Section 6: Pulsatile and Phasic Insulin Release in Normal and Diabetic Men

Pulsatile Insulin Secretion: Detection, Regulation, and Role in Diabetes

Niels Pørksen,[1] Malene Hollingdal,[1] Claus Juhl,[1] Peter Butler,[2] Johannes D. Veldhuis,[3] and Ole Schmitz[1]

Insulin concentrations oscillate at a periodicity of 5–15 min per oscillation. These oscillations are due to coordinate insulin secretory bursts, from millions of islets. The generation of common secretory bursts requires strong within-islet and within-pancreas coordination to synchronize the secretory activity from the β-cell population. The overall contribution of this pulsatile mechanism dominates and accounts for the majority of insulin release. This review discusses the methods involved in the detection and quantification of periodicities and individual secretory bursts. The mechanism by which overall insulin secretion is regulated through changes in the pulsatile component is discussed for nerves, metabolites, hormones, and drugs. The impaired pulsatile secretion of insulin in type 2 diabetes has resulted in much focus on the impact of the insulin delivery pattern on insulin action, and improved action from oscillatory insulin exposure is demonstrated on liver, muscle, and adipose tissues. Therefore, not only is the dominant regulation of insulin through changes in secretory burst mass and amplitude, but the changes may affect insulin action. Finally, the role of impaired pulsatile release in early type 2 diabetes suggests a predictive value of studies on insulin pulsatility in the development of this disease. *Diabetes* 51 (Suppl. 1): S245–S254, 2002

In 1922, Karen Hansen (1) examined serial measurements of blood glucose concentrations and reported rapid and slower oscillations in the peripheral concentrations of this substrate. Half a century later, rapid oscillations in the peripheral insulin concentrations (2–6) and the islet hormones glucagon (3,5,7,8) and somatostatin (8–10) were demonstrated. Numerous studies have reported the importance of this release pattern for optimal insulin action (9,11–19,20–25), for overall insulin secretion (26–35), and for possible development of disease (36–43). Studies on pulsatile insulin secretion may therefore be important to appreciate how overall insulin release is regulated and how the underlying mechanisms can impact insulin action. Having established the physiology of pulsatile insulin release processes, this knowledge may be employed to search for defects in disease states, and on a mechanistic basis to evaluate different hypotheses of the etiology 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 2 diabetes mellitus (44,45,47,48,50,53) and early type 1 diabetes mellitus (54). Finally, diurnal (meal-related) oscillations are an apparent (55–59) and probably important (44) 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 the important aspects of oscillatory β-cell function reported in vitro.

Assessment of pulsatile insulin secretion. The purpose of assessment of pulsatile insulin secretion may differ, and hence the method for analysis may be chosen according to what information is being sought. Secretion is determined from concentrations of either insulin or C-peptide and accurate measurements with minimal noise are therefore crucial. Insulin is secreted into the portal vein circulation and undergoes partial (40–80%) hepatic extraction before dilution into the systemic insulin pool (60–63). In animal models, acutely and chronically implanted portal vein catheters have been used for sampling purposes (31,64,65). Sampling downstream the pancreaticoduodenal vein may increase pulse amplitude to 100–500% (31,64) versus 30% in the peripheral circulation. Most human studies will for obvious reasons rely on peripheral sampling.

Sampling intensity and duration. Sampling every minute (5,6,9,13,27,28,30–33,38,39,41,64,66) or every second minute (4,15,21,26,67–69) for 1–2 h has been used 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 demonstrate a decrease in

From the [1]Department of Endocrinology and Metabolism M, Aarhus University Hospital, Aarhus, Denmark; the [2]Department of Endocrinology and Diabetes, University of Southern California, Los Angeles, California; and the [3]Department of Medicine and NSF Center for Biological Timing, Charlottesville, Virginia.

Address correspondence and reprint requests to porksen@dadlnet.dk.

Accepted for publication 21 June 2001.

GLP-1, glucagon-like peptide-1; IGF-1, insulin-like growth factor-1; LH, leutinizing hormone.

The symposium and the publication of this article have been made possible by an unrestricted educational grant from Servier, Paris.

pulse detection when reducing the sampling intensity from 60 s per sample (31,64), and the referenced publications tend to detect fewer pulses when sampling every second is used versus every minute (Table 1), whereas sampling every 30 s (31,64) failed to improve pulse detection. The duration of sampling may also interfere with the ability to estimate the characteristics of pulsatile insulin release. Most studies are time and cost consuming, and the ideal sampling duration should be the shortest duration to give reliable data. With deconvolution analysis and pulse detection analysis, 40–60 min (64) seems to result in good data whereas regularity statistics (autocorrelation [minimum two times pulse interval] and spectral analysis [$N > 100$]) as well as regularity statistics (approximate entropy: $N > 40$) improve with increased sampling duration (5,6,9,26,67).

Insulin versus C-peptide measurements. Insulin and C-peptide are coreleased in a one-to-one molar ratio (75), but insulin undergoes substantial (40–80%) and variable (60,62,76,77,79) hepatic insulin extraction (reviewed in ref. 78). C-peptide is not cleared by the liver (79). The hepatic insulin extraction favors the use of C-peptide to calculate the overall insulin secretory rates but the longer half-life of C-peptide (~35 min) (80) versus insulin (5–8 min) (33) favors the use of insulin to examine pulsatility, since the longer half-life of C-peptide will dampen oscillations and decrease signal-to-noise ratio oscillations correspondingly.

Assay and biological noise. Sampling and assaying will add noise to the biological signals. During and after sampling, hemolysis may occur, and proteases may degrade insulin in syringe and tube, which is referred to as biological noise. The measurement of the insulin concentration is further subject to assay variability, and a reliable, sensitive, and specific assay is crucial in order to minimize the variability due to measurements.

Analytical strategy. The problem with biological time series is often the presence of *1*) trends and *2*) noise.

Trends and subtrends. The purpose of analysis of time series may differ and the analytical strategy should be chosen to address the question being asked. Some statistics require that data must be detrended to avoid the influence of subpatterns usually accounted for by the use of a high-pass filter: first differencing, or analysis of residuals after subtraction of a fitted line employing linear (regression), moving average or other models to fit the data curve. Noise may be filtered by a low-pass filter (i.e., smoothing procedures), where the chosen window lengths and weighing of data points may be important for the outcome. The detrending procedures and smoothing procedures will affect the specific patterns in the data that will be examined, and care should be taken to assure that biological variability (i.e., biological oscillations) is not dismissed due to the smoothing and detrending.

Pulse detection. Analysis of peripheral insulin concentrations may 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 has been based on predetermined statistical criteria to allow concentration changes to be considered significant, and this approach is the basis of commonly used and well-established methods, such as pulsar (7,15,71,81) and cluster analysis (64,74,82) and ULTRA (68,69,83). However, each concentration change per se must pass significance criteria for detection, and relative low amplitude biological signals may not be detected, as may be seen when increased frequency causes overlapping pulses. The methods quantify the secondary concentration events, but not secretion.

Periodicity analysis. The frequency of the insulin pulses may be estimated by spectral analysis or autocorrelation analysis that evaluates the concentration time series for regular periodicities. Spectral analysis may be rather robust to noise, since more random data variability will likely not influence the detected periodicity. However, the method, as commonly used, is not directly suitable to quantify the significance of any periodicity with a single value. Autocorrelation analysis examines replicability of patterns and will give frequency of concentration changes, significance for this pattern, and a correlation coefficient. However, these two methods are restricted to evaluate periodicities and do not directly give information on secretion. Lack of significant periodicities does not exclude the presence of a more irregular pulsatile release pattern or preserved regularity with impaired relative amplitude.

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 application of approximate entropy (41,84–86), which measures the logarithmic likelihood that runs of patterns are reproduced on the next incremental comparison. The method has proven useful in a number of studies on hormonal secretion including growth hormone (87), luteinizing hormone (LH) (88), aldosterone (89), together with cortisol and ACTH (90,91), and recently for analysis of pulsatile insulin secretion (41,92) to discriminate pathophysiology versus health. The method is robust to noise and absolute differences in data. Derived values from approximate entropy analysis do not provide information on the variables involved in pulsatile insulin release, i.e., basal secretion, pulse mass, etc.

Detection and quantification of pulsatile insulin secretion by deconvolution. Insulin concentration data can be deconvolved to detect and quantify insulin secretory bursts, as described in ref. 93. Insulin secretion calculated by the use of C-peptide concentrations is more robust to intra- and interindividual variation in kinetic parameters (80). When performing deconvolution analysis, it is important to adjust basal secretion to allow accommodation of most troughs. Likewise, the secretory burst half duration should be adjusted to fit individual obvious secretory bursts, consisting of series of data points building up to a peak and down to a trough. Using deconvolution (93) involves subjective elements, although defined peaks are tested for significance, and all data analysis must be performed in a blinded manner. The technique requires no assumptions on frequency, amplitude, and stationarity.

Contribution of pulsatile insulin release to the overall insulin secretion. Peripheral insulin concentrations oscillate (2–6) due to pulsatile insulin secretion (4), but to appreciate this, the net contribution of the secretory

TABLE 1
The impact of sampling site, sampling intensity, species, peptide measured, and analytical strategy on detected pulse frequency

First author	Reference	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	(3)	*Science*	1977	Baboon	Fasted	Insulin	Peripheral vein	1 min	Autocorrelation	9
Lang	(6)	*N Engl J Med*	1979	Human	Fasted	Insulin	Superior caval vein	1 min	Autocorrelation	13
Hansen	(67)	*Am J Physiol*	1981	Baboon	Fasted	Insulin	Peripheral vein	2 min	Autocorrelation, spectral analysis	
Hansen	(26)	*Am J Physiol*	1982	Baboon	Fasted+ postprandial	Insulin	Peripheral vein	2 min	Autocorrelation, spectral analysis	9–12
Lang	(5)	*Diabetes*	1982	Human	Fasted	Insulin	Peripheral vein	1 min	Autocorrelation	11–13
Goodner	(70)	*Science*	1982	Baboon	Fasted	Insulin	Artery	2 min	Autocorrelation	9
Jaspan	(71)	*Am J Physiol*	1986	Canine	Fasted	Insulin, C-peptide	Portal and hepatic	1–2 min	Spectral analysis	10–14
Matthews	(9)	*Endocrinology*	1987	Canine	Fasted, halothane	Insulin	Portal vein	1 min	Autocorrelation	10?–30
O'Rahilly	(39)	*N Engl J Med*	1988	Human	Fasted	Insulin	Peripheral vein	1 min	Autocorrelation, Fourier	13–14
Chou	(72)	*Life Sciences*	1991	Rat	Fasted	Insulin	Peripheral vein	3 min	Clifton	13
Goodner	(73)	*Am J. Physiol*	1991	Baboon	Fasted, sedation	Insulin	Peripheral vein	2 min	Clifton	10
Peiris	(21)	*J Clin Endocrinol*	1992	Human	No	Insulin	Peripheral vein	2 min	Pulsar	7–12
Balks	(74)	*J Clin Endocrinol Metab*	1992	Human	Basal and postprandial	Insulin, C-peptide	Peripheral vein	2 min	Deconvolution and cluster	12
Storch	(66)	*Dtsch Med Wochenshr*	1993	Human	Basal and postprandial	Insulin, C-peptide	Portal vein	1 min	Cluster	4.1–6.5
O'Meara	(69)	*Am J Physiol*	1993	Human	Fasted	Insulin, C-peptide	Peripheral vein	2 min	Ultra, pulsefit, cluster, autocorrelatin, spectral analysis	11
Porksen	(64)	*Am J Physiol*	1995	Canine	No	Insulin	Portal and periheral veins	1–4 min	Cluster, deconvolution	6.5–9
Engdahl	(13)	*J Appl Physiol*	1995	Human	No	Insulin	Peripheral vein	1 min	Deconvolution	10
Porksen	(30)	*Diabetes*	1996	Canine	Yes	Insulin	Portal vein	1 min	Deconvolution	5–7
Hunter	(15)	*Diabetes*	1996	Human	No	Insulin	Peripheral vein	2 min	Pulsar	7–8
Porksen	(33)	*Am J Physiol*	1997	Human	Yes	Insulin	Peripheral vein	0.5–3.0	Deconvolution	4.7

bursts is important, since time-invariant release may be dominant despite the presence of concentration oscillations. Furthermore, the relation between the in vivo observed secretory bursts and the in vitro observed pulsatile release from the isolated pancreas (8,9,73) and the isolated periperfused islets (94,95), as well as episodic β-cell depolarization (96–98), β-cell glycolysis (97,99), and β-cell increase in calcium remains to be established (97, 98,100,102). As for the coupling of cyclic metabolism to pulsatile in vitro release, this has been addressed in detail in an excellent review by Tornheim (104). Mathematical models have been employed to improve understanding of the mechanisms underlying the coordinate pulsatile release (105,106). Comparison of exact measures of secretory burst shape, duration, frequency, and secretory process orderliness of in vitro and in vivo insulin secretory bursts may further underline the linkage from intracellular β-cell events to large amplitude in vivo pulsatile insulin release.

Configuration. The shape of the individual secretory bursts from individual islets and from in vivo secretion are almost superimposable (corrected for amplitude), suggesting that the in vivo pulse is generated by simultaneous secretion from the intrapancreatic islet population, as is evident when comparing the human in vivo (31,33) and in vitro (97) pulse shape.

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 frequency from the isolated perfused pancreas has been reported to be 6–10 min per pulse (7–9,42,107). Estimates of the latter are not confounded by hepatic insulin extraction, and dilution into the systemic insulin pool and the pulses are thus more easily detectable in this model. Similarly, studies on pancreas-transplanted subjects with shunts from the pancreas to the inferior caval vein show an increase in frequency in pancreas-transplanted subjects versus healthy control subjects (8 vs. 12 min per oscillation) (68). Finally, the reported frequency of 3–5 min per pulse in the isolated perifused islet (68,97,108–110) (10–17 min per pulse in refs. 94,111,112) suggests that there is a hierarchy of regulatory mechanisms with the fastest frequency in individual islets (3–5 min), followed by the perfused pancreas (6–10 min), and finally the in vivo insulin secretion (5–15 min). Studies comparing sampling intensities, sampling sites, and mathematical strategies for the detection of insulin secretory bursts suggest that studies sampling every 2 min may underestimate the frequency, and that analysis of data based on pulses limited by a significant increase and decrease in insulin concentrations in the peripheral circulation will underestimate the frequency of insulin secretory bursts when secretory bursts tend to overlap (15,21,26,69,72). Portal vein sampling in dogs (30,64) and humans (66) reveals a frequency of ~6 min per pulse, which may be detected using better assays to analyze frequently sampled peripheral plasma (33).

Overall contribution. In order 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. Inserting catheters into the portal vein up- and downstream to the pancreaticoduodenal vein(s) in dogs has been used to directly measure cross-pancreatic insulin changes (insulin secretion), and pulse detection analysis reveals that at least 70% of all insulin is released during distinct punctuated insulin secretory bursts (64), which is independently confirmed by deconvolution of portal vein data (64). The similarities of portal vein insulin concentration oscillations in dogs (30,64) and humans (66) suggest a similar contribution in humans. Patients with liver cirrhosis have similar pulsatile insulin release patterns based upon human portal vein sampling (unpublished data). A similar resolution from deconvolution of peripheral insulin concentration time series into mainly high-frequency insulin secretory bursts is reported (33) using new insulin assays.

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

The neuronal control of in vivo pulsatile insulin secretion. As can be seen from the above data, coordination is needed among the islets to permit the release from ~1 million islets scattered throughout the pancreas to appear as common bursts. The observation of in vivo pulses implies that most β-cells secrete in pulses and at the same time. In vitro studies on individual β-cells show that they secrete in pulses (102) and that the pulsatile release pattern is probably linked to cyclic glycolysis (99), with cyclic generation of lactate (18), cyclic oxygen consumption (97), resulting in β-cell depolarization via ATP-dependent potassium channels, causing influx of calcium and subsequently (ATP-dependent) exocytosis (rev. in refs. 104,113). Thus, the necessary mechanisms for the pulsatile release is within the individual β-cells (104,114), and the ability to act as pacemaker resides within the β-cell (109). A single islet consists of hundreds to a few thousand individual β-cells, along with α-cells and δ-cells. This cell population has also a common and coordinate pulsatile release pattern of insulin (94,97,111,115), and therefore intra-islet coordination of release from the individual β-cells must occur. This coordination may be through electrophysiological coupling among β-cells as demonstrated in ref. 116, and studies on intracellular calcium reveal that, following increase in intracellular calcium in a β-cell, an intra-islet spreading of the calcium influx is observed (98,117,118). The observation that insulin secretion from the isolated perfused pancreas is pulsatile has led to the hypothesis of an intrapancreatic pacemaker (8) that initiates the secretory pulses, probably via an extensive intrapancreatic neuronal network.

A coordinating role of ganglionic nicotinic receptors has been postulated from studies on the isolated perfused pancreas with nerve blockers (hexamethonium, α-bungarotoxin, or curarine) (119). Preserved pulsatile insulin secretion after pancreas transplantation (at increased frequency) supports this hypothesis (68), whereas cholinergic, α-adrenergic, and β-adrenergic blocking agents did not perturb the detected periodicity in humans (120) or in monkeys (66).

Transplantation of islets into the liver results in re-

innervation of islets after 4–14 weeks (121,122), and the release pattern from islets dispersed throughout the liver has been studied at different times along the time-course of re-innervation (29), at 2, 7, 28, and 200 days posttransplantation. There was no coordinate release at 2 and 7 days, whereas, along with starting re-innervation at 28 days, two of five livers released insulin as coordinate pulses. In contrast, when re-innervation was fully established, 14 of 14 livers released insulin as common coordinate insulin secretory bursts (29), indicating that innervation is a possible mechanism for intra-islet coordination.

In addition, the overall insulin secretion may be regulated by nerve blockers (67,120) and neurotransmitters as galanine (123), epinephrine (124,125), GABA (126), dopamine (127), as well as nitric oxide activity (128), and, since overall insulin release is pulsatile, the impact of these agents is probably involved in the regulation of pulses, although this has not yet been studied in detail.

Metabolic control of in vivo pulsatile insulin secretion. In vitro studies suggest an increase in secretory burst mass by glucose with no impact on the secretory burst frequency (108,109). Cyclic β-cell glycolysis involves a positive feedback stimulation of phosphofructokinase by its product, fructose-1,6-biphosphate, as reviewed in ref. 104, and impaired pulsatile secretion in phosphofructokinase-deficient subjects is reported (129). In dogs (30), the frequency of the insulin secretory burst has been reported to increase with hyperglycemia (7–8 min/pulse to ~5 min/pulse), along with a much more pronounced increase in the secretory burst mass, whereas similar frequencies and a marked increase in amplitude have been reported after meal ingestion (74). The increase in frequency in vivo from 7–8 min/pulse at basal state to 4–5 min/pulse at hyperglycemia observed by us versus similar frequencies at different levels of hyperglycemia (~4 min/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 hyperglycemia. If this frequency dampening operating system is not present in vitro, this would explain the difference in frequency generally reported when comparing in vivo to in vitro release of insulin. A classic feedback loop involving glucose and insulin may be involved in the generation of normal in vivo pulsatile insulin secretion. Studies on entrainment of the ultradian insulin release pattern show that oscillatory glucose infusion may entrain oscillatory insulin release (52). In vitro studies on rapid entrainment show that even the rapid pulsatile insulin secretion may be induced by a rapid oscillatory glucose infusion in the isolated perfused rat pancreas (42) and in the isolated rat islets (94). In vivo, a similar ability of punctuate glucose infusions to control pulsatile insulin secretion has been reported (130,131). Studies using square wave infusions of modest doses of glucose (6 mg/kg/min over 1 min) may induce a very pronounced pulsatile insulin release, with little or no breakthrough insulin release, so that the release process is entirely controlled by these rather small changes in circulating glucose concentrations (~0.3 mmol/l). Furthermore, changes in frequency of the glucose induction control the frequency between 7 and 12 min per pulse (131). This seems contradictory to the hypothesis of a pacemaker control that initiates the pulses, but it is possible that a specialized neuronal pacemaker is under the influence of glucose changes.

In addition to glucose, amino acids and fatty acids are important for both insulin secretion and overall fuel homeostasis. The impact of amino acids on insulin pulsatility has not been studied. Free fatty acids are known to stimulate insulin secretion in vitro following short-term exposure, but also to inhibit glucose-stimulated insulin release after long-term exposure (132). Free fatty acids influence the release of insulin related to its oxidation, but also serve a role as metabolic coupling factor (together with malonyl-CoA) for regulation of glucose-stimulated insulin release (133,134).

The pulsatile secretory pattern was preserved in obesity (with elevated free fatty acids), with similar relative amplitude of insulin oscillations (135), although a relationship between waist-hip ratio and frequency of pulsatile insulin secretion suggests a role for the pulse frequency for insulin action (21). Recent data on pulsatile insulin release in type 2 diabetic versus in healthy control subjects linked the orderliness of the release pattern estimated by approximate entropy to the concentration of free fatty acids, and in both healthy subjects and type 2 diabetic individuals, the release orderliness correlated positively with the concentrations of free fatty acids (23). This, together with the data in fasting and obesity mentioned above, strongly suggest that lipotoxicity is not a mechanism for impaired pulsatile release of insulin repeatedly reported in type 2 diabetes, but may even suggest a role for free fatty acids to improve the pulsatile release pattern, possibly through enhanced sensitivity to oscillating glucose concentrations. Recent data suggest that free fatty acids also exhibit an oscillatory pattern in humans (136).

Hormonal control of in vivo pulsatile insulin secretion. The pulsatile pattern of release seems important not only for insulin but also for the pituitary (93,137,138), adrenal (139), and parathyroid (140) hormones, for glucagon-like peptide-1 (GLP-1) (141), leptin (142), and other islet hormones (3,5,8,9,143). Numerous hormones are important in controlling the overall release of insulin. The colocalization of the somatostatin-secreting δ-cells within the population of insulin-secreting cells may enable a paracrine influence on the insulin release. The mechanism by which somatostatin inhibits insulin secretion is through the amount of insulin released per secretory burst, leading to reduced insulin pulses in the portal vein (32). The latter seems to be important for the relative extraction of insulin in the liver, which likely reflects the binding of insulin to its receptors, since a linear relationship of insulin amplitude to hepatic insulin extraction was observed (32). In contrast, somatostatin has no impact on the frequency of secretory bursts, either at basal or postprandial conditions (32). Like somatostatin, GLP-1 is a gut hormone secreted upon luminal stimuli and acting on β-cells to regulate the prandial insulin profile. It is considered an important and potent secretagogue that (as an analogue) could be a future therapeutic agent for the treatment of type 2 diabetes. GLP-1 causes amplification of the insulin secretory burst mass, whereas frequency and orderliness of the secretory process is not affected (27). This is in line with

the expected actions of a hormone that sensitizes to prevailing glucose concentrations and is of importance for a possible therapeutic agent, since deterioration of the secretory process by induction of disorderliness or impairment of the pulsatility would impact negatively on β-cell performance. Of possible therapeutic importance is the demonstration of preserved action in type 2 diabetes with amplification of the insulin secretory burst mass at constant low-dose infusion of GLP-1 (144). The pituitary-islet interaction is represented by insulin-like growth factor-1 (IGF-1) with the presence of IGF-1 receptors on β-cells. In humans, IGF-1 is shown to act on insulin secretion via inhibition of the secretory burst mass, and with no impact on secretory burst frequency (28), which is analogous to the effects of IGF-1 on pulsatile secretion of growth hormone (137).

Impact of drugs on insulin pulsatility. Since pulsatile secretion may be important for both the action of insulin and for appropriate β-cell function, the impact of sulfonylurea on the secretory pattern is important. In humans, an amplification of peripheral insulin oscillations with no change in frequency has been reported (120), and, in dogs, 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 (34). This seems important, since a deterioration of the physiological release process is undesirable for a therapeutic agent for type 2 diabetes. It is also of note that drugs that exert actions directly on the ATP-dependent potassium channels, whereby the glycolytic steps involved in pulsatile secretion are bypassed, do not seem to impair the coordination of secretion from ~1 million intrapancreatically dispersed islets. The preserved or improved pulsatile secretion during tolbutamide infusion strongly suggests that the in vivo coordinating mechanisms are strong and override the stimulus on individual islets. Similar effects are observed for the nonsulfonylurea secretagogue repaglinide (145), as is seen during GLP-1 infusion in type 2 diabetic individuals, suggesting therapeutic options for improving defects in pulsatile insulin release in type 2 diabetes.

Other oral antidiabetic drugs act as insulin sensitizers to reduce the insulin requirements and to improve metabolic control. Ultradian pulses are shown to improve in type 2 diabetic subjects treated with the glucose sensitizer troglitazone (146). Furthermore, in vitro studies on entrainment of rapid pulsatile secretion from pancreata of Zucker diabetic fatty rats clearly demonstrate improved responsiveness of the β-cells to the glucose oscillations in rats treated with pioglitazone (43), thus indicating a role for persistent increased demand on β-cell performance in the development of pulsatile release defects.

Pulsatile insulin release in diabetes and prediabetes. In type 2 diabetes mellitus, the secretion of insulin is impaired, which, together with insulin resistance, characterizes the metabolic derangement of the disease. The defective release of insulin involves decreased diurnal oscillations (55), impaired ultradian oscillations (50), and reduced entrainment of ultradian oscillations (46). In 1981, an impairment of the rapid pulsatile secretion was demonstrated in type 2 diabetic subjects (38), resulting in much focus on the possible role of insulin oscillations on insulin action, but also on possible primary defects in β-cell activity to cause type 2 diabetes. The latter was supported by the observation of defective oscillatory insulin release in first-degree relatives of type 2 diabetic subjects with impaired glucose tolerance (39). Nevertheless, secretory dysfunction in both type 2 diabetes and glucose intolerance may be secondary to the glucose toxicity. Therefore, the presence of increased nonstationarity and disorderliness of rapid pulsatile insulin secretion in glucose-tolerant first-degree relatives of type 2 diabetic subjects is further suggestive of a primary defect in insulin secretion in type 2 diabetes (41). This is paralleled by impaired entrainment of ultradian oscillations in women with polycystic ovary syndrome and with (but not without) a family history of type 2 diabetes (45). It seems likely that early secretory defects should manifest themselves in the complex cascade of metabolic events that is involved in pulsatile secretion. To detect early defects, a test that examines for subtle impairment seems necessary, in contrast to the intravenous glucose tolerance test that evaluates the response to a sudden rise in glucose from ~5 to ~25 mmol/l glucose. Examining for pulsatile secretion has so far proved better at detecting β-cell dysfunction in metabolically normal but predisposed individuals compared with traditional, more crude tests for β-cell function, as in ref. 41, where different tests were applied to examine β-cell function in glucose-tolerant genetically predisposed healthy subjects. Increased disorderliness of insulin release accompanied by attenuated secretory burst mass is observed in older individuals, linking aging to β-cell dysfunction, and maybe suggesting that the defects that occurs in type 2 diabetes could be linked to aging effects (147,148).

The use of minimal repeated glucose infusions to test for the ability to adapt pulsatile insulin secretion to periodical trivial glucose excursions has proven to markedly improve the ability to separate the pathophysiological pulsatile release of insulin in type 2 diabetic individuals versus physiology in matched control subjects assessed by autocorrelation analysis, spectral analysis, and approximate entropy (149) and by spectral analysis (130). This supports the use of more refined methods as entrainment or pulse induction when examining for the predictive value of apparent β-cell dysfunction versus insulin resistance for the development of type 2 diabetes mellitus in (genetically) predisposed individuals. Defects in insulin secretion observed in conditions with known decreased β-cell mass (150,151) are important in order to provide a linkage between islet mass, secretory capacity, and physiological pulsatile release patterns.

CONCLUSIONS

The detection of high-frequency insulin oscillations correspond 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. The detected pattern is similar to release from the isolated pancreas and isolated islets and to metabolic events in individual β-cells. The mechanism governing the coordination of in vivo pulsatile insulin secretion from 1 million islets scattered throughout the pancreas is be-

lieved to be neurogenic, due to preserved pulsatility in the isolated perfused pancreas. However, the presence of glucose oscillations preceding insulin oscillations indicate that glucose may control the pulsatile pattern in vivo, supported by entrainment of oscillatory insulin secretion in islets, the isolated perfused pancreas, and in vivo by episodic glucose infusions.

The importance of the release pattern in regulating physiological insulin secretory patterns is paralleled by a probably important role of impaired pulsatile secretory pattern in developing type 2 diabetes, either as a very early marker of β-cell dysfunction or maybe even as an etiologically inherited β-cell defect. The mechanisms for development of pathophysiological pulsatile insulin secretory patterns of type 2 diabetes may yield insight into the causes of the β-cell secretory dysfunction of the disease, including whether these characteristics may develop as a consequence of the overworked β-cell, metabolic derangements, or whether they represent genetically inherited defects of β-cell function.

REFERENCES

1. Hansen KM: Oscillations in the blood sugar of fasting normal persons. *Acta Endocrinol (Copenh)* 1923
2. Anderson GE, Kologlu Y, Papadopoulus C: Fluctuations in postabsorptive blood glucose in relation to insulin release. *Metabolism* 16:586–596, 1967
3. Goodner CJ, Walike BC, Koerker DJ, Ensinck JW, Brown AC, Chideckel EW, Palmer J, Kalnasy L: Insulin, glucagon, and glucose exhibit synchronous, sustained oscillations in fasting monkeys. *Science* 195:177–179, 1977
4. Koerker DJ, Goodner CJ, Hansen BW, Brown AC, Rubenstein AH: Synchronous, sustained oscillation of C-peptide and insulin in the plasma of fasting monkeys. *Endocrinology* 102:1649–1652, 1978
5. Lang DA, Matthews DR, Burnett M, Ward GM, Turner RC: Pulsatile, synchronous basal insulin and glucagon secretion in man. *Diabetes* 31:22–26, 1982
6. Lang DA, Matthews DR, Peto J, Turner RC: Cyclic oscillations of basal plasma glucose and insulin concentrations in human beings. *N Engl J Med* 301:1023–1027, 1979
7. Stagner J, Samols E: Comparison of insulin and glucagon pulsatile secretion between the rat and dog pancreas in-vitro [published erratum appears in *Life Sci* 45:463, 1989]. *Life Sci* 43:929–934, 1988
8. Stagner JI, Samols E, Weir GC: Sustained oscillations of insulin, glucagon, and somatostatin from the isolated canine pancreas during exposure to a constant glucose concentration. *J Clin Invest* 65:939–942, 1980
9. Matthews DR, Hermansen K, Connolly AA, Gray D, Schmitz O, Clark A, Orskov H, Turner RC: Greater in vivo than in vitro pulsatility of insulin secretion with synchronized insulin and somatostatin secretory pulses. *Endocrinology* 120:2272–2278, 1987
10. Samols E, Stagner JI: Islet somatostatin—microvascular, paracrine, and pulsatile regulation. *Metabolism* 39:55–60, 1990
11. Bratusch Marrain PR, Komjati M, Waldhausl W: Pulsatile insulin delivery: physiology and clinical implications. *Diabet Med* 4:197–200, 1987
12. Bratusch Marrain PR, Komjati M, Waldhausl WK: Efficacy of pulsatile versus continuous insulin administration on hepatic glucose production and glucose utilization in type I diabetic humans. *Diabetes* 35:922–926, 1986
13. Engdahl JH, Veldhuis JD, Farrell PA: Altered pulsatile insulin secretion associated with endurance training. *J Appl Physiol* 79:1977–1985, 1995
14. Goodner CJ, Sweet IR, Harrison HCJ: Rapid reduction and return of surface insulin receptors after exposure to brief pulses of insulin in perifused rat hepatocytes. *Diabetes* 37:1316–1323, 1988
15. Hunter SJ, Atkinson AB, Ennis CN, Sheridan B, Bell PM: Association between insulin secretory pulse frequency and peripheral insulin action in NIDDM and normal subjects. *Diabetes* 45:683–686, 1996
16. Matthews DR, Naylor BA, Jones RG, Ward GM, Turner RC: Pulsatile insulin has greater hypoglycemic effect than continuous delivery. *Diabetes* 32:617–621, 1983
17. Paolisso G, Salvatore T, Sgambato S, Torella R, Varricchio M, D'Onofrio F: Metabolic effects of pulsatile insulin infusion in the elderly. *Acta Endocrinol (Copenh)* 123:19–23, 1990
18. Paolisso G, Scheen AJ, Giugliano D, Sgambato S, Albert A, Varricchio M, D'Onofrio F, Lefebvre PJ: Pulsatile insulin delivery has greater metabolic effects than continuous hormone administration in man: importance of pulse frequency. *J Clin Endocrinol Metab* 72:607–615, 1991
19. Paolisso G, Sgambato S, Gentile S, Memoli P, Giugliano D, Varricchio M, D'Onofrio F: Advantageous metabolic effects of pulsatile insulin delivery in noninsulin-dependent diabetic patients. *J Clin Endocrinol Metab* 67:1005–1010, 1988
20. Paolisso G, Sgambato S, Passariello N, Scheen A, D'Onofrio F, Lefebvre PJ: Greater efficacy of pulsatile insulin in type I diabetics critically depends on plasma glucagon levels. *Diabetes* 36:566–570, 1987
21. Peiris AN, Stagner JI, Vogel RL, Nakagawa A, Samols E: Body fat distribution and peripheral insulin sensitivity in healthy men: role of insulin pulsatility. *J Clin Endocrinol Metab* 75:290–294, 1992
22. Schmitz O, Arnfred J, Nielsen OH, Beck Nielsen H, Orskov H: Glucose uptake and pulsatile insulin infusion: euglycaemic clamp and [3-3H]glucose studies in healthy subjects. *Acta Endocrinol Copenh* 113:559–563, 1986
23. Schmitz O, Juhl CB, Hollingdal M, Veldhuis JD, Porksen N, Pincus SM: Irregular circulating insulin concentrations in type 2 diabetes mellitus: an inverse relationship between circulating free fatty acid and the disorderliness of insulin time-series in diabetic and healthy individuals. *Metabolism* 50:41–46, 2001
24. Schmitz O, Pedersen SB, Mengel A, Porksen N, Bak J, Moller N, Richelsen B, Alberti KG, Butler PC, Orskov H: Augmented effect of short-term pulsatile versus continuous insulin delivery on lipid metabolism but similar effect on whole-body glucose metabolism in obese subjects. *Metabolism* 43:842–846, 1994
25. Ward GM, Walters JM, Aitken PM, Best JD, Alford FP: Effects of prolonged pulsatile hyperinsulinemia in humans: enhancement of insulin sensitivity. *Diabetes* 39:501–507, 1990
26. Hansen BC, Jen KL, Koerker DJ, Goodner CJ, Wolfe RA: Influence of nutritional state on periodicity in plasma insulin levels in monkeys. *Am J Physiol* 242: R255–R260, 1982
27. Porksen N, Grofte B, Nyholm B, Holst JJ, Pincus SM, Veldhuis JD, Schmitz O, Butler PC: Glucagon-like peptide 1 increases mass but not frequency or orderliness of pulsatile insulin secretion. *Diabetes* 47:45–49, 1998
28. Porksen N, Hussain MA, Bianda TL, Nyholm B, Christiansen JS, Butler PC, Veldhuis JD, Froesch ER, Schmitz O: IGF-I inhibits burst mass of pulsatile insulin secretion at supraphysiological and low IGF-I infusion rates. *Am J Physiol* 272: E352–E358, 1997
29. Porksen N, Munn S, Ferguson D, O'Brien T, Veldhuis J, Butler P: Coordinate pulsatile insulin secretion by chronic intraportally transplanted islets in the isolated perfused rat liver. *J Clin Invest* 94:219–227, 1994
30. Porksen N, Munn S, Steers J, Veldhuis JD, Butler PC: Effects of glucose ingestion versus infusion on pulsatile insulin secretion: the incretin effect is achieved by amplification of insulin secretory burst mass. *Diabetes* 45:1317–1323, 1996
31. Porksen N, Munn S, Steers J, Vore S, Veldhuis J, Butler P: Pulsatile insulin secretion accounts for 70% of total insulin secretion during fasting. *Am J Physiol* 269:E478–E488, 1995
32. Porksen N, Munn SR, Steers JL, Veldhuis JD, Butler PC: Effects of somatostatin on pulsatile insulin secretion: elective inhibition of insulin burst mass. *Am J Physiol* 270:E1043–E1049, 1996
33. Porksen N, Nyholm B, Veldhuis JD, Butler PC, Schmitz O: In humans at least 75% of insulin secretion arises from punctuated insulin secretory bursts. *Am J Physiol* 273:E908–E914, 1997
34. Porksen NK, Munn SR, Steers JL, Schmitz O, Veldhuis JD, Butler PC: Mechanisms of sulfonylurea's stimulation of insulin secretion in vivo: selective amplification of insulin secretory burst mass. *Diabetes* 45:1792–1797, 1996
35. Sturis J, O'Meara NM, Shapiro ET, Blackman JD, Tillil H, Polonsky KS, Van Cauter E: Differential effects of glucose stimulation upon rapid pulses and ultradian oscillations of insulin secretion. *J Clin Endocrinol Metab* 76:895–901, 1993
36. Bingley PJ, Matthews DR, Williams AJ, Bottazzo GF, Gale EA: Loss of regular oscillatory insulin secretion in islet cell antibody positive nondiabetic subjects. *Diabetologia* 35:32–38, 1992
37. Goodner CJ, Koerker DJ, Weigle DS, McCulloch DK: Decreased insulin- and glucagon-pulse amplitude accompanying beta-cell deficiency induced by streptozocin in baboons. *Diabetes* 38:925–931, 1989
38. Lang DA, Matthews DR, Burnett M, Turner RC: Brief, irregular oscillations of basal plasma insulin and glucose concentrations in diabetic man. *Diabetes* 30:435–439, 1981

39. O'Rahilly S, Turner RC, Matthews DR: Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes. *N Engl J Med* 318:1225–1230, 1988
40. Paolisso G, Sgambato S, Torella R, Varricchio M, Scheen A, D'Onofrio F, Lefebvre PJ: Pulsatile insulin delivery is more efficient than continuous infusion in modulating islet cell function in normal subjects and patients with type 1 diabetes. *J Clin Endocrinol Metab* 66:1220–1226, 1988
41. Schmitz O, Porksen N, Nyholm B, Skjaerbaek C, Butler PC, Veldhuis JD, Pincus SM: Disorderly and nonstationary insulin secretion in relatives of patients with NIDDM. *Am J Physiol* 272:E218–E226, 1997
42. Sturis J, Pugh WL, Tang J, Ostrega DM, Polonsky JS, Polonsky KS: Alterations in pulsatile insulin secretion in the Zucker diabetic fatty rat. *Am J Physiol* 267:E250–E259, 1994
43. Sturis J, Pugh WL, Tang J, Polonsky KS: Prevention of diabetes does not completely prevent insulin secretory defects in the ZDF rat. *Am J Physiol* 269:E786–E792, 1995
44. Byrne MM, Sturis J, O'Meara NM, Polonsky KS: Insulin secretion in insulin-resistant women with a history of gestational diabetes. *Metabolism* 44:1067–1073, 1995
45. Ehrmann DA, Sturis J, Byrne MM, Karrison T, Rosenfield RL, Polonsky KS: Insulin secretory defects in polycystic ovary syndrome: relationship to insulin sensitivity and family history of non-insulin-dependent diabetes mellitus. *J Clin Invest* 96:520–527, 1995
46. O'Meara NM, Sturis J, Van Cauter E, Polonsky KS: Lack of control by glucose of ultradian insulin secretory oscillations in impaired glucose tolerance and in non-insulin-dependent diabetes mellitus. *J Clin Invest* 92:262–271, 1993
47. Polonsky KS, Sturis J, Bell GI: Seminars in Medicine of the Beth Israel Hospital, Boston. Non-insulin-dependent diabetes mellitus: a genetically programmed failure of the beta cell to compensate for insulin resistance. *N Engl J Med* 334:777–783, 1996
48. Scheen AJ, Sturis J, Polonsky KS, Van Cauter E: Alterations in the ultradian oscillations of insulin secretion and plasma glucose in aging. *Diabetologia* 39:564–572, 1996
49. Simon C, Brandenberger G, Follenius M: Ultradian oscillations of plasma glucose, insulin, and C-peptide in man during continuous enteral nutrition. *J Clin Endocrinol Metab* 64:669–674, 1987
50. Sturis J, Polonsky KS, Shapiro ET, Blackman JD, O'Meara NM, Van Cauter E: Abnormalities in the ultradian oscillations of insulin secretion and glucose levels in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 35:681–689, 1992
51. Sturis J, Scheen AJ, Leproult R, Polonsky KS, Van Cauter E: 24-hour glucose profiles during continuous or oscillatory insulin infusion: demonstration of the functional significance of ultradian insulin oscillations. *J Clin Invest* 95:1464–1471, 1995
52. Sturis J, Van Cauter E, Blackman JD, Polonsky KS: Entrainment of pulsatile insulin secretion by oscillatory glucose infusion. *J Clin Invest* 87:439–445, 1991
53. Ryan EA, Imes S, Liu D, McManus R, Finegood DT, Polonsky KS, Sturis J: Defects in insulin secretion and action in women with a history of gestational diabetes. *Diabetes* 44:506–512, 1995
54. O'Meara NM, Sturis J, Herold KC, Ostrega DM, Polonsky KS: Alterations in the patterns of insulin secretion before and after diagnosis of IDDM. *Diabetes Care* 18:568–571, 1995
55. Polonsky KS, Given BD, Hirsch LJ, Tillil H, Shapiro ET, Beebe C, Frank BH, Galloway JA, Van Cauter E: Abnormal patterns of insulin secretion in non-insulin-dependent diabetes mellitus. *N Engl J Med* 318:1231–1239, 1988
56. Polonsky KS, Given BD, Van Cauter E: Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. *J Clin Invest* 81:442–448, 1988
57. Shapiro ET, Tillil H, Polonsky KS, Fang VS, Rubenstein AH, Van Cauter E: Oscillations in insulin secretion during constant glucose infusion in normal man: relationship to changes in plasma glucose. *J Clin Endocrinol Metab* 67:307–314, 1988
58. Van Cauter E, Blackman JD, Roland D, Spire JP, Refetoff S, Polonsky KS: Modulation of glucose regulation and insulin secretion by circadian rhythmicity and sleep. *J Clin Invest* 88:934–942, 1991
59. Van Cauter E, Shapiro ET, Tillil H, Polonsky KS: Circadian modulation of glucose and insulin responses to meals: relationship to cortisol rhythm. *Am J Physiol* 262:E467–E475, 1992
60. Eaton RP, Allen RC, Schade DS: Hepatic removal of insulin in normal man: dose response to endogenous insulin secretion. *J Clin Endocrinol Metab* 56:1294–1300, 1983
61. Eaton RP, Friedman N, Allen RC, Schade DS: Insulin removal in man: in vivo evidence for a receptor-mediated process. *J Clin Endocrinol Metab* 58:555–559, 1984
62. Jaspan J, Polonsky K: Glucose ingestion in dogs alters the hepatic extraction of insulin: in vivo evidence for a relationship between biologic action and extraction of insulin. *J Clin Invest* 69:516–525, 1982
63. Ishida T, Chou J, Lewis RM, Hartleay CJ, Entman M, Field JB: The effect of ingestion of meat on hepatic extraction of insulin and glucagon and hepatic glucose output in conscious dogs. *Metabolism* 32:558–567, 1983
64. Porksen N, Munn S, Steers J, Veldhuis JD, Butler PC: Impact of sampling technique on appraisal of pulsatile insulin secretion by deconvolution and cluster analysis. *Am J Physiol* 269:E1106–E1114, 1995
65. Valdeolmillos M, Gomis A, Sanchez Andres JV: In vivo synchronous membrane potential oscillations in mouse pancreatic beta-cells: lack of coordination between islets. *J Physiol (Lond)* 493:9–18, 1996
66. Storch MJ, Rossle M, Kerp L: [Pulsatile insulin secretion into the portal vein in liver cirrhosis]. *Dtsch Med Wochenschr* 118:134–138, 1993
67. Hansen BC, Pek S, Koerker DJ, Goodner CJ, Wolfe RA, Schielke GP: Neural influences on oscillations in basal plasma levels of insulin in monkeys. *Am J Physiol* 240:E5–E11, 1981
68. O'Meara NM, Sturis J, Blackman JD, Byrne MM, Jaspan JB, Thistlethwaite JR, Polonsky KS: Oscillatory insulin secretion after pancreas transplant. *Diabetes* 42:855–861, 1993
69. O'Meara NM, Sturis J, Blackman JD, Van Cauter E, Polonsky KS: Analytical problems in detecting rapid insulin secretory pulses in normal humans. *Am J Physiol* 264:E231–E238, 1993
70. Goodner CJ, Hom FG, Koerker DJ: Hepatic glucose production oscillates in synchrony with the islet secretory cycle in fasting rhesus monkeys. *Science* 215:1257–1260, 1982
71. Jaspan JB, Lever E, Polonsky KS, Van Cauter E: In vivo pulsatility of pancreatic islet peptides. *Am J Physiol* 251:E215–26, 1986
72. Chou HF, McGivern R, Berman N, Ipp E: Oscillations of circulating plasma insulin concentrations in the rat. *Life Sci* 48:1463–1469, 1991
73. Goodner CJ, Koerker DJ, Stagner JI, Samols E: In vitro pancreatic hormonal pulses are less regular and more frequent than in vivo. *Am J Physiol* 260: E422–E429, 1991
74. Balks HJ, Schmidt A, Prank K, Hemmer F, von zur Muhlen A, Brabant G: Temporal pattern of pancreatic insulin and C-peptide secretion and of plasma glucose levels after nutritional stimulation. *J Clin Endocrinol Metab* 75:1198–1203, 1992
75. Horwitz DL, Starr JI, Mako ME, Blackard WG, Rubenstein AH: Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *J Clin Invest* 55:1278–1283, 1975
76. Ferrannini E, Wahren J, Faber OK, Felig P, Binder C, DeFronzo RA: Splanchnic and renal metabolism of insulin in human subjects: a dose-response study. *Am J Physiol* 244:E517–27, 1983
77. Morishima T, Bradshaw C, Radziuk J: Measurement using tracers of steady-state turnover and metabolic clearance of insulin in dogs. *Am J Physiol* 248: E203–E208, 1985
78. Duckworth WC: Insulin degradation: mechanisms, products, and significance. *Endocr Rev* 9:319–345, 1988
79. Polonsky K, Jaspan J, Pugh W, Cohen D, Schneider M, Schwartz T, Moossa AR, Tager H, Rubenstein AH: Metabolism of C-peptide in the dog: in vivo demonstration of the absence of hepatic extraction. *J Clin Invest* 72:1114–1123, 1983
80. Van Cauter E, Mestrez F, Sturis J, Polonsky KS: Estimation of insulin secretion rates from C-peptide levels: comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes* 41:368–377, 1992
81. Stagner J, Nakagawa A, Peiris A, Samols E, Vogel R: Islet hormone pulse intervals are dependent upon sampling frequency. *Life Sci* 50:769–774, 1992
82. Veldhuis JD, Johnson ML: Cluster analysis: a simple, versatile, and robust algorithm for endocrine pulse detection. *Am J Physiol* 250:E486–E493, 1986
83. Blackman JD, Polonsky KS, Jaspan JB, Sturis J, Van Cauter E, Thistlethwaite JR: Insulin secretory profiles and C-peptide clearance kinetics at 6 months and 2 years after kidney-pancreas transplantation. *Diabetes* 41:1346–1354, 1992
84. Pincus S, Singer BH: A recipe for randomness. *Proc Natl Acad Sci U S A* 95:10367–10372, 1998
85. Pincus SM: Approximate entropy as a measure of system complexicity. *Proc Natl Acad Sci U S A* 88:2297–2301, 1991
86. Pincus SM, Keefe DL: Quantification of hormone pulsatility via an approximate entropy algorithm. *Am J Physiol* 262:E741–54, 1992
87. Hartman ML, Pincus SM, Johnson ML, Matthews DH, Faunt LM, Vance ML, Thorner MO, Veldhuis JD: Enhanced basal and disorderly growth

hormone secretion distinguish acromegalic from normal pulsatile growth hormone release. *J Clin Invest* 94:1277–1288, 1994
88. Pincus SM, Mulligan T, Iranmanesh A, Gheorghiu S, Godschalk M, Veldhuis JD: Older males secrete luteinizing hormone and testosterone more irregularly, and jointly more asynchronously, than younger males. *Proc Natl Acad Sci U S A* 93:14100–14105, 1996
89. Siragy HM, Vieweg WV, Pincus S, Veldhuis JD: Increased disorderliness and amplified basal and pulsatile aldosterone secretion in patients with primary aldosteronism. *J Clin Endocrinol Metab* 80:28–33, 1995
90. Roelfsema F, Pincus SM, Veldhuis JD: Patients with Cushing's disease secrete adrenocorticotropin and cortisol jointly more asynchronously than healthy subjects. *J Clin Endocrinol Metab* 83:688–692, 1998
91. van den Berg G, Pincus SM, Veldhuis JD, Frolich M, Roelfsema F: Greater disorderliness of ACTH and cortisol release accompanies pituitary-dependent Cushing's disease. *Eur J Endocrinol* 136:394–400, 1997
92. Meneilly GS, Ryan AS, Veldhuis JD, Elahi D: Increased disorderliness of basal insulin release, attenuated insulin secretory burst mass, and reduced ultradian rhythmicity of insulin secretion in older individuals. *J Clin Endocrinol Metab* 82:4088–4093, 1997
93. Veldhuis JD, Carlson ML, Johnson ML: The pituitary gland secretes in bursts: appraising the nature of glandular secretory impulses by simultaneous multiple-parameter deconvolution of plasma hormone concentrations. *Proc Natl Acad Sci U S A* 84:7686–7690, 1987
94. Chou HF, Ipp E: Pulsatile insulin secretion in isolated rat islets. *Diabetes* 39:112–117, 1990
95. Hayek A: Preservation of insulin release periodicity from individual islets of obese Zucker rats. *Horm Metab Res* 13:119–120, 1981
96. Cook DL: Isolated islets of Langerhans have slow oscillations of electrical activity. *Metabolism* 32:681–685, 1983
97. Longo EA, Tornheim K, Deeney JT, Varnum BA, Tillotson D, Prentki M, Corkey BE: Oscillations in cytosolic free Ca2+, oxygen consumption, and insulin secretion in glucose-stimulated rat pancreatic islets. *J Biol Chem* 266:9314–9319, 1991
98. Santos RM, Rosario LM, Nadal A, Garcia Sancho J, Soria B, Valdeolmillos M: Widespread synchronous $[Ca^{2+}]_i$ oscillations due to bursting electrical activity in single pancreatic islets. *Pflugers Arch* 418:417–422, 1991
99. Chou HF, Berman N, Ipp E: Oscillations of lactate released from islets of Langerhans: evidence for oscillatory glycolysis in beta-cells. *Am J Physiol* 262: E800–E805, 1992
100. Bergsten P: Slow and fast oscillations of cytoplasmic Ca^{2+} in pancreatic islets correspond to pulsatile insulin release. *Am J Physiol* 268:E282–7, 1995
101. Gylfe E, Grapengiesser E, Hellman B: Propagation of cytoplasmic Ca^{2+} oscillations in clusters of pancreatic beta-cells exposed to glucose. *Cell Calcium* 12:229–240, 1991
102. Hellman B, Gylfe E, Bergsten P, Grapengiesser E, Lund PE, Berts A, Tengholm A, Pipeleers DG, Ling Z: Glucose induces oscillatory Ca^{2+} signalling and insulin release in human pancreatic beta cells. *Diabetologia* 37 (Suppl. 2):S11–S20, 1994
103. Hellman B, Gylfe E, Grapengiesser E, Lund PE, Berts A: Cytoplasmic Ca^{2+} oscillations in pancreatic beta-cells. *Biochim Biophys Acta* 1113: 295–305, 1992
104. Tornheim K: Are metabolic oscillations responsible for normal oscillatory insulin secretion? *Diabetes* 46:1375–1380, 1997
105. Berman N, Chou HF, Berman A, Ipp E: A mathematical model of oscillatory insulin secretion. *Am J Physiol* 264:R839–R851, 1993
106. Sherman A: Contributions of modeling to understanding stimulus-secretion coupling in pancreatic beta-cells. *Am J Physiol* 271:E362–E372, 1996
107. Stagner JI, Samols E: Perturbation of insulin oscillations by nerve blockade in the in vitro canine pancreas. *Am J Physiol* 248:E516–E521, 1985
108. Bergsten P, Hellman B: Glucose-induced amplitude regulation of pulsatile insulin secretion from individual pancreatic islets. *Diabetes* 42:670–674, 1993
109. Cunningham BA, Deeney JT, Bliss CR, Corkey BE, Tornheim K: Glucose-induced oscillatory insulin secretion in perifused rat pancreatic islets and clonal beta-cells (HIT). *Am J Physiol* 271:E702–E710, 1996
110. Westerlund J, Hellman B, Bergsten P: Pulsatile insulin release from mouse islets occurs in the absence of stimulated entry of Ca2+. *J Clin Invest* 97:1860–1863, 1996
111. Marchetti P, Scharp DW, Mclear M, Gingerich R, Finke E, Olack B, Swanson C, Giannarelli R, Navalesi R, Lacy PE: Pulsatile insulin secretion from isolated human pancreatic islets. *Diabetes* 43:827–830, 1994
112. Rosario LM, Atwater I, Scott AM: Pulsatile insulin release and electrical activity from single ob/ob mouse islets of Langerhans. *Adv Exp Med Biol* 211:413–425, 1986
113. Misler S, Barnett DW, Gillis KD, Pressel DM: Electrophysiology of stimulus-secretion coupling in human beta-cells. *Diabetes* 41:1221–1228, 1992
114. Palti Y, David GB, Lachov E, Mida YH, Schatzberger R: Islets of Langerhans generate wavelike electric activity modulated by glucose concentration. *Diabetes* 45:595–601, 1996
115. Bergsten P, Grapengiesser E, Gylfe E, Tengholm A, Hellman B: Synchronous oscillations of cytoplasmic Ca^{2+} and insulin release in glucose-stimulated pancreatic islets. *J Biol Chem* 269:8749–8753, 1994
116. Meissner HP: Electrophysiological evidence for coupling between beta cells of pancreatic islets. *Nature* 262:502–504, 1976
117. Gilon P, Henquin JC: Influence of membrane potential changes on cytoplasmic Ca^{2+} concentration in an electrically excitable cell, the insulin-secreting pancreatic B-cell. *J Biol Chem* 267:20713–20720, 1992
118. Valdeolmillos M, Nadal A, Soria B, Garcia-Sancho J: Fluorescence digital image analysis of glucose-induced $[Ca^{2+}]_i$ oscillations in mouse pancreatic islets of Langerhans. *Diabetes* 42:1210–1214, 1993
119. Stagner JI, Samols E: Role of intrapancreatic ganglia in regulation of periodic insular secretions. *Am J Physiol* 248:E522–E530, 1985
120. Matthews DR, Lang DA, Burnett MA, Turner RC: Control of pulsatile insulin secretion in man. *Diabetologia* 24:231–237, 1983
121. Griffith RC, Scharp DW, Hartman BK, Ballinger WF, Lacy PE: A morphologic study of intrahepatic portal-vein islet isografts. *Diabetes* 26:201–214, 1977
122. Korsgren O, Jansson L, Andersson A, Sundler F: Reinnervation of transplanted pancreatic islets. A comparison among islets implanted into the kidney, spleen, and liver. *Transplantation* 56:138–143, 1993
123. Hermansen K: Effects of galanin on the release of insulin, glucagon and somatostatin from the isolated, perfused dog pancreas. *Acta Endocrinol (Copenh)* 119:91–98, 1988
124. Campfield LA Smith FJ: Neural control of insulin secretion: interaction of norepinephrine and acetylcholine. *Am J Physiol* 244:R629–R634, 1983
125. Natali A, Gastaldelli A, Galvan AQ, Sironi AM, Ciociaro D, Sanna G, Rosenzweig P, Ferrannini E: Effects of acute alpha 2-blockade on insulin action and secretion in humans. *Am J Physiol* 274:E57–E64, 1998
126. Kawai, Unger RH: Effects of gamma-aminobutyric acid on insulin, glucagon, and somatostatin release from perfused dog pancreas. *Endocrinology* 113:111–113, 1983
127. Arneric SP, Chow SA, Long JP, Fischer LJ: Inhibition of insulin release from rat pancreatic islets by drugs that are analogues of dopamine. *Diabetes* 33:888–893, 1984
128. Shankar R, Zhu JS, Ladd B, Henry D, Shen HQ, Baron AD: Central nervous system nitric oxide synthase activity regulates insulin secretion and insulin action. *J Clin Invest* 102:1403–1412, 1998
129. Ristow M, Carlqvist H, Hebinck J, Vorgerd M, Krone W, Pfeiffer A, Muller-Wieland D, Ostenson CG: Deficiency of phosphofructo-1-kinase/muscle subtype in humans is associated with impairment of insulin secretory oscillations. *Diabetes* 48:1557–1561, 1999
130. Mao CS, Berman N, Roberts K, Ipp E: Glucose entrainment of high-frequency plasma insulin oscillations in control and type 2 diabetic subjects. *Diabetes* 48:714–721, 1999
131. Porksen N, Juhl C, Hollingdal M, Pincus SM, Sturis J, Veldhuis JD, Schmitz O: Concordant induction of rapid in vivo pulsatile insulin secretion in healthy humans by recurrent punctuated glucose infusions. *Am J Physiol* 278:E162–E170, 2000
132. Zhou YP, Grill VE: Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. *J Clin Invest* 93:870–876, 1994
133. McGarry JD, Dobbins RL: Fatty acids, lipotoxicity and insulin secretion. *Diabetologia* 42:128–138, 1999
134. Prentki M, Vischer S, Glennon MC, Regazzi R, Deeney JT, Corkey BE: Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. *J Biol Chem* 267:5802–5810, 1992
135. Sonnenberg GE, Hoffman RG, Mueller RA, Kissebah AH: Splanchnic insulin dynamics and secretion pulsatilities in abdominal obesity. *Diabetes* 43:468–477, 1994
136. Getty L, Panteleon AE, Mittelman SD, Dea MK, Bergman RN: Rapid oscillations in omental lipolysis are independent of changing insulin levels in vivo. *J Clin Invest* 106:421–430, 2000
137. Bermann M, Jaffe CA, Tsai W, DeMott Friberg R, Barkan AL: Negative feedback regulation of pulsatile growth hormone secretion by insulin-like growth factor I: involvement of hypothalamic somatostatin. *J Clin Invest* 94:138–145, 1994
138. Brabant G, Prank K, Hoang-Vu C, Hesch RD, von zur Muhlen A: Hypo-

thalamic regulation of pulsatile thyrotopin secretion. *J Clin Endocrinol Metab* 72:145–150, 1991
139. Schofl C, Becker C, Prank K, von zur Muhlen A, Brabant G: Twenty-four-hour rhythms of plasma catecholamines and their relation to cardiovascular parameters in healthy young men. *Eur J Endocrinol* 137:675–683, 1997
140. Prank K, Nowlan SJ, Harms HM, Kloppstech M, Brabant G, Hesch RD, Sejnowski TJ: Time series prediction of plasma hormone concentration: evidence for differences in predictability of parathyroid hormone secretion between osteoporotic patients and normal controls. *J Clin Invest* 95:2910–2919, 1995
141. Balks HJ, Holst JJ, von zur Muhlen A, Brabant G: Rapid oscillations in plasma glucagon-like peptide-1 (GLP-1) in humans: cholinergic control of GLP-1 secretion via muscarinic receptors. *J Clin Endocrinol Metab* 82:786–790, 1997
142. Sinha MK, Sturis J, Ohannesian J, Magosin S, Stephens T, Heiman ML, Polonsky KS, Caro JF: Ultradian oscillations of leptin secretion in humans. *Biochem Biophys Res Commun* 228:733–738, 1996
143. Juhl C, Porksen N, Sturis J, Prange A, Veldhuis J, Pincus S, Fineman MS, Schmitz O: High frequency oscillations in circulating amylin concentrations in healthy humans. *Am J Physiol* 2000 (In Press)
144. Juhl CB, Schmitz O, Pincus S, Holst JJ, Veldhuis J, Porksen N: Short-term treatment with GLP-1 increases pulsatile insulin secretion in type II diabetes with no effect on orderliness. *Diabetologia* 43:583–588, 2000
145. Juhl C, Porksen N, Sturis J, Hollingdal M, Pincus S, Veldhuis J, Dejgaard A, Schmitz O: Repaglinide amplifies insulin secretory burst mass without affecting burst frequency (Abstract). *Diabetologia* 42 (Suppl. 1):A4, 1999
146. Meneilly GS, D Elahi: Physiological importance of first-phase insulin release in elderly patients with diabetes. *Diabetes Care* 21:1326–1329, 1998
147. Cavaghan MK, Ehrmann DA, Byrne MM, Polonsky KS: Treatment with the oral antidiabetic agent troglitazone improves beta cell responses to glucose in subjects with impaired glucose tolerance. *J Clin Invest* 100:530–537, 1997
148. Meneilly GS, Veldhuis JD, Elahi D: Disruption of the pulsatile and entropic modes of insulin release during an unvarying glucose stimulus in elderly individuals. *J Clin Endocrinol Metab* 84:1938–1943, 1999
149. Hollingdal M, Juhl C, Porksen N, Sturis J, Veldhuis JD, Pincus S, Polonsky KS, Schmitz O: Glucose pulse induction markedly improves detection of a disrupted oscillatory insulin secretion in type 2 diabetes (Abstract). *Diabetologia* 42 (Suppl. 1):A78, 1999
150. Leahy JL, Bonner-Weir S, Weir GC: Abnormal glucose regulation of insulin secretion in models of reduced B-cell mass. *Diabetes* 33:667–673, 1984
151. Madsbad S, Christiansen E, Tibell A, Tyden G, Rasmussen K, Burcharth F: Beta-cell dysfunction following successful segmental pancreas transplantation. Danish-Swedish Study Group of Metabolic Effect of Pancreas Transplantation. *Transplant Proc* 26:469–470, 1994