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Reviews

The in vivo regulation of pulsatile insulin secretion

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Abstract

The presence of oscillations in peripheral insulin concentrations has sparked a number of studies evaluating the impact of the insulin release pattern on the action of insulin on target organs. These have convincingly shown that equal amounts of insulin presented to target organs have improved action when delivered in a pulsatile manner. In addition, impaired (not absent) pulsatility of insulin secretion has been demonstrated in Type II (non-insulin-dependent) diabetes mellitus, suggesting a possible mechanism to explain impaired insulin action in Type II diabetes. Whereas the regulation of overall insulin secretion has been described in detail, the mechanisms by which this regulation affects the pulsatile insulin secretory pattern, and the relative and absolute contribution of changes in the characteristics of pulsatile insulin release have not been reviewed previously. This review will focus on the importance of the secretory bursts to overall insulin release, and on how insulin secretion is adjusted by changes in these secretory bursts. Detection and quantification of secretory bursts depend on methods, and the methodology involved in studies dealing with pulsatile insulin secretion is described. Finally, data suggest that impaired pulsatile insulin secretion is an early marker for beta-cell dysfunction in Type II diabetes, and the role of early detection of impaired pulsatility to predict diabetes or to examine mechanisms to cause beta-cell dysfunction is mentioned. [Diabetologia (2002) 45: 3–20]

Keywords Insulin, pulsatility oscillation, diabetes, physiology, secretion, regulation, C peptide.

In 1922, Karen Hansen [1] measured two series of blood glucose concentrations and reported oscillations in the peripheral concentrations of this substrate, results which were validated by simultaneous sampling from two sites to exclude the possibility that assay variability was a cause of the observed variations. Furthermore she studied the glucose concentrations in diabetic patients, and described both rapid and slower oscillations [1]. Half a century later the observation of rapid oscillations was shown to correlate with oscillations in the peripheral insulin concentrations [2–6], with glucose concentration increases slightly out of phase with insulin secretion pulses [7]. The pulsatile secretion of insulin was shown to coincide with islet pulsatile release of glucagon [4, 6, 8, 9], and somatostatin [9–11]. A number of studies have reported importance of this release pattern for optimal insulin action [10, 12–21, 21–25], for overall insulin secretion [26–35], and for possible development of disease [36–43]. Studies on pulsatile insulin secretion could therefore be important for appreciating how insulin release is regulated overall and how

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Abbreviations: ApEn, Approximate entrophy; LH, luteinizing hormone; ACTH, adrenocorticotropin; GABA, gamma-amino-butyrate; GLP-1, glucagon-like-peptide-1; IGF-1, insulin-like-growth-factor-1; IGF-BP1, insulin-like-growth-factor-1-binding-protein

the underlying mechanisms impact on insulin action. Once the physiology of pulsatile insulin release processes is understood, this knowledge could be employed to analyse defects in disease states, and on a mechanistic basis to evaluate different hypotheses of the cause of the defects observed at disease.

In addition to the rapid pulsatile insulin release pattern, an ultradian oscillatory pattern has been described [44–50], and associated with improved insulin action [46, 51, 52], and, in case of impairment, with Type II (non-insulin-dependent) diabetes mellitus [44, 45, 47–49], and early Type I (insulin-dependent) diabetes mellitus [53]. Finally, diurnal (meal related) oscillations are an apparent [54–58] and, probably, an important [47] contributor to the complexity of insulin release, that assures normal physiological release to meet the needs of glucose homeostasis.

This review will mainly focus on the rapid oscillatory insulin secretory pattern, and will relate the observations of in vivo pulsatility to important aspects of oscillatory beta-cell function reported in vitro.

Impact of pulsatile insulin secretion on insulin action

The demonstration of pulsatile insulin secretion has lead to a number of studies that specifically addressed the importance of this insulin release pattern on insulin action. The target organs are mainly liver, muscle and adipose tissues. Studies have been designed to assess the impact of insulin release patterns on each of these insulin sensitive target organs. Anatomically, the most obvious candidate organ for a pulsatile versus constant release is the liver. Because insulin is released into the portal vein, any change in insulin secretion will cause changes of portal vein insulin concentrations. Through fenestrated sinusoids and the space of Disse, these changes will result in changes in the concentrations that the hepatocytes see and impact on liver metabolism. . The impact of pulsatile versus constant insulin delivery on hepatic insulin action is improved when insulin is presented to the liver in a pulsatile manner. Because portal vein insulin delivery is hard to control in vivo, studies on the isolated perfused liver are needed, and in these studies, specifically designed to address this, an improved ability to suppress glucagon-induced hepatic glucose production has been reported when insulin is delivered in a pulsatile manner [59]. Similarly, in Type I diabetic patients, a model to evaluate insulin actions without confounders from other hormones, showed that hepatic glucose production was reduced by 25–30 % when the same amounts of insulin were delivered in a pulsatile rather than constant way [23]. The improved insulin action could be attributed to insulin-insulin receptor kinetics, where insulin binding causes internalization of the insulin receptor for a brief period, allowing it to resurface before the next insulin secretory burst arises. This could explain why the impact of pulsatile delivery is frequency-dependent [16].

In contrast, pulsatile insulin delivery has not been reported to change [60] or improve peripheral glucose uptake [12, 18, 20, 21, 23, 24]. Furthermore, adipose tissue shows improved insulin sensitivity when insulin is administered in a pulsatile fashion [12, 13] as reviewed previously [19, 61]. Conversely, the insulin secretory pattern is associated with changes in insulin sensitivity [12, 13, 62, 62] and is altered in the elderly [63, 64]. Effects of pulsatile insulin delivery on glucose uptake in insulin-sensitive tissues seem to require some time to occur, and most studies have been extended to 6 or 7 h to show a difference. Considering the rather quick effects on liver metabolism in the open loop isolated perfused liver, a direct effect on the liver and an indirect effect on peripheral glucose metabolism could be possible. This also seems likely considering the very dampened insulin oscillations that occur once insulin reaches muscle insulin receptors. The amplitude is about 30 % in the arterial circulation, and before reaching the muscular or adipose tissue beds, the insulin signal needs to cross the capillaries and diffuse to the target organ, thus further reducing the relative signal (pulse amplitude). Furthermore, the recent emphasis on muscle lipid content to cause insulin resistance supports this notion.

The improved insulin action with physiologically pulsatile insulin delivery has further underscored the importance of impaired insulin pulsatility in Type II diabetes whose hallmark is impaired insulin action. Therefore, strategies to affect beta-cell function in Type II diabetes could influence insulin action, by the same mechanisms by which the oscillatory insulin release is affected.

Methods for assessing pulsatile insulin secretion

There are different reasons for assessing pulsatile insulin secretion, and the method for analysis has to be chosen accordingly. In general the secretion is determined using concentrations of either insulin or C-peptide. For this reason, to detect a secretory burst and to prevent the detection of assay or biological variability as secretory pulses, it is crucial to optimize the methods involved in sampling, concentration measurements and data analysis to discriminate between signal and background noise.

Sampling site. Insulin is secreted into the portal vein circulation, and undergoes partial (40 to 80 % [65–68]) hepatic extraction before dilution into the systemic insulin pool. Detection of pulsatile insulin release is further hampered by a time-delayed dampening of the insulin pulses [69]. Portal-vein sampling

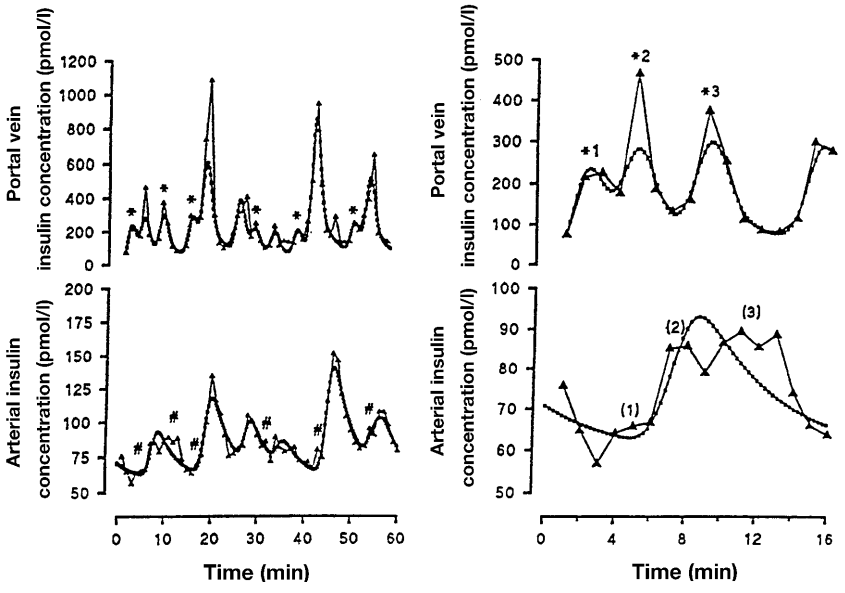

Fig. 1. Simultaneously measured portal vein (top panels), and arterial (bottom panels) insulin concentration time series, at overnight fasting conditions in a dog. The full line shows the concentration profile that arises from deconvolution analysis, assuming detection of distinct secretory bursts, superimposed on time invariant basal insulin release. Note that in the peripheral circulation (bottom panels) some of the secretory bursts detected in the portal vein (*) are missing, although inspection of raw data suggests the presence of overlapping pulses (#). This is highlighted in the right side panels demonstrating three portal vein pulses that merge into one three-phasic pulse in the peripheral circulation (1, 2, 3)

can overcome most of the pulse detection problems referred to above (Fig. 1; and Fig. 2). In animal models, short-term (hours) and chronically (weeks) implanted portal-vein catheters have been used for sampling purposes, providing an opportunity for applying portal-vein sampling to studies on in vivo pulsatile insulin release [26, 69, 70]. Sampling from the portal vein results in an increase in the insulin concentration oscillations from approximately 30 % in the peripheral circulation to 80 to 500 % [26, 69] in the portal vein and consequently the ratio of signal (pulse oscillations) to noise (assay variability, dilution by saline flush, variable protease degradation of insulin in samples etc) is markedly improved with portal-vein sampling. This has been achieved in humans [71] and could be used specifically for those who need an implantation of transjugular intrahepatic portosystemic shunt [72, 73, 73].

Sampling intensity and duration. Traditionally sampling every minute [4, 5, 10, 14, 26–29, 34–37, 43, 69, 73] or every second minute [3, 13, 15, 33, 74–76] for 1–2 h has been done to study the rapid insulin oscillations. Sampling from the portal vein or a peripheral vein for insulin measurements every 30, 60, 120, and 240 s demonstrates a decrease in pulse detection when the sampling intensity is reduced from 60 s for each sample [26, 69]. Furthermore, there is a tendency for fewer pulses to be detected in those studies sampling every second as opposed to every minute (Table 1) (Figure 3). In contrast, no further improvement of the pulse detection has been achieved by sampling every 30 s [26, 69]. The high frequency sampling and replicate measurements are costly so sampling duration should be short. The repetitive nature of pulsatile insulin secretion [4, 5, 10, 33, 76] would allow a reliable measurement of pulse frequency and amplitude using a sampling duration of 40 to 60 min [69] if deconvolution analysis or simple pulse detection algorithms are used. However, analysis based on autocorrelation (minimum 2 times pulse interval) and on spectral analysis ($n > 100$) as well as regularity statistics (approximate entropy: $n > 40$) in general improve with increased sampling duration and therefore most studies using periodicity statistics are carried out over a longer duration [4, 5, 10, 33, 76].

Insulin versus C-peptide measurements. Insulin and C-peptide are co-released in a 1 to 1 molar ratio [77]. Whereas insulin undergoes substantial (40–80 %) and variable [66, 78–80] hepatic-insulin extraction [81], C-peptide is presumably not cleared by the liver [82], which resulted in a predominance of the use of C-peptide when calculating the overall insulin secretory rates. C-peptide kinetics are similar under different conditions and thus suited for a comparison of insulin release among different groups [83]. However, the half-life of C-peptide is markedly longer (~ 35 min) [83] than the half-life of insulin (5–8 min) [35], and this longer half-life of C-peptide will dampen oscillations and reduce signal to noise ratio oscillations correspondingly and result in an improved de-

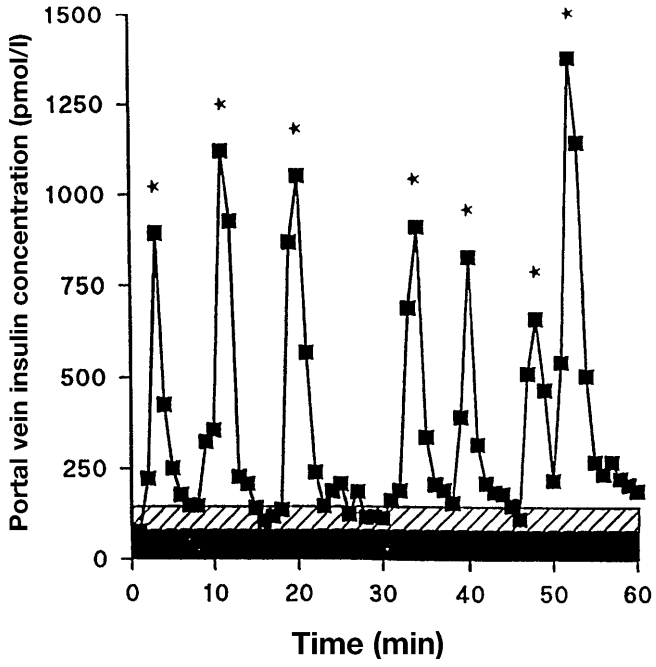

Fig. 2. Portal vein insulin concentrations in an overnight fasted dig. Note the very large insulin pulses. The hatched and solid areas are arterial insulin concentrations (measured every 10 min) showing that between pulses, no insulin is released in such a case

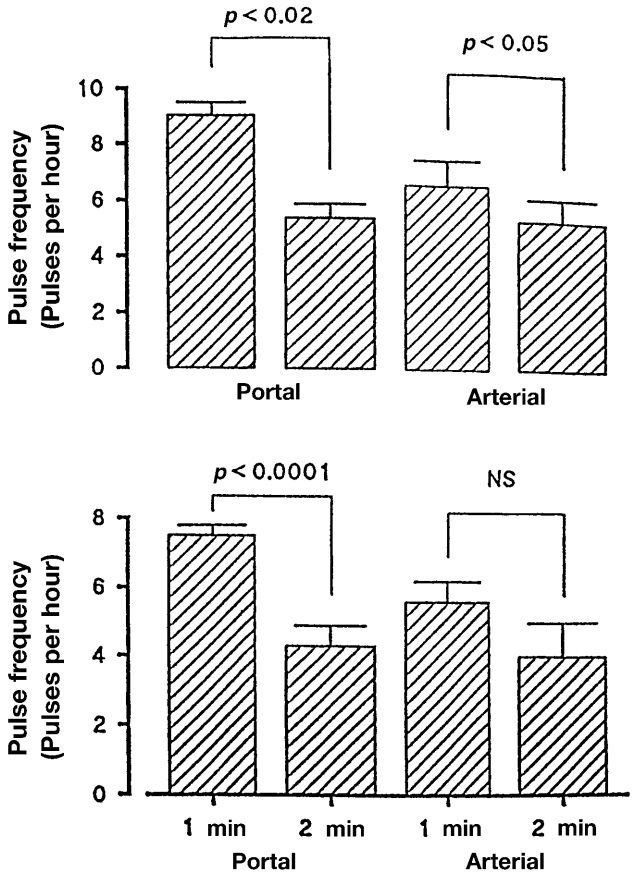

Fig. 3. Detected pulse frequency by deconvolution analysis (top panel) and by cluster analysis (bottom panel) when sampling every 1 min comparing with every 2 min in the portal vein (left) rather than the artery (right)

tection of secretory bursts by use of insulin as opposed to C-peptide measurements. Amylin, which has been shown to have synchronous oscillations in the peripheral circulation [84], and a combined analysis of insulin with amylin or C-peptide or both could improve the analytical ability to detect coordinate secretory events.

Assay and biological noise. Sampling could involve some variability because saline infusion and flushing could be necessary to assure sampling catheter patency, resulting in dilution, which can be minimized through dead-space withdrawal. During and following the sampling process a variable haemolysis could occur, and proteases could be involved in degrading insulin in syringes and tubes, a process referred to as biological noise [69]. Measurements of insulin concentrations are also subject to assay variability, and a reliable, sensitive and specific assay is crucial to minimize the variability due to measurements. Considered together, the noise from sampling and assay procedures should be compared to the biological signal detected because concentrations change due to the pulsatile release. This supports the use of portal-vein sampling with improved signal but also highlights the necessity to optimize sampling and analysis of blood for insulin concentrations. A specific problem with assays can be the detection of pro-insulin, which has a longer half-life, and occurs at greater concentrations in the circulation in early Type II diabetes [85, 86] and at states with beta-cell loss [87]. Therefore, to discriminate between pathophysiology in Type II diabetes and states of impaired beta-cell function, the use of an assay that does not cross react with pro-insulin is helpful.

Analytical strategy. The problems with biological time series are often connected with the presence of trends and noise.

Trends and subtrends. An analytical strategy needs to be chosen to address the question asked (i.e. what is the frequency that is examined for). The influence of subpatterns on high frequency oscillations can be avoided by detrending as with first differencing, or analysis of residuals after subtraction of a fitted line employing linear regression, for example, by moving average or other models to fit the data curve. The solution to the problem of noise could be a low pass filter as with smoothing procedures, where the chosen window lengths and weighing of data points could be important for the outcome. These procedures affect the specific patterns in the data to be examined. When several patterns occur together techniques

Table 1.

First author	Ref ID	Journal	Year	Species	Stimulation	Peptide	Sampling site	Intensity min/sample	Analysis	Periodicity (min/pulse)
Anderson	[2]	Metabolism	1967	Canine	No	Insulin	Pancreatic and hepatic veins	15–30 s	Compare with SD	4 (1.5–6) ?
Goodner	[6]	Science	1977	Baboon	Fasted	Insulin	Periphe ral vein	1 min	Autocorrelation	9
Lang	[5]	N Engl J Med	1979	Human	Fasted	Insulin	Superior caval vein	1 min	Autocorrelation	13
Hansen	[76]	Am J Physiol	1981	Baboon	Fasted	Insulin	Peripheral vein	2 min	Autocorrelation, spectral analysis	
Hansen	[33]	Am J Physiol	1982	Baboon	Fasted + postprandial	Insulin	Peripheral vein	2 min	Autocorr elation, spectral analysis	9–12
Lang	[4]	Diabetes	1982	Human	Fasted	Insulin	Peripheral vein	1 min	Autocorrelation	11–13
Goodner	[7]	Science	1982	Baboon	Fasted	Insulin	Artery	2 min	Autocorrelation	9
Jaspan	[71]	Am J Physiol	1986	Canine	Fasted	Insulin, C-peptide	Portal and Hepatic vein	1–2 min	Spectral analysis	10–14
Matthews	[10]	Endocrinology	1987	Canine	Fasted, halothane	Insulin	Portal vein	1 min	Autocorrelation	10 ? – 30
O'Rahilly	[43]	N Engl J Med	1988	Human	Fasted	Insulin	Peripheral vein	1 min	Autocorrelation, Fourier	13–14
Chou	[121]	Life Sciences	1991	Rat	Fasted	Insulin	Peripheral vein	3 min	Clifton	13
Goodner	[101]	Am J Physiol	1991	Baboon	Fasted, sedation	Insulin	Peripheral Vein	2 min	Clifton	10
Peiris	[15]	J Clin Endocrinol Metab	1992	Human	No	Insulin	Peripheral vein	2 min	Pulsar	7–12
Balks	[90]	J Clin Endocrinol Metab	1992	Human	Basal and postprandial	Insulin, C-peptide	Peripheral vein	2 min	Deconvolution and Cluster	12
Storch	[73]	Dtsch Med Wochenschr	1993	Human	Bas al and postprandial	Insulin, C-peptide	Portal vein	1 min	Cluster	4.1–6.5
O'Meara	[74]	Am J Physiol	1993	Human	Fasted	Insulin, C-peptide	Peripheral vein	2 min	Ultra, Pulsefit, Cluster, Autocorrelation, Spectral analysis	11
Porksen	[69]	Am J Physiol	1995	Canine	No	Insulin	Portal and Peripheral veins	1–4 min	Cluster, Deconvolution	6.5–9
Engdahl	[14]	J Appl Physiol	1995	Human	No	Insulin	Peripheral vein	1 min	Deconvolution	10
Porksen	[27]	Diabetes	1996	Canine	Yes	Insulin	Portal vein	1 min	Deconvolution	5–7
Hunter	[13]	Diabetes	1996	Human	No	Insulin	Peripheral vein	2 min	Pulsar	7–8
Porksen	[35]	Am J Physiol	1997	Human	Yes	Insulin	Peripheral vein	0.5– 3.0	Deconvolution	4.7

could be used to separate these patterns and evaluate them independently [36]. The rapid and ultradian insulin oscillations data set could contain periodicities of about 7 and 120 min, and at minutely sampled intervals the slower ultradian oscillations could be detrended by first differencing the data, or by subtracting a moving average that is close to the frequency studied (i.e. for insulin 7–11 point moving average), before analysing for the rapid frequency pattern. In contrast, smoothing procedures by 3–11 point moving average tend to remove signals of the rapid frequency. Inspection of raw data before any filtering procedure to allow time for readers to gain intuitive impressions of data signals and see the raw data.

Pulse detection. Analysis of peripheral insulin concentrations can give rise to detection of the individual concentration pulses and valleys ("basal concentrations"), and subsequently mean relative and absolute amplitudes and mean frequency. This detection method is based on predetermined statistical criteria to allow concentration changes to be considered significant; this approach is the basis of well established methods, such as Pulsar [8, 13, 71, 88] and Cluster analysis [69, 89, 90], and ULTRA [74, 75, 91]. The drawback is that each concentration change has to pass the significance criteria to be detected, and relative low biological signals might not be detected, as well as impairment of pulse detection due to frequency increase, which is a possible problem, when increasing frequency of pulses tend to cause overlap. Due to low signal-to-noise ratio, the estimated pulse frequency decreases to the extent that pulses are not detected. The method will quantify the secondary concentration events.

Periodicity analysis. The frequency of the insulin pulses can be estimated by spectral analysis, or autocorrelation analysis which evaluates the concentration time series for regular periodicities. Spectral analysis may be rather unaffected by noise because more random data variability will probably not influence the detected periodicity. The ability to estimate if the spectral power in a single data set reflects significant periodicity can be estimated by observing analysed data and subsequently subjecting it to random shuffling before re-analysis giving a mean spectral power and SD of randomly distributed values, allowing comparison of observed spectral density to data obtained from randomly distributed data. Autocorrelation analysis also detects periodicity in data. The method examines replicability of patterns. The method will give frequency of concentration changes, significance for this pattern, and a correlation coefficient. However, these two methods are restricted to the evaluation of periodicities of peripheral concentration changes and do not directly give information on secretion apart from a likely estimate of the frequency of the insulin secretory bursts. Lack of significant periodicities can be due both to large variability in frequency or amplitude of truly present secretory bursts, which can be detected by pulse detection algorithm, or they can be due to relatively small pulses occurring at a regular interval. The latter could in part be overcome by increasing sampling duration.

Regularity statistics. The pulsatile insulin release pattern could also be characterized in further detail to examine the reproducibility of the subordinate patterns in the data set. A validated mathematical approach is application of approximate entropy (ApEn) [36, 92–94]. Approximate entropy is a recently introduced regularity statistics that measures the logarithmic likelihood that runs of patterns will be reproduced on next incremental comparison. The method has proved to be useful in a number of studies on hormonal secretion including growth hormone [95], LH [96], aldosterone [97], cortisol and ACTH [98, 99], and recently insulin [36, 64] to discriminate between pathophysiology and health. The method is robust to noise and to absolute differences in data. Derived values from approximate entropy analysis do not provide information on basal secretion, pulse mass/amplitude or frequency.

Detection and quantification of pulsatile insulin secretion by deconvolution. Insulin concentration time series can be analysed by deconvolution to detect and quantify insulin secretory bursts. Deconvolution of insulin concentration data can be carried out with a multi-parameter technique [100]. This requires the assumption that insulin concentrations measured in samples collected at known intervals result from five determinable and correlated parameters. These are, (i) a finite number of discrete insulin secretory bursts occurring at specific times and (ii) having individual amplitudes (maximal rate of secretion attained within a burst); (iii) a common half-duration (duration of an algebraically Gaussian secretory pulse at half-maximal amplitude), which is superimposed upon; (iv) a basal time-invariant insulin secretory rate; and finally, a bi-exponential hormonal (C-peptide/insulin) disappearance model in the systemic circulation with known or estimated half-lives. Assuming the foregoing hormonal disappearance values, the number, locations, amplitudes and half-duration of insulin secretory bursts, as well as a non-negative basal insulin secretory rate for each data set is estimated by non-linear least-squares fitting of the multi-parameter convolution integral for each insulin time series. Insulin secretion calculated by use of C-peptide concentrations is more robust to intra-individual and inter-individual variation in kinetic parameters [83]. When doing deconvolution analysis it is important to adjust basal secretion to allow accommodation of troughs. Deconvolution involves subjective elements,

although defined peaks are tested for significance and all data analysis must be done in a blinded manner. The technique requires no assumptions about frequency, amplitude and stationarity.

Contribution of pulsatile insulin release to the overall insulin secretion

Peripheral insulin concentrations oscillate [2–6], because of pulsatile insulin secretion [3]. However, the net contribution of the secretory bursts is important, because the presence of concentration oscillations does not exclude the dominance of a time-invariant (basal) release pattern. Furthermore, the relation between the in vivo observed secretory bursts and the in vitro observed pulsatile release from the isolated pancreas [9, 10, 101] and the isolated peri-perfused islets [102, 103], as well as episodic beta-cell depolarization [104–106], beta-cell glycolysis [105, 107], and beta-cell increase in calcium remains to be established [104, 105, 108–111]. The coupling of cyclic metabolism to pulsatile in vitro release has been addressed in an excellent review by Tornheim [112]. Mathematical models have been used to better understand the mechanisms underlying coordinate pulsatile release [113, 114]. Comparisons of exact measures of secretory burst shape, duration, frequency and secretory process orderliness of in vitro and in vivo insulin secretory bursts could further underline the link between intracellular beta-cell events, to large amplitude in vivo pulsatile insulin release.

Configuration. If an in vivo pulse consists of the summation of about one million simultaneous insulin secretory bursts from individual islets then a similar burst shape and duration is to be expected. In contrast, a broader in vivo than in vitro pulse configuration is expected if the in vivo pulse consists of the summation of release from islets that secrete at different times, because each islet in itself has a secretory burst duration of 2 to 3 min [115]. If therefore, the shapes of the individual secretory bursts from individual islets and from in vivo secretion are super-imposible (corrected for amplitude) then this suggests that the in vivo pulse is generated by simultaneous secretion from the intra-pancreatic islet population (Fig. 4). Comparisons between human in vivo [26, 35] and in vitro [105] pulse shape suggests that the in vivo pulse reflects simultaneous secretion from all islets.

Frequency. A large variety of frequencies of in vivo pulsatile insulin secretion has been reported, varying from 4 to 15 min per pulse (Table 1). In contrast, the in vitro periodicity from the isolated perfused pancreas has been reported to be 6–10 min per pulse [8–10, 39, 116]. The latter are not confounded by he-

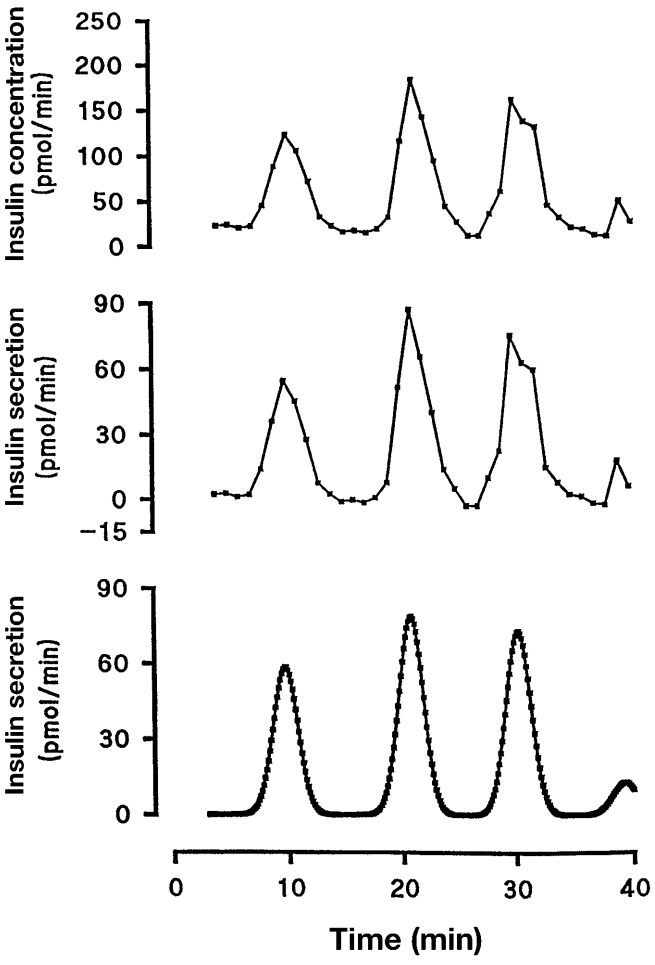

Fig. 4. Measured portal-vein insulin concentrations (top panel) and calculated insulin secretory rates from deconvolution analysis (bottom panel) and by cross-pancreatic sampling, multiplying by portal-vein plasma flow. Note that in this case no basal (non-pulsatile) insulin secretion is detected and this is observed very clearly by both methods independently

patic insulin extraction or dilution into the systemic insulin pool to make pulse detection easier. Similarly, studies on pancreas-transplanted subjects with shunts from the pancreas to the inferior caval vein, show increases in frequency versus healthy controls (8 versus 12 min per oscillation) [75]. Finally the reported frequency of 3 to 5 min per pulse in the isolated perifused islet [105, 115, 117, 118] (10–17 min per pulse in [102, 119, 120]), suggests that there is a hierarchy of regulatory mechanisms with the fastest frequency in individual islets (3 to 5 min), followed by the perfused pancreas (6–10 min) and finally the in vivo insulin secretion (5 to 15 min). However, the open loop systems usually employed in in vitro rather than the closed loop system in vivo, with confounding effects of systemic dilution and hepatic insulin extraction, could cause a relatively low estimate of the in vivo pulse frequency. Studies comparing sampling intensities, sampling sites and mathe-

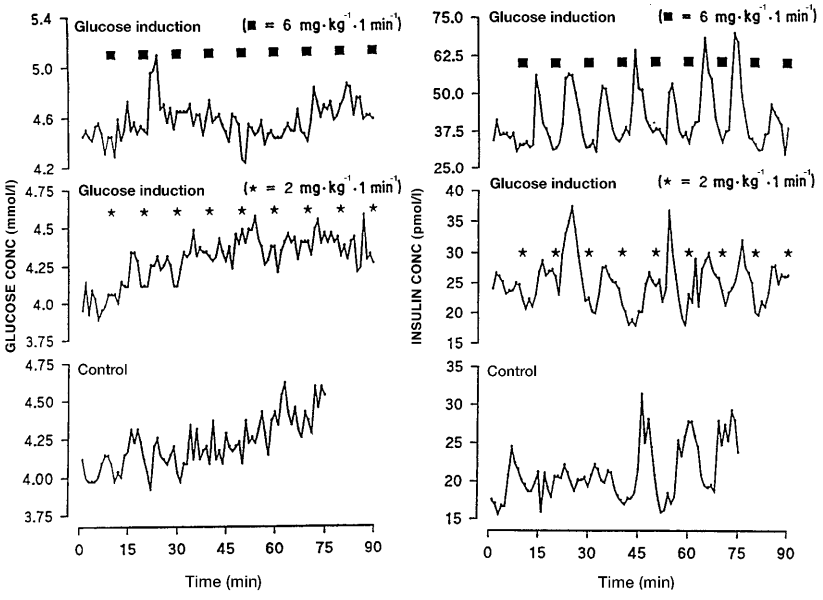

Fig. 5. Glucose (left panels) and insulin (right panels) when pulsatile insulin secretion was controlled (entrained) by punctuate glucose infusions over 1 min giving 6 mg/kg over 1 min (top panels), or 2 mg/kg over 1 min (middle panel, every 10 min starting at time 10 min, compared with basal (non-entrained) concentrations in the bottom panels. Note the large apparent amplitude of peripheral insulin oscillations occurring with each glucose infusion, despite very minimal glucose excursions in the top panel and a more irregular pattern in the middle and bottom panels

matical strategies for the detection of insulin secretory bursts, suggest that studies sampling every 2 min and pulse detection analysis tend to underestimate the frequency, when secretory bursts tend to overlap [13, 15, 33, 74, 121]. Portal-vein sampling in dogs [27, 69], and in humans [73], shows a frequency of secretory bursts of approximately 6 min per pulse. A similar frequency is detected using a new insulin ELISA technique on minutely sampled peripheral blood, and using deconvolution [35]. It thus appears that refined sampling protocols and highly sensitive analytical tools, yield a periodicity of about 6 min. This is similar to the frequency observed in the isolated perfused pancreas [8–10, 39, 116] and suggests that the pacesetting mechanism resides within the pancreas and is not a circulating or central nervous system controlled mechanism and that frequency could be reduced by in vivo operating mechanisms. Yet it has still convincingly been shown in other studies, that a periodicity of about 13 min occurs at some states (see Table 1), and the question of periodicity is not well established.

Overall contribution. To assess the contribution of pulsatile to overall insulin secretion it is necessary to measure or calculate the pulsatile and time-invariant components of insulin secretion rates. In vivo, the insulin secretory rates are usually determined by use of deconvolution of peripheral insulin or C-peptide concentrations. The accuracy of estimated secretion could be improved by sampling from the portal vein. Inserting catheters into the portal vein upstream and downstream of the pancreatico duodenal vein in dogs has been used to directly measure crosspancreatic insulin changes (insulin secretion) (Fig. 4; Fig. 5). Combined with pulse detection analysis to allow separation of insulin release into basal and pulsatile release shows that at least 70 % of all insulin is released during distinct punctuated insulin secretory bursts [69]. Application of a mathematically independent analysis, employing deconvolution of the portal-vein insulin concentration time series (using measured portal-vein insulin kinetics) confirms the estimate that at least 70 % of insulin secretion arises from the pulsatile component of insulin secretion [69]. The similarities of portal-vein insulin concentration oscillations in dogs [27, 69] and humans [73] suggest a similar contribution in humans. After improvement of sampling techniques, insulin assays [122] and statistical analysis [35] a similar resolution from deconvolution of peripheral insulin concentration time series into mainly high frequency insulin secretory bursts and little (if any) non-pulsatile secretion is reported [35].

It therefore appears that in vivo, insulin secretion arises from a punctuate (likely Gaussian distributed) inter-islet coordinate high frequency (~ 5–8 min/pulse) series of secretory bursts, that dominates the overall insulin release. The similarity to in vitro secretory patterns indicates that the in vivo observed pattern is a summation of islet activity reported in vitro.

The neuronal control of in vivo pulsatile insulin secretion

Observation of in vivo pulses implies that most beta-cells secrete in pulses and at the same time. In vitro studies on individual beta-cells show that they secrete in pulses [109], and that the pulsatile release pattern is probably linked to cyclic glycolysis [107], with cyclic generation of lactate [107], cyclic oxygen consumption [105], resulting in beta-cell depolarization through ATP-dependent potassium channels, causing influx of calcium, and subsequently (ATP-dependent) exocytosis as reviewed in [112] and [123]. Thus the necessary mechanisms for the pulsatile release is within the individual beta-cells [112, 124], and the ability to act as pacemaker resides within the beta-cell [115]. A single islet consists of hundreds to a few thousands of individual beta-cells, along with α-cells and δ-cells. This cell population has also got a common and coordinate pulsatile release pattern of insulin [102, 105, 119, 125], and therefore intra-islet coordination of release from the individual beta cells must occur. This coordination could be through electro-physiological coupling among beta cells [126]. Studies on intracellular calcium show that an intra-islet spreading of the calcium influx is observed following an increase in intracellular calcium in a beta cell [104, 127, 128]. Alternatively, an agent that can diffuse between cells provides intra-islet synchronization of insulin release. Still an inter-islet coordinating mechanism is needed for in vivo pulsatile insulin secretion. The observation that insulin secretion from the isolated perfused pancreas is pulsatile has led to the hypothesis of an intra pancreatic pacemaker [9] that initiates the secretory pulses, probably through an extensive intra pancreatic neuronal network.

To examine the possible role of intrapancreatic nerves in regulating the coordination, various nerve blockers have been infused in the isolated perfused pancreas to observe whether pulsatile release pattern was disturbed. A significant impairment of the pulsatile release was observed when infusing post-synaptic nicotinic receptor antagonists (hexamethonium, α-bungarotoxin, or curarine) in the isolated perfused pancreas [129], indicating an intra-pancreatic coordinating role of ganglionic nicotinic receptors. This is supported by the deterioration of pulsatile insulin release by infusion of the nerve blocker tetrodotoxin [116]. In addition, an intrinsic intra-islet neuronal controlling mechanism has been postulated based on increased frequency of insulin pulses from single islets exposed to tetrodotoxin [130]. The presence of preserved pulsatile insulin secretion after pancreas transplantation, albeit at increased frequency, further indicates the presence of an intra-pancreatic coordinating mechanism [75]. In vivo, cholinergic, alpha-adrenergic and beta-adrenergic blocking agents did not perturb the detected periodicity in humans [131] or in monkeys [76], thus failing to support significance of these receptors on controlling pulsatile insulin release in vivo.

A different strategy has been the transplantation of islets into the liver, where re-enervation of islets is known to occur after 4 to 14 weeks [132, 133], to study the release pattern from islets dispersed throughout the liver at different times along the time-course of re-innervation [30]. In this study, 2000 islets were transplanted using the intra-hepatic method, by intra-portal injection, resulting in dispersed islets. Open loop perfusion studies on the insulin secretion from these islets were done at 2, 7, 28 and 200 days, and showed no coordinate release at 2 (1/5) and 7 (0/5) days whereas, along with beginning re-innervation at 28 days, two out of five livers released insulin as coordinate pulses. In contrast, at fully established re-innervation all 12 livers released insulin as common coordinate insulin secretory bursts [30]. This indicates that innervation is a possible mechanism for intra-islet coordination.

In addition, the overall insulin secretion could be regulated by nerve blockers [76, 131] and neurotransmitters such as galanine [134], epinephrine [135, 136], GABA [137] dopamine [138], as well as nitric oxide [139], and because overall insulin release is pulsatile, the impact of these agents is probably involved in the regulation of pulses, although they have not been studied. In addition, sensory nerve blocking markedly increases insulin release [140], suggesting an inhibitory role of sensory nerves, and this could explain the loss of auto-feedback inhibition of insulin on insulin release in humans with pancreas transplant [141].

It appears that some intra-islet and inter-islet coordination is necessary for in vivo pulsatile insulin secretion, and that the intra-islet electro-physiological coupling provides a mechanism for the former, whereas intra-pancreatic neuronal coordination probably governs the inter-islet coordination. Therefore, the neuronal input probably has a pacemaker and coordinator function.

Metabolic control of in vivo pulsatile insulin secretion

The concept of the beta cell as a fuel sensor [142] imports significance to the mechanism by which glucose exerts its effects on the in vivo insulin release processing. In vitro studies suggest an increase in secretory burst mass by glucose with no impact on the secretory burst frequency [115, 118]. Examining data on in vitro insulin release at increasing glucose concentrations indicate increasing pulsatile release, whereas the basal release could be constant and glucose-unaffected [115, 118]. The mechanism by which glucose stimulates insulin release seems to involve cyclic glycolysis

[105, 107], which generates oscillating intracellular concentrations of ATP [143], closure of ATP-dependent potassium channels and depolarization [120], increase in intracellular calcium [104, 105, 127], and subsequently an (ATP-dependent ?) exocytotic process. The cyclic glycolysis involves a positive feedback stimulation of phosphofructokinase by its product, fructose-1,6-biphosphate [112]. It seems plausible that similar mechanisms could be involved in vivo, as indicated from impaired pulsatile secretion in phosphofructokinase defects [144], implying that effects on secretory burst mass, but not frequency, are to expected. However, in dogs [27], the frequency of insulin secretory burst has been reported to increase with hyperglycaemia (7–8 min/pulse to ~ 5 min/pulse), along with a much more pronounced increase in the secretory burst mass by us, whereas others find similar frequencies and marked increase in amplitude after, rather than before, meal ingestion [90]. The increase in frequency in vivo from 7–8 min/pulse at basal state to 4–5 min/pulse at hyperglycaemia observed by us, compared with similar frequencies at different levels of hyperglycaemia (~ 4 minutes/pulse) observed in vitro could be due to an in vivo operating system dampening basal frequency to every 7–8 min per pulse, allowing the interval to shorten to every 4–5 min at hyperglycaemia. If this frequency dampening operating system is not present in vitro, this would explain the difference in frequency generally reported when comparing in vivo with in vitro release of insulin. In addition, because pulse detection ability could decrease with increasing pulse frequency, hyperglycaemia in Type II diabetes mellitus could be involved in the differences in detected pulsatile secretion. An interesting classic feed-back loop that involves glucose and insulin could also be involved in the generation of normal in vivo pulsatile insulin secretion, apart from the mechanisms described above. It has been shown that an ultradian oscillatory glucose infusion could entrain oscillatory insulin release [51]. In vitro studies on rapid entrainment show that even the rapid pulsatile insulin secretion could be induced by a rapid oscillatory glucose infusion in the isolated perfused rat pancreas [39], and in the isolated rat islets [102]. In vivo, a similar ability of punctuate glucose infusions to control pulsatile insulin secretion has been reported [145, 146]. Studies using square wave infusions of modest doses of glucose (6 mg/kg/min over 1 min) could induce a very pronounced pulsatile insulin release, with little or no breakthrough insulin secretion. Thus, the release process is entirely controlled by these rather small changes in circulating glucose concentration (~ 0.3 mmol/l) (Fig. 6). Furthermore, changes in frequency of the glucose induction control the frequency between 7 to 12 min per pulse [146]. This observation, that small doses of glucose could determine the frequency of pulsatile insulin release, seems contradictory to the hypothesis of a pacemaker control that initiates the pulses. However, it appears possible that the hypothesized specialized neuronal pacemaker is also under the influence of glucose changes. This is supported by the observation that frequency increases with hyperglycaemia.

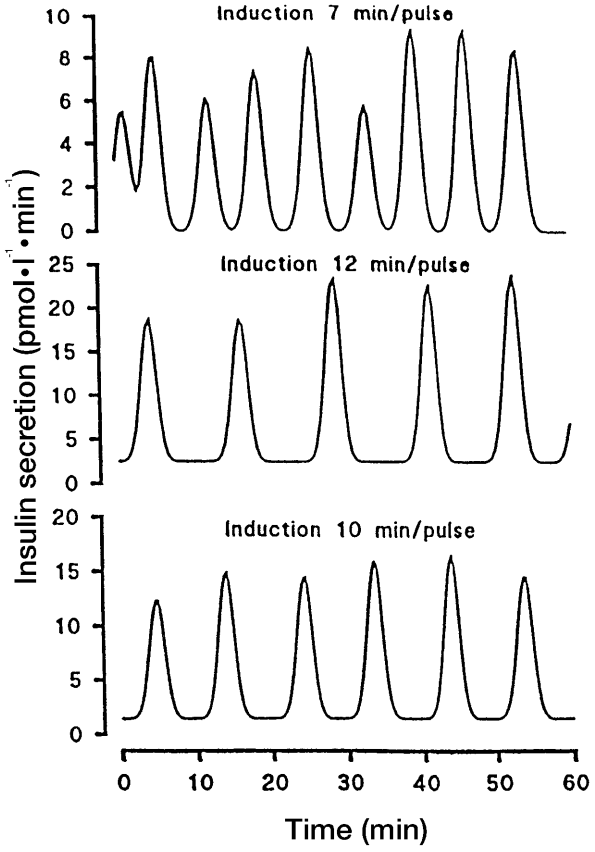

Fig. 6. Calculated (deconvolved) pulsatile insulin secretory rates when entraining insulin pulsatility by glucose infusion every 7 (top panel) 12 (middle panel) or 10 (bottom panel) min. Note again, the apparent minimal insulin secretion between pulses and that each glucose infusion is followed by an insulin pulse, in this case with no false positive or false negative pulses

In addition to glucose, other nutrients such as amino acids and fatty acids are important for both insulin secretion and overall fuel homeostasis. Whereas the impact of various amino acids on overall insulin secretion has been studied, no studies have addressed the impact on pulsatility. Because the mechanism for stimulation could vary among amino acids, in vivo studies are further troubled as infusion of amino acids usually results in stimulation of growth hormone and glucagon secretion, which could exert confounding effects.

Non-esterified fatty acids are known to stimulate insulin secretion in vitro following short-term exposure, but inhibiting glucose-stimulated insulin release after long-term exposure [147]. Long chain non-esterfied fatty acids influence the release of insulin related to oxidation but also serve as a metabolic coupling

factor (together with malonyl-CoA) for the regulation of glucose-stimulated insulin release [143, 148].

If non-esterified fatty acids potentiate the response of beta-cell secretory response to changes in glucose, an improved insulin release to glucose concentration feed back mechanism would be expected with increased concentrations of non-esterified fatty acids. In contrast, to the extent that non-esterified fatty acids compete with glucose for oxidation, an impaired ability to respond to glucose oscillations could occur. The pulsatile secretory pattern was preserved in obesity (with elevated non-esterified fatty acids), with similar relative amplitude of insulin oscillations [149], although a relation between waist-to-hip ratio and frequency of pulsatile insulin secretion suggests a role for the pulse frequency for insulin action [15].

Recent data on pulsatile insulin release in patients with Type II diabetes compared with healthy subjects, linked the orderliness of the release pattern estimated by approximate entropy, to the concentration of non-esterified fatty acids, and in both healthy subjects and in Type II diabetic individuals the release orderliness correlated positively with the concentrations of non-esterified fatty acids [150]. This, together with data in fasting and obesity, mentioned above, strongly suggest lipotoxicity is not a mechanism for impaired pulsatile release of insulin repeatedly reported in Type II diabetes, but could even suggest a role for non-esterified fatty acids to improve the pulsatile release pattern, possibly through improved sensitivity to oscillating glucose concentrations.

Non-esterified fatty acids also show an oscillatory pattern in dogs [151] and in humans [152]. This could provide a feedback mechanism for insulin to non-esterified fatty acid oscillatory coupling system. Using glucose infusions to induce pulsatile insulin secretory bursts at determined intervals shows independence of the oscillations in non-esterified fatty acids from insulin oscillations. Moreover, in long-term fasting there is a discrepancy between detected frequencies of 4 min per pulse for non-esterified fatty acids and 6–7 min for glucose and insulin. This indicates that there is probably another oscillatory system, possibly adrenergic/sympathetic, that controls the rapid changes in non-esterified fatty acids.

In addition, synchronous fluctuations have been shown of plasma insulin and lactate concentrations at a periodicity of about 85 min per oscillation.

Hormonal control of in vivo pulsatile insulin secretion

The pulsatile pattern of release seems to be important not only for insulin but also for the pituitary [100, 153, 154], adrenal [155] and parathyroid [156] hormones, for GLP-1 [157], leptin [158], and other islet hormones [4, 6, 9, 10, 84]. Because both growth hormone and the islet hormones are important for metabolic control, an interplay between the hormones might be necessary to optimize their release and action. Such interplay could involve actions on the secretory profiles of the other hormones in the same way as for IGF-1 on growth hormone [154]. Numerous hormones are important for controlling insulin release. Somatostatin secreting δ-cells within the population of insulin secreting cells allows paracrine influence on insulin release. Somatostatin inhibits insulin secretion through the amount of insulin released per secretory burst, leading to reduced insulin pulses in the portal vein [28]. The latter seems to be important for the relative extraction of insulin in the liver. This probably reflects the binding of insulin to its receptors, because a linear relation of insulin amplitude to hepatic insulin extraction was observed [28]. In contrast, somatostatin has no impact on the frequency of secretory bursts, either at basal, or at postprandial conditions [28].

Like somatostatin, GLP-1 is a gut hormone, secreted upon luminal stimuli, and acting on beta cells to regulate the prandial insulin profile. It is a potent secretagogue that could become a future therapeutic agent for treatment of Type II diabetes. GLP-1 is considered a beta-cell glucose sensitizer that exerts actions together with increased glucose concentrations [159]. The mechanism for stimulating the release of insulin is through amplification of secretory burst mass which does not, however, affect the frequency and orderliness of the secretory process [34] (Fig. 7). This is in line with the expected actions of a hormone that sensitizes the beta cell to glucose. It is important that a possible therapeutic agent does not impair pulsatility. Of possible therapeutic importance is the demonstration of preserved action in Type II diabetes with amplification of the insulin secretory burst mass at constant low dose infusion of GLP-1.

The pituitary-islet interaction is represented by IGF-1 that mediates growth hormone actions. The existence of IGF-1 receptors on beta cells suggests a role for IGF-1 in modulating insulin secretion, whereas inhibition of hepatic production of IGF-BP1 by insulin increases free IGF-1 concentrations, creating what seems to be a negative feedback loop between these structurally similar hormones. In humans, IGF-1 is shown to act on insulin secretion by inhibition of the secretory burst mass, and with no impact on secretory burst frequency [29], which is analogous to the effects of IGF-1 on pulsatile secretion of growth hormone [154].

Finally, angiotensin II suppresses insulin secretion by reducing insulin secretory burst mass without affecting the regularity as assessed by approximate entropy or the frequency [160]. Therefore, the hormones studied so far all exert their effects by regulating the secretory burst mass but numerous other hormones not studied could have different actions on the insulin release process.

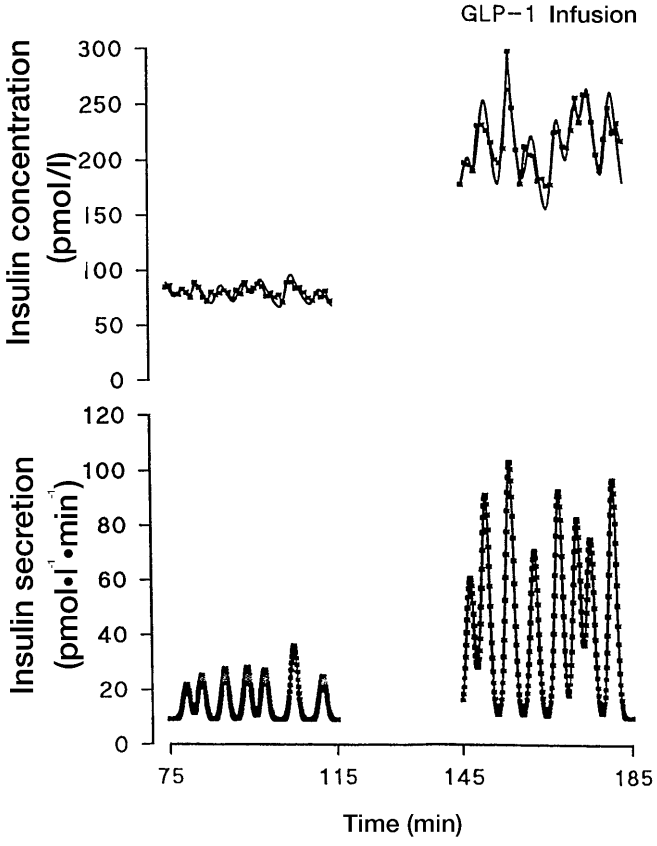

Fig. 7. An example of how overall insulin secretion is regulated via changes in the pulsatile component, in this case before (left panels) compared with during (right panels) infusion of the insulinotropic hormone GLP-1. The top panels show concentrations and best fit curves from deconvolution, whereas the bottom panels show calculated secretory rates. Note the massive amplification of the insulin secretory burst amplitude, with no apparent change in frequency

Impact of drugs on insulin pulsatility

Because pulsatile secretion could be important for both insulin action and beta-cell functions, the impact of sulphonylurea on the secretory pattern is important. In humans, an amplification of peripheral insulin oscillations with no change in frequency has been reported [131]. In a canine model, tolbutamide infusion results in a massive amplification of secretory burst mass with no effects on frequency or basal secretion and with no deterioration of the pulsatile pattern [32]. This seems important because a deterioration of the physiological release process is not desirable for a therapeutic agent for Type II diabetes, as mentioned for GLP-1 above. Of note, drugs that exert actions directly on the ATP-dependent potassium channels bypassing the glycolytic steps involved in pulsatile secretion, do not seem to impair the coordination of secretion of the about one million intra-pancreatically dispersed islets. The preserved or even improved pulsatile secretion during tolbutamide infusion strongly suggests that the in vivo coordinating mechanisms are strong and override the stimulus of individual islets. The influence of sulphonylurea is yet to be established in Type II diabetes. A novel non-sulphonylurea insulin secretagogue, repaglinide, as tolbutamide amplifies the secretory burst mass, without affecting the frequency of insulin oscillations [161]. Similarly, GLP-1 infusion in Type II diabetic individuals improves the secretory burst mass thus suggesting therapeutical options for improvement of pulsatile insulin release in Type II diabetes.

Other oral anti-diabetic drugs act as insulin sensitizers, reduce the insulin need and improve metabolic control. The effects of these drugs on insulin secretion are probably indirect but could mirror the influence of insulin resistance and metabolic control on beta-cell performance. No studies on these factors have been done but ultradian pulses have been shown to improve in periodicity in Type II diabetic patients treated with troglitazone [162]. Furthermore, in vitro studies on entrainment of rapid pulsatile secretion from pancreases of Zucker rats, clearly show improved responsiveness of the beta cells to the glucose oscillations by pioglitazone [38].

Pulsatile insulin release in diabetes and prediabetes

In Type II diabetes mellitus, the secretion of insulin is impaired, and this, together with insulin resistance, characterizes the metabolic derangement of the disease. The defective release of insulin involves reduced diurnal oscillations [57], impaired ultradian oscillations [49], and their reduced entrainment [46] as well as impaired rapid pulsatile secretion [37]. The resulting hypothesis of impaired insulin oscillations as a primary beta-cell defect in Type II diabetes was supported by the observation of defective oscillatory insulin release in first degree relatives of such patients [43]. Still, secretory dysfunction in both Type II diabetes and in glucose intolerance could be secondary to the glucose toxicity [2].

To further investigate if beta-cell dysfunction could be primary in Type II diabetes and parallel defects need to be identified in the complex cascade of metabolic events involved in pulsatile insulin secretion. To detect early defects, a test that examines subtle impairments seems necessary. It has to be superior to the intravenous glucose tolerance test that evaluates the response to a sudden rise in plasma glucose from about 5 mmol/l to 25 mmol/l [2]. Of note, increased disorderliness of insulin release accompanied by attenuated secretory burst mass is observed in older individuals, linking ageing to beta-cell dysfunction and suggesting that the defects that occur in Type II diabetes could be linked to ageing [63, 163].

Minimal repeated glucose infusions used to test for the adaptability of pulsatile insulin secretion to peri-

odical trivial glucose excursions have been shown to improve markedly the ability to separate abnormal pulsatile insulin release in Type II diabetic individuals from that of matched control subjects by both autocorrelation analysis, spectral analysis, approximate entropy [164] and by spectral analysis [145]. These findings support the use of more refined methods for the prediction of apparent beta-cell dysfunction in Type II diabetes mellitus and genetically predisposed individuals, where more simple insulin secretion tests have little predictive value when using first phase insulin secretion [165]. The loss of regular pulsatile insulin patterns in Type II diabetes mellitus as well as in persons with slightly increased glucose concentrations could be due to an increase in frequency [27, 166] and a decrease in relative amplitude [31]. Similarly, increased frequency [167] as well as decreased regularity measured by approximate entropy [168] has been observed more frequently in obesity and in states of increasing insulin resistance [15].

Conclusion

The detection of high-frequency insulin oscillations corresponds to serial secretory insulin bursts. These bursts are the dominant mechanism for insulin secretion at basal states and the release of insulin is regulated through changes in frequency and/or mass of these secretory bursts. This secretory pattern is similar to that of the isolated pancreas, isolated islets and metabolic events in individual beta cells. The mechanism governing the coordination of in vivo pulsatile insulin secretion from the million islets scattered throughout the pancreas is believed to be neurogenic. However, the presence of plasma glucose oscillations preceding that of insulin indicates that glucose could control the pulsatile pattern in vivo, supported by entrainment of oscillatory insulin secretion in islets, the isolated perfused pancreas and in vivo in response to episodic glucose infusions.

Changes in the secratory pattern of insulin are of major importance in the development of Type II diabetes, either as a very early marker of beta-cell dysfunction, or as an aetiologically inherited beta-cell defect. The analysis of the mechanisms responsible for the development of pathological pulsatile insulin-secretory patterns of Type II diabetes should give a better insight into the causes of the beta-cell dysfunction in this disease and associated genetically inherited defects.

Sources. This review is based on the relevant literature published in English during the period 1922 to 2001. The sources available to the author were integrated with sources identified through PubMed searches, using searches for "insulin and oscillations", "insulin and pulsatility or pulsatile", "pulsatile secretion", "calcium oscillations", and "oscillations and secretion".

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