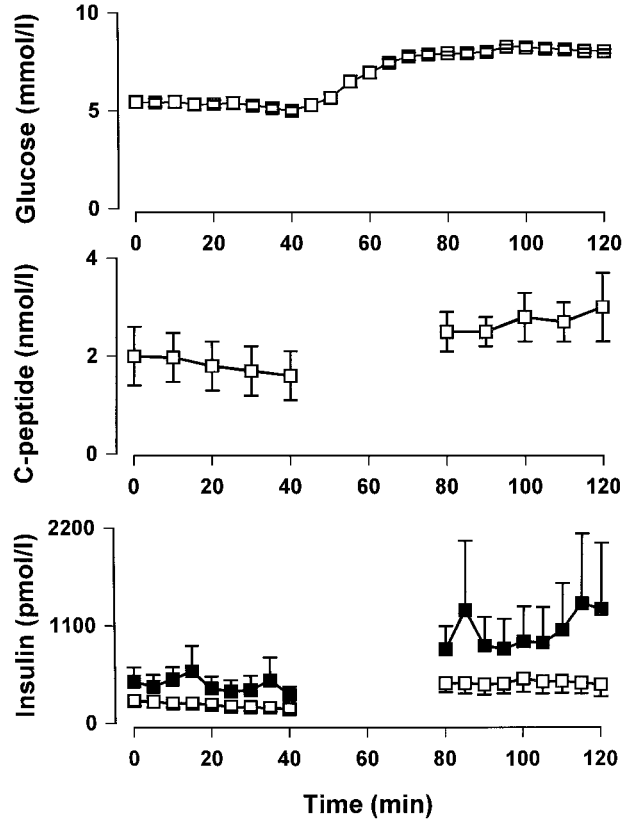

FIG. 1. Mean arterialized plasma glucose (*top panel*), C peptide (*middle panel*), and both arterialized (□) and portal vein (■) insulin concentrations. The time 0–40 min corresponds to the basal intensive sampling period. The hyperglycemic clamp was initiated at 40 min, and steady state hyperglycemia was achieved by 80 min, when the second intensive sampling period was undertaken.

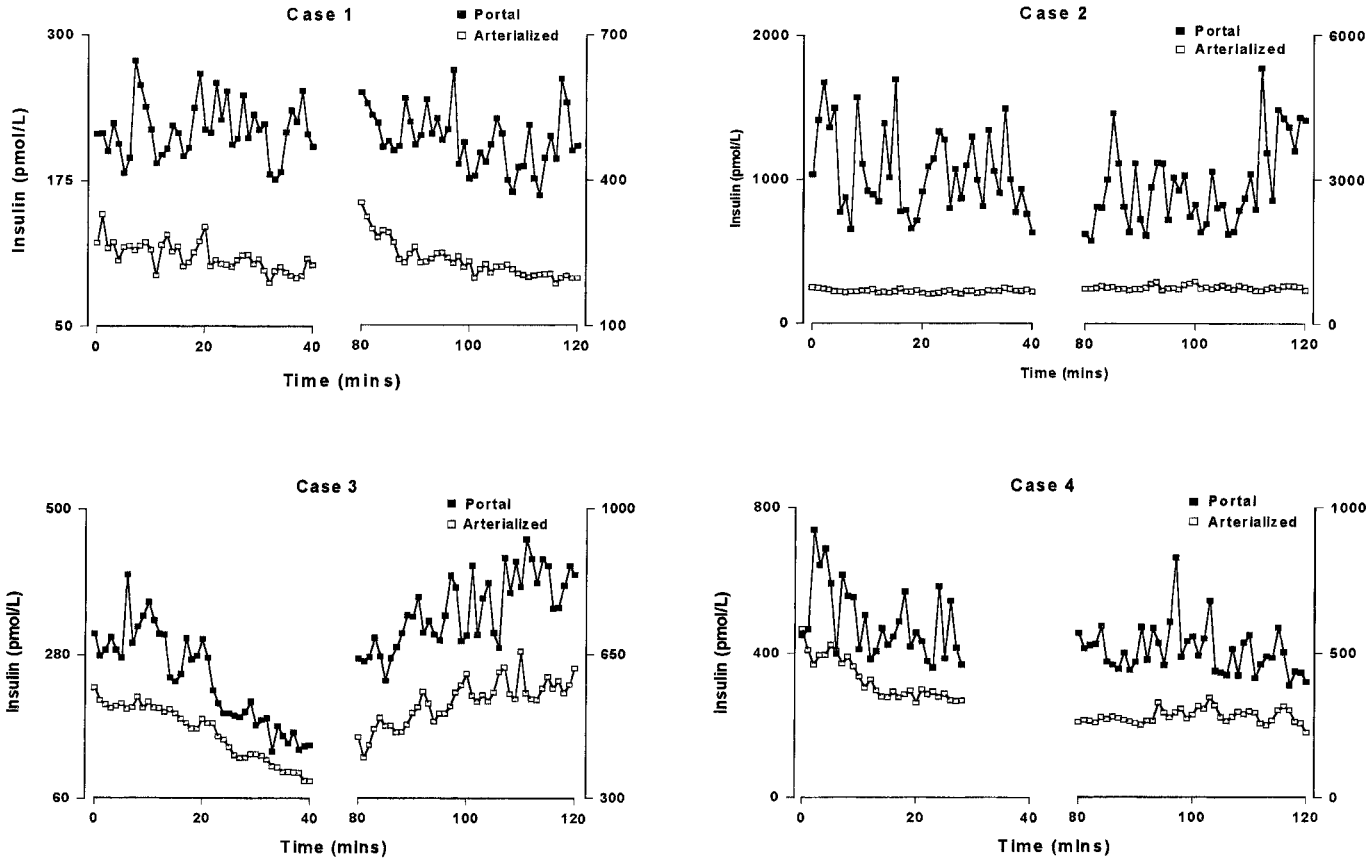

FIG. 2. Plasma insulin concentration profiles from the basal (0–40 min) and hyperglycemic clamp (80–120 min) periods in four cases obtained from the arterialized (□) and portal vein (■) sampling sites. Note that the scales are adjusted in the *left* and *right panels* to accommodate the insulin concentration range observed. In all cases, oscillations in insulin concentrations are much greater in the portal vein than in the arterialized sampling site.

was performed with a multiparameter technique that requires the following assumptions. 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 either regular or randomly dispersed times and having a) individual amplitudes (maximal rate of secretion attained within a burst) and mass (integral of the calculated secretory event) and b) a common half-duration (duration of an algebraically Gaussian secretory pulse at half-maximal amplitude), which are superimposed on c) a basal time-invariant insulin secretory rate; 2) a biexponential insulin disappearance model in the systemic circulation, consisting of earlier directly estimated half-lives of 2.8 and 5.0 min and a fractional slow component of 0.28 in healthy fasting humans; and 3) a biexponential insulin disappearance model in the portal circulation, consisting of half-lives of 1.0 and 3.0 min and a fraction slow component of 0.667. These parameters achieved the statistically best fit (maximally reduced fitted variance) of the portal vein insulin concentration profile. All secretory rates were expressed as mass units of insulin (picomoles) released per unit distribution volume (liters) per unit time (minutes). As the volume of distribution of insulin is unknown in the portal sampling site in humans and undoubtedly differs from that in the systemic circulation, insulin secretion rates obtained at these two sampling sites cannot be directly compared.

Statistical analysis

Data are presented as the mean ± SEM. Statistical comparison between groups was made using Student's two-tailed *t* test. To examine the relationship between insulin concentration fluctuations observed in the portal vein and systemic circulation, cross-correlation was per-

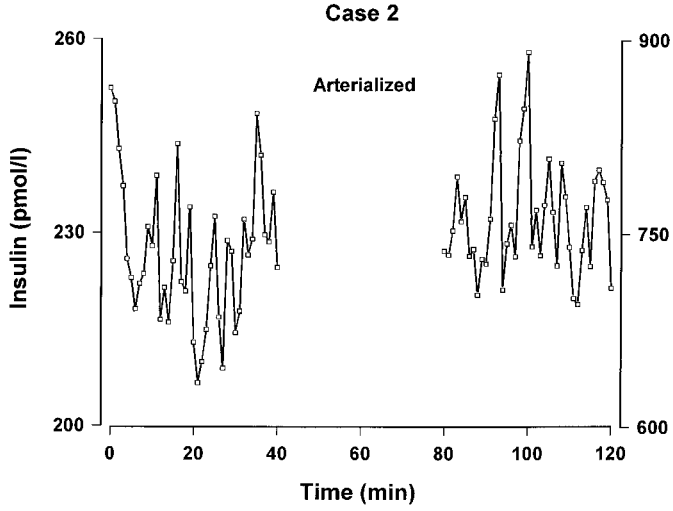

FIG. 3. Plasma insulin concentration profile observed from the arterialized sampling catheter in case 2 in the basal state (0–40 min) and during hyperglycemia (80–120 min). Note that the scale has been adjusted for each sampling period to maximize the visualization of oscillations. In comparison with the arterialized insulin concentration profiles in Fig. 2, the expanded scale clearly illustrates the prominent oscillations in insulin concentration in the both basal and stimulated states.

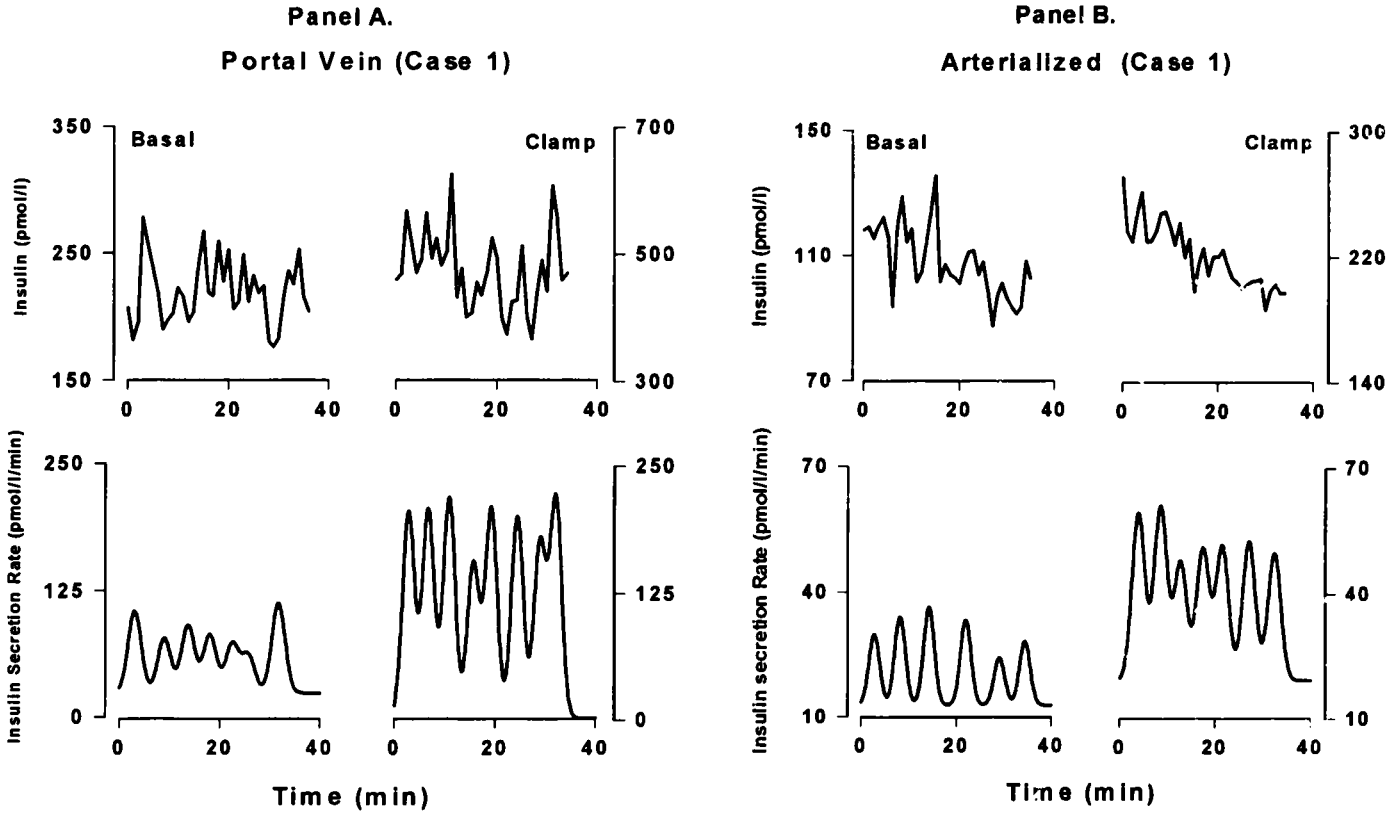

FIG. 4. A, The portal vein insulin concentration profile and corresponding deconvolved insulin secretion rates obtained during the intensive sampling periods in the basal state (*left panels*) and during the hyperglycemic clamp periods (*right panels*) for case 1. B, The arterialized insulin concentration profile and corresponding deconvolved insulin secretion rates obtained during the intensive sampling periods in the basal state (*left panels*) and during the hyperglycemic clamp periods (*right panels*) for case 1. Note that the insulin secretion rate is (mass units per volume of distribution)/time units. As the volume of distribution is unknown in the portal vein, these secretion units cannot be directly compared with those obtained from peripheral sampling.

formed (19). Cross-correlation analysis relates each portal vein insulin concentration to a corresponding value in the matching arterial series. This procedure consists of linear correlations carried out repeatedly at various time lags between the paired concentrations. Thus, at zero time lag, each portal vein plasma insulin concentration is compared with a time delayed measure (*e.g.* lag time minus 2 min) in the systemic circulation sample. By this means, an array of correlations can be collected that depend on the time matching of the two series.

To examine the relationship between pulses identified in the portal vein and systemic circulation we also performed peak concordance on these pulses from both sites. Peak concordance is a statistically independent (from cross-correlation) method to establish the relationship between detected pulses in two sampling sites. After identifying discrete insulin secretory bursts in the two time series (portal and peripheral), exact coincidence was defined by simultaneous pulse concordance (*i.e.* peak maxima occurred within one half-sampling interval of each other). Lagged coincidence was defined accordingly [*e.g.* with portal (+lags) or peripheral (−lags] peaks occurring first. The hypergeometric probability density (joint binomial distribution) was used to estimate the expected number of randomly concordant pulses, and the probability of falsely refuting the null hypothesis of pure chance concordance of the observed coincidences.

Results

Mean plasma glucose, insulin, and C peptide concentrations (Fig. 1)

The mean arterialized plasma glucose concentrations during the basal (fasting) and stimulated (hyperglycemic clamp) sampling periods were 5.3 ± 0.3 and 8.1 ± 0.1 mmol/L, respectively ($P < 0.001$). As expected, there was a rise in the mean arterialized (basal *vs.* stimulated, 209 ± 7.4 *vs.* 456 ± 16.8 pmol/L, $p < 0.001$) and mean portal vein (basal *vs.* stimulated, 440 ± 25.3 *vs.* 1020.7 ± 72.3 pmol/L; $P < 0.001$) insulin concentration after the increase in the plasma glucose concentration from 5.3 to 8 mmol/L during the hyperglycemic clamp. The mean arterialized C peptide concentration also increased with hyperglycemia (basal *vs.* stimulated, 1.8 ± 0.1 *vs.* 2.7 ± 0.1 nmol/L; $P < 0.001$), confirming an increase in insulin secretion in response to the glucose stimulus. Throughout the study in each subject, the portal vein insulin concentration was higher than the corresponding arterialized insulin concentration in both basal ($P < 0.001$) and stimulated ($P < 0.001$) sampling periods (Fig. 1).

Portal vein blood flow

The mean portal vein blood flow values in the two cases in which it was measured were 1.1 and 0.8 L/min. There was no change in the portal vein blood flow between the basal state and the hyperglycemic clamp.

Insulin concentration profiles (Figs. 2 and 3)

Inspection of the plasma insulin concentration profiles from the individual patients indicated the presence of re-

TABLE 2. Liver function studies

Type of cirrhosis	Alk phos (40–125 U/L)	GGT (5–35 u/L)	Bilirubin (2–17 μmol/L)	Albumin (36–47 g/L)	ALT (10–40 μL)	INR (2–4.5)
Alcoholic	125	124	56	32	14	1.4
Alcoholic	80	20	17	43	27	1
Alcoholic	146	100	42	34	56	1.3
Alcoholic	135	61	147	35	35	1.7
Alcoholic	92	54	17	41	33	1.2

ALT, Alanine amino transferase; Alk Phos, alkaline phosphatase; GGT, γ-glutamyl trasferase; INR, international normalized ratio. Normal ranges in *parentheses*.

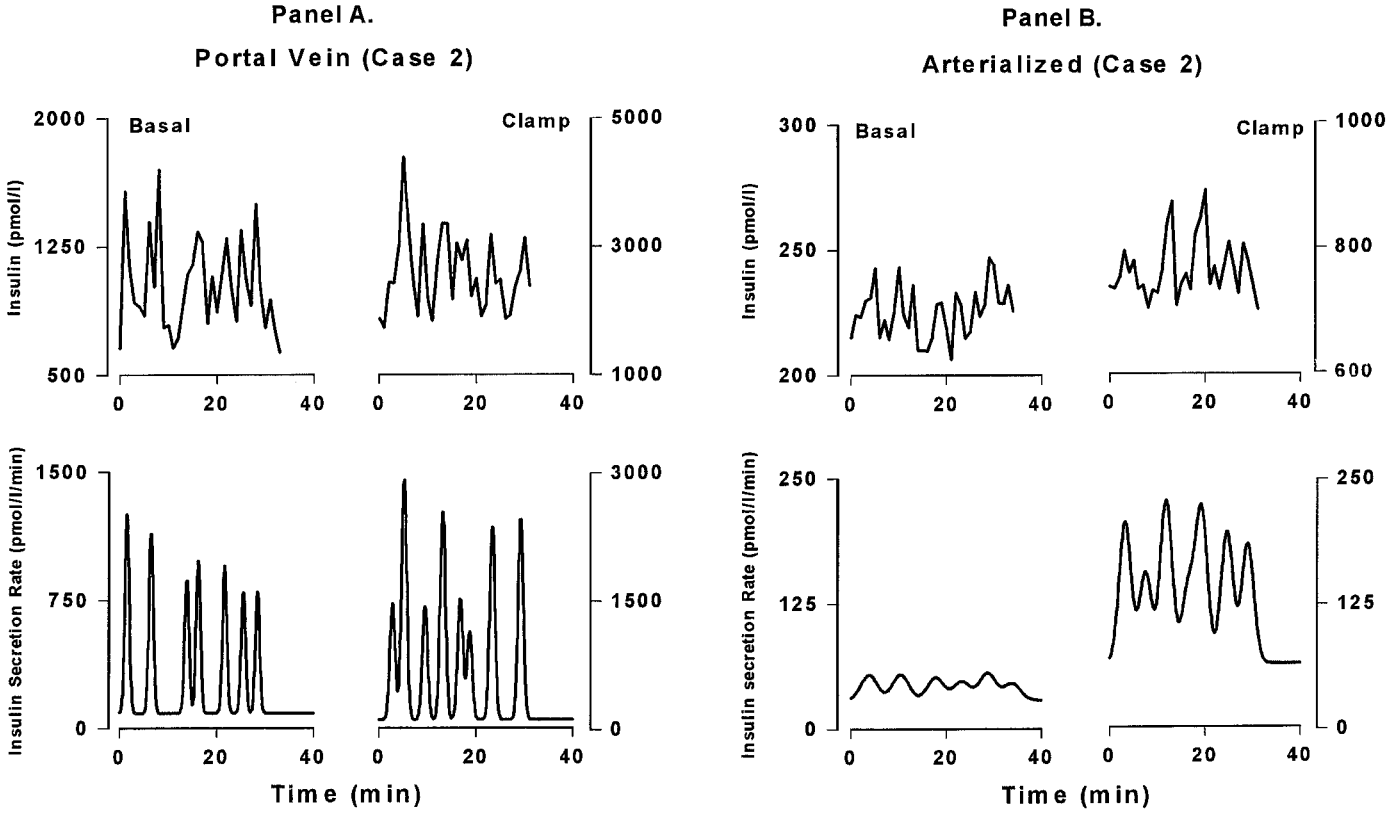

FIG. 5. A, The portal vein concentration profile and corresponding deconvolved insulin secretion rates obtained during the intensive sampling periods in the basal state (*left panels*) and during the hyperglycemic clamp periods (*right panels*) for case 2. B, The arterialized insulin concentration profile and corresponding deconvolved insulin secretion rates obtained during the intensive sampling periods in the basal state (*left panels*) and during the hyperglycemic clamp periods (*right panels*) for case 2.

current oscillations in both arterial and portal circulations in all patients. The magnitude of these oscillations was much larger in the portal circulation (Fig. 2) in both the basal and stimulated states compared with the corresponding profiles from the systemic circulation. The amplitude of the oscillations increased during hyperglycemia (Fig. 2). The range of the insulin pulse amplitude observed in the portal circulation was 100-1000 pmol/L in the basal state and increased to 200-3000 pmol/L during the hyperglycemic clamp. This contrasts with the corresponding range of pulse amplitudes observed in the systemic circulation of 10–30 and 40–100 pmol/L in the basal and stimulated states, respectively (Fig. 3).

Pulse detection

When the insulin concentration profiles were subjected to deconvolution, insulin pulses were invariably identified in both the portal and systemic circulation (Figs. 4 and 5). The pulse mass increased in response to hyperglycemia in both portal (basal *vs.* stimulated, 418 ± 155 *vs.* 1078 ± 368 pmol/L; $P < 0.05$) and systemic (basal *vs.* stimulated, 75 ± 10 *vs.* 241 ± 61 pmol/L; $P < 0.05$) circulations (Fig. 6). Furthermore, the measured pulse mass/volume of distribution was about 5-fold larger in the portal circulation in both basal and stimulated states. The pulse amplitude determined by deconvolution was also markedly (7- to 8-fold) larger in the portal circulation in both basal (portal *vs.* systemic, 254 ± 158 *vs.* 23 ± 2.7 pmol/L·min) and stimulated (portal *vs.* systemic, 524 ± 337 *vs.* 72 ± 19.8 pmol/L·min) states, confirming the impression gained by inspection of the insulin concentration profiles. The corresponding four pulse half-duration estimates were similar, with a global mean of 2.9 min. The interpulse interval (Fig. 6) determined by direct portal vein sampling was similar in the basal sampling period and during the clamp (Fig. 6). The pulse interval detected by sam-

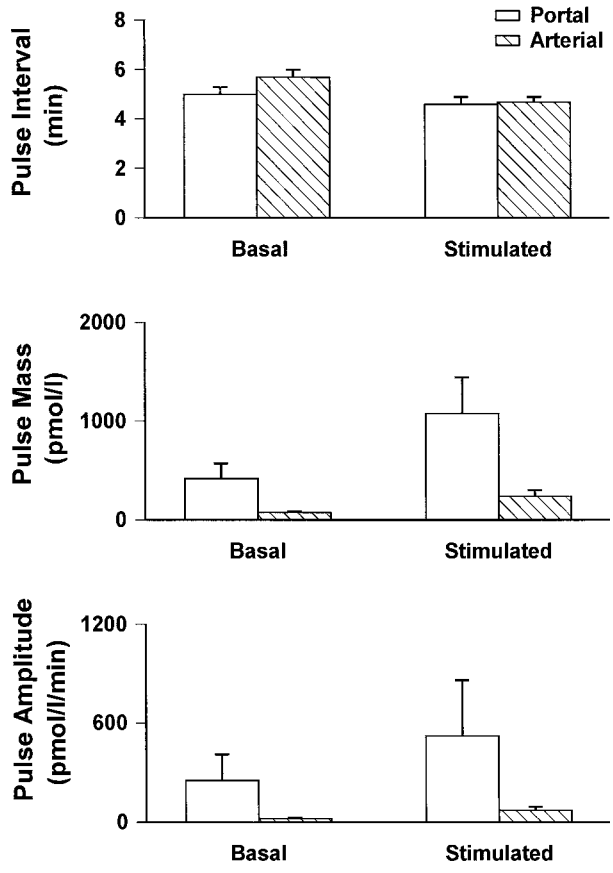

FIG. 6. The mean insulin interpulse interval (*top panel*), pulse mass (*middle panel*), and pulse amplitude (*bottom panel*) during the basal sampling period and the stimulated hyperglycemic period evaluated by sampling from the portal vein (*open panels*) *vs.* the arterial catheter (*shaded panels*). Units are minutes (pulse interval), picomoles per L (mass), and picomoles per L/min (amplitude).

pling from the systemic circulation was slightly, but not significantly, higher than that observed in the portal circulation.

In the portal circulation, the calculated proportion of insulin derived from discrete insulin secretory bursts was 64 ± 1.6% in the basal state and 93 ± 2.9% during the hyperglycemic clamp. In contrast, in sampling from the systemic circulation, the calculated proportion of insulin secreted in the pulsatile mode was apparently lower than that from the simultaneous sample measurements obtained from the portal circulation (basal state, portal *vs.* systemic, 66 ± 1.8% *vs.* 35 ± 4.1%; $P < 0.05$) and stimulated (portal *vs.* systemic, 93 ± 2.9% *vs.* 56 ± 1.2%; $P < 0.01$). Both autocorrelation and peak concordant analysis independently revealed a relationship between insulin oscillations in the portal vein and the systemic circulation (Figs. 7 and 8).

Discussion

The present study uses direct and high frequency sampling from the portal vein to affirm that insulin is secreted in humans in discrete secretory bursts, and that hyperglycemia enhances insulin secretion by selective amplification of the mass of insulin contained within each secretory burst. Furthermore, we could establish that the prehepatic frequency of insulin pulses is approximately 5 min, which, in fact, corresponds to that observed in the portal vein of dogs *in vivo* (5, 6) and from isolated perfused islets *in vitro* (14, 15).

Our conclusion that most insulin secreted into the portal circulation is derived from pulses is contingent upon the assumptions required for the deconvolution method employed (see *Materials and Methods*). It should also be noted that the half-life for insulin used in these studies for portal vein insulin concentration deconvolution was obtained by a best-fit approach to the portal vein insulin concentration profiles. Direct measurement of the volume of distribution or half-life at this site by a bolus injection upstream of the TIPSS sampling catheter in a mesenteric vein is not practicable in conscious humans. Nonetheless, similar studies in dogs with surgically implanted catheters arrived at the same conclusion that most insulin is secreted in discrete insulin bursts (6). Also, a prior study in humans sampling from the systemic circulation came to the same conclusion (11).

There has been some disagreement in the literature about the frequency of pulsatile insulin secretion *in vivo*, with estimates ranging from about 6 (5, 6) to about 20 (2–4, 7, 8) min. When we measured pulsatile insulin release directly from the canine portal vein, the interpulse interval averaged 6 min, based on a conventional insulin immunoassay and a deconvolution program specifically validated for insulin pulse detection (5, 6). Although this pulse frequency corresponded to that observed in perfusion of single islets *in vitro*, it was 3 times greater than that observed previously in humans by sampling from the systemic circulation (2–4, 7, 8). We postulated that the reason for this large discrepancy was the relative loss of insulin signal when insulin pulses are examined in the systemic compared with the portal circulation (6).

At least in the canine model, we confirmed that the sampling site was crucial when a conventional insulin assay was employed (5), as simultaneous estimates in the portal vein and the systemic circulation revealed insulin interpulse intervals of 6.7 and 9 min, respectively (5). The insulin pulse detection was further obscured by using a 2-min sampling regimen in the systemic circulation, as employed in some previous reports (7). The disadvantages of sampling from the systemic circulation were especially evident when the only available insulin assays were conventional immunoassays (20). Even multiple replicates by these assays, which have cross-reactivity with proinsulin and insulin split products, do not allow for fully reliable detection of high frequency insulin pulses in the systemic circulation. However, the introduction of the more sensitive and specific ELISA assays for insulin (17) should theoretically overcome some of these problems. Recently, using an ELISA for insulin measurements, we reported an insulin pulse frequency of approximately 8 min when sampling in the systemic circulation in humans (11, 12). The present study now confirms directly that the systemic sampling route can provide an accurate estimate of insulin pulse frequency *in vivo* in humans when the insulin concentration is measured by a sensitive and specific ELISA method and submitted to appropriate deconvolution-based analysis.

Sampling directly from the portal circulation revealed a

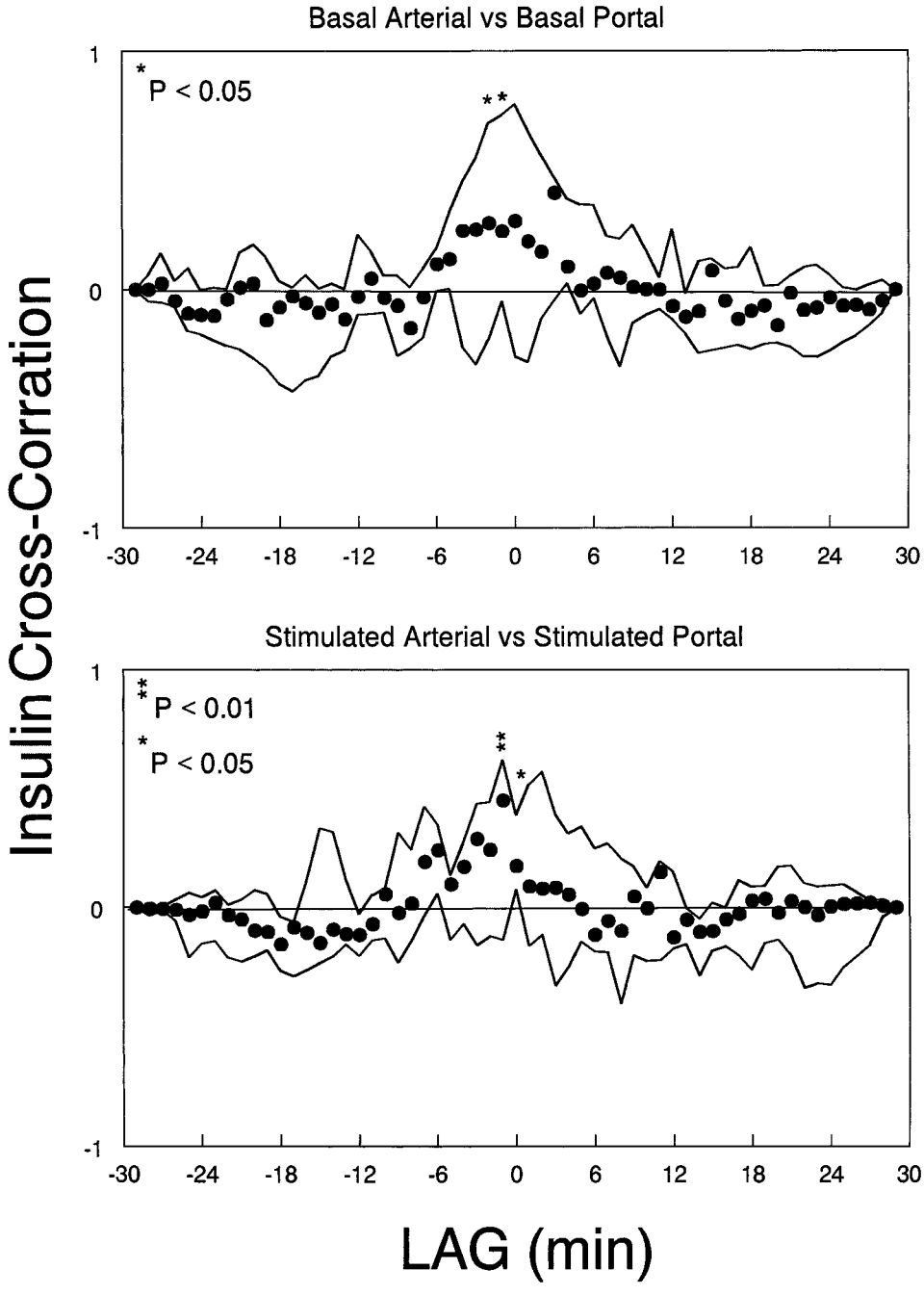

FIG. 7. Cross-correlation analysis of arterial and portal vein insulin concentration data in the basal and stimulated states. These data illustrate that changes in serial portal vein insulin concentrations precede those in the systemic circulation by 1–3 min ($P < 0.05$ and $P < 0.01$).

remarkable magnitude of insulin oscillations at this site. In the present study the amplitude of insulin concentrations in the portal vein was about 200-1000 pmol/L in the basal state and increased to about 500-5000 pmol/L during hyperglycemia. These values in the human are comparable with those observed in the canine portal vein (5, 6) and presumably reflect the insulin concentration wavefront to which the liver is exposed. Although the present study subjects were necessarily only those with portal hypertension requiring treatment, we selected subjects with normal or near-normal liver function tests who were clinically well and had normal prestudy OGTTs. Moreover, the range of insulin oscillations observed in the portal vein in these subjects is comparable to that previously observed in healthy dogs (5, 6), and the portal vein blood flow, when available, was in the normal reported range for humans. Although some studies have reported that pulsatile insulin delivery (to the peripheral circulation) enhances insulin sensitivity, none has yet recapitulated the magnitude of insulin oscillations to which the liver is exposed *in vivo* during stimulated enhanced insulin secretion (21, 22). The question remains, therefore, what the physiological importance is, if any, of exposing the liver to such dramatic oscillations of insulin concentration. As the magnitude of peripheral insulin pulses is decreased in patients

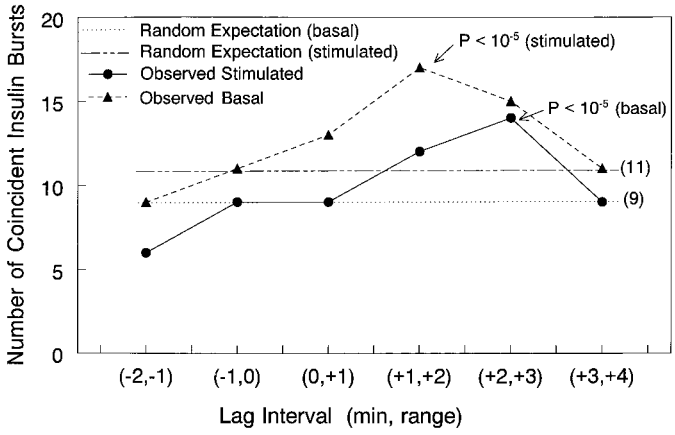

FIG. 8. Peak concordance analysis of pulses detected in the portal vein and systemic circulation. Observed number of coincident insulin secretory bursts detected by simultaneous sampling in the portal and peripheral circulation. The *x*-axis gives the lag interval between the individually identified portal and peripheral insulin pulses. A lag interval of $(-2, -1)$ denotes that pulses occurred centered within this absolute time lag (*i.e.* the pulses in the portal blood followed those of insulin in the peripheral blood by no less than 1 min and no more than 2 min). Thus, at a lag interval of $(+1, +2)$, insulin pulses in the portal blood preceded those in the peripheral blood by no less than 1 min and no more than 2 min (*right side* of graph). The expected number of purely random coincidences was calculated via the hypergeometric probability distribution in both the basal and stimulated states (*interrupted lines*). The observed number of coincidences significantly exceeded expectation values at 1- to 3-min lag times, wherein portal insulin pulses appeared first. The maximal coincidence was 13 events in the basal state, and 17 events in the stimulated state. The corresponding expected values were 9 and 11 given the different pulse frequencies at these times, with respective P values less than 10^{-5}.

with type 2 diabetes (23), it is likely that the liver in these patients is exposed to even more attenuated oscillations in insulin pulse amplitude, which may contribute to the hepatic insulin resistance characteristic of this disease (24).

The remarkable transhepatic attenuation of the insulin pulse signal from the portal circulation to the systemic circulation observed here is comparable to that observed previously in the dog (5). Although the attenuation may reflect up to an approximately 4-fold dilution of portal venous blood by the systemic venous drainage, the insulin pulse amplitude is damped to a much greater extent than this (~10-fold). One potential explanation for this is preferential hepatic insulin clearance of insulin pulses. We previously noted that the hepatic clearance of portal vein insulin in the dog is related in an ascending monotonic (quadratic) manner to the pulse amplitude of the insulin concentration wavefront presented to the liver (25). Taken together, these preliminary observations provoke the need for additional studies to address this possibility of a link between pulse waveform and hepatic insulin clearance. In one prior study in which blood was sampled from the portal vein of patients with cirrhosis, comparable large oscillations in insulin concentration were observed, although no quantification of pulsatile insulin release or corresponding arterial sampling was available (26).

In summary, direct sampling from the portal vein in humans establishes an insulin interpulse interval of about 5 min and thereby resolves the discrepant insulin pulse frequency previously reported in humans. A comparable insulin interpulse interval can also be detected by high frequency peripheral sampling with the use of a highly sensitive insulin assay and appropriately validated pulse detection. Direct portal vein sampling is consistent with prior studies in dogs and humans, which reported that the majority of insulin is secreted in insulin bursts. Finally, we confirm that the liver in humans, like that in dogs, is exposed to dramatic insulin concentration oscillations that far exceed the magnitude evaluated to date in clinical studies of insulin action *in vivo*.

Acknowledgments

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