

# Failure of Physiological Plasma Glucose Excursions to Entrain High-Frequency Pulsatile Insulin Secretion in Type 2 Diabetes

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**Insulin is released in high-frequency pulsatile bursts at intervals of 6–13 min. Intrapancreatic mechanisms are assumed to coordinate pulsatile insulin release, but small oscillations in plasma glucose concentrations may contribute further. To gain additional insight into  $\beta$ -cell (patho)physiology, we explored the ability of repetitive small glucose infusions (6 mg/kg over 1 min every 10 min) to modify rapid pulsatile insulin secretion in 10 type 2 diabetic individuals (plasma glucose  $9.3 \pm 1.0$  mmol/l, HbA<sub>1c</sub>  $7.9 \pm 0.5\%$ , mean  $\pm$  SE) and 10 healthy subjects. All subjects were investigated twice in randomly assigned order: during saline and during glucose exposure. Blood was collected every minute for 90 min to create a plasma insulin concentration time-series for analysis using 3 complementary algorithms: namely, spectral analysis, autocorrelation analysis, and approximate entropy (ApEn). During saline infusion, none of the algorithms were able to discriminate between diabetic and control subjects ( $P > 0.20$ ). During glucose entrainment, spectral density peaks (SP) and autocorrelation coefficients (AC) increased significantly ( $P < 0.001$ ), and ApEn decreased ( $P < 0.01$ ), indicating more regular insulin time-series in the healthy volunteers. However, no differences were observed in the diabetic individuals between the glucose and saline conditions. Furthermore, in spite of identical absolute glucose excursions ( $\sim 0.3$  mmol/l) glucose pulse entrainment led to a complete (SP:  $4.76 \pm 0.62$  [range 2.08–7.60] vs.  $17.24 \pm 0.93$  [11.70–20.58],  $P < 0.001$ ; AC:  $0.01 \pm 0.05$  [0.33–0.24] vs.  $0.64 \pm 0.05$  [0.35–0.83],  $P < 0.001$ ) or almost complete (ApEn:  $1.59 \pm 0.02$  [1.48–1.67] vs.  $1.42 \pm 0.05$  [1.26–1.74],  $P < 0.005$ ) separation of the insulin time-series in diabetic and control subjects. Even elevating the glucose infusion rate in the diabetic subjects to achieve comparable relative (and hence higher absolute) glucose excursions ( $\sim 4.9\%$ ) failed to entrain pulsatile insulin secretion in this group. In con-**

**clusion, the present study demonstrates that failure to respond adequately with regular oscillatory insulin secretion to recurrent high-frequency and (near)-physiological glucose excursion is a manifest feature of  $\beta$ -cell malfunction in type 2 diabetes. Whether the model will be useful in unmasking subtle (possible pre-diabetic) defects in  $\beta$ -cell sensitivity to glucose drive remains to be determined. *Diabetes* 49:1334–1340, 2000**

**S**imilar to many other hormones, insulin is secreted in an oscillatory manner (1–4). Recent studies have demonstrated that the majority, if not all, of normal insulin secretion occurs as pulsatile bursts (5,6). In healthy humans, high-frequency insulin concentration pulses recurring at intervals between 6 and 13 min have been reported (4,6) along with oscillations of larger amplitude and a longer periodicity of  $\sim 120$  min (7,8). The cellular mechanisms that generate and regulate oscillatory insulin secretion by the  $\beta$ -cell are elusive, although a pivotal role of phosphofruktokinase M has been suggested recently (9,10). Analogously, coordination within and between islets is complex and only partly understood, but intraislet communication through gap junctions and via the neuronal network within the pancreas is likely of importance (11).

Concentrations of metabolites and fuel substrates may oscillate. Rapid oscillations in blood glucose concentrations were initially demonstrated  $>70$  years ago (12). More recent data showing a temporal relationship between insulin oscillations and plasma glucose excursions (3,4) indicate that a feedback loop may exist between  $\beta$ -cells and endogenous glucose production and contribute to regulation of the insulin release process. Whereas it is established that the ability of glucose to entrain ultradian insulin secretion patterns is disturbed in diabetic and prediabetic individuals (13,14), the ability of  $\beta$ -cells to sense subtle and high-frequency glucose excursions has been examined only minimally. The cardinal role of glucose as a regulator of high-frequency insulin pulsatility was recognized in *in vitro* animal models using perfused islets or pancreases from Wistar rats and Zucker diabetic fatty (ZDF) rats (15,16)—the latter being a model for type 2 diabetes. These studies demonstrated that small and frequent glucose oscillations in perfusate with amplitudes between  $\sim 0.5$  and  $1.5$  mmol/l were able to govern insulin secretory oscillations consistently in islets and pancreases of the Wistar rats in contrast to islets and pancreases from the ZDF rats, which exhibited an impaired pulsatility. The ability

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J.S. holds stock in Novo Nordisk.

ApEn, approximate entropy; FFA, free fatty acid.

of minute plasma glucose excursions ( $\sim 0.3$  mmol/l =  $\sim 5\%$ ) to entrain oscillatory insulin secretion has recently been recognized in healthy humans (17). In the latter investigations, large amplitude insulin pulses ( $\sim 100\%$ ) were triggered at a lag time of 2 min by the subtle glucose oscillations ( $\sim 5\%$ ) during minimal glucose infusions, and the periodicity was easily modified by periodicity shifts between 7 and 12 min.

Type 2 diabetes is characterized by a broad array of abnormalities in insulin secretion, among which is disruption of high-frequency pulsatile insulin secretion (18). To elucidate the nature of this  $\beta$ -cell abnormality in more detail and consequently to enhance our knowledge of human  $\beta$ -cell pathophysiology, we explored the ability of frequent and near-physiological glucose excursions to govern the insulin release process in 10 type 2 diabetic individuals versus 10 healthy control subjects. To this end, we used a recently developed pulse induction protocol in humans (17), wherein small amounts of glucose (6 mg/kg) are given as a bolus every 10 min, and serum insulin concentration time-series are assessed by complementary time-series analyses (spectral analysis, autocorrelation analysis, and approximate entropy).

## RESEARCH DESIGN AND METHODS

**Subjects.** Ten healthy subjects and 10 type 2 diabetic individuals participated in the study. All participants were Caucasian. The diabetic subjects were all negative for anti-GAD (Brahms Diagnostica, Berlin, Germany). Average duration of diabetes was 4 years (range 0.5–10). Of the type 2 diabetic subjects, 7 were treated with diet only, whereas 3 were treated with diet and sulfonylurea. Sulfonylurea treatment was withdrawn at least 36 h before study days. Apart from sulfonylurea treatment, no participants were treated with any medication known or believed to influence insulin secretion or glucose metabolism. The pertinent clinical data are depicted in Table 1. The protocol was approved by the Ethical Committee of Aarhus, Denmark, and the study was performed in accordance with the Helsinki Declaration. All subjects were on an isocaloric diet and were not allowed to be involved in severe exercise 3 days before each study day.

**Design.** Subjects were admitted to the Clinical Research Unit at Medical Department M., University Hospital of Aarhus. After an overnight fast ( $\sim 10$  h), at 0800, 2 catheters (Venflon 17 G/45 mm; BOC Ohmeda AB, Helsingborg, Sweden) were placed in the antecubital veins for sampling and infusion purposes. A 30-min resting period was allowed before the protocols were initiated.

Subjects underwent, in a randomly ordered sequence, infusion with either saline (protocol 1) or glucose (200 g/l) (protocol 2). At 0 min, blood was drawn for determination of baseline glucose, insulin, C-peptide, free fatty acid (FFA), and HbA<sub>1c</sub> (in the diabetic participants), after which the intermittent glucose or saline infusion was started. Glucose was given in a dose of 6 mg/kg over

1 min followed by a 9-min pause every 10 min over 90 min. Infusion rates of saline (placebo) corresponded to those of glucose. For later measurements of serum insulin and plasma glucose, blood (1.5 ml) was collected every minute from 1 to 90 min. The sampling catheter was perfused with saline (0.9%, 1.0 ml/min), and 20 s before sampling, the saline infusion was stopped and 1.0 ml blood was drawn and discarded. To assure patency of the sampling catheter, blood was collected at a constant rate by use of a 2-ml syringe from 5 s before to 10 s after each minute, after which saline infusion (1.0 ml/min) was repeated until 20 s before the next sampling. Dead-space of the cannula and a 3-way stopcock (Connecta; BOC Ohmeda, Helsingborg, Sweden) was in total 0.38 ml. This sampling procedure has previously been evaluated in our laboratory, and variation due to sampling has been estimated as  $< 4\%$  (6).

In addition, in 8 of the diabetic subjects, a third examination was accomplished using a higher glucose infusion based on the degree of fasting glycemia (protocol 3) to ensure comparable relative fractionated glucose excursions between control and diabetic subjects. The amount of glucose infused for 1 min every 10 min was calculated as fasting plasma glucose divided by 5 mmol/l multiplied by 6 mg/kg and was on average 10.6 mg/kg (range 7.3–14.6). Blood sampling procedures were as described above.

**Assays.** Plasma glucose concentrations were measured in duplicate by the glucose oxidation method (Beckman Instruments, Palo Alto, CA). Serum insulin concentrations were measured in duplicate by a 2-site immunospecific insulin enzyme-linked immunosorbent assay, which uses 2 monoclonal antibodies (Dako Diagnostics, Cambridgeshire, U.K.) specific for human insulin. The detection range of this insulin enzyme-linked immunosorbent assay was 5–600 pmol/l. The intra-assay coefficient of variation was 2.8 and 2.4% at concentrations of 150 and 350 pmol/l, respectively. There was no cross-reactivity with proinsulin, and with split (32,33)- and des(31,32)-proinsulin, the antibodies cross-reacted (45 and 66%) with split (65,66)-proinsulin and des(64,65)-proinsulin, respectively, whereas C-peptide, IGF-I, IGF-II, and glucagon did not cross-react (19). Serum C-peptide measurements were performed using a commercially available kit (K6218; Dako Diagnostics). Finally, serum FFA concentrations were determined by a colorimetric method using a commercial kit (Wako Chemicals, Neuss, Germany).

**Analytical strategy.** Spectral analysis, autocorrelation analysis, and approximate entropy analysis require stationary time-series. An 11-point centered unweighted moving average was calculated and subtracted from the original time-series. Analyses were performed on the residuals. This detrending method was used to preserve pulsatility with a periodicity near 10 min, which would be expected spontaneously and after the glucose entrainment protocol.

**Spectral analysis.** To quantify the degree of periodicity in the series, spectral analysis was performed. A Tukey window of 25 data points was used, and the spectra were normalized using the assumption that the total variance in each time-series was 100%. The effect parameter was chosen to be 10 min, in as much as the stimulus was given every 10 min.

**Auto- and cross-correlation analyses.** The periodic nature of individual insulin and glucose profiles was assessed by autocorrelation analysis, and the relationship between the profiles of glucose and insulin was quantified by cross-correlation analysis. In the autocorrelation analyses, the correlation coefficients between the time-series and a copy of itself at lag times of 0, 1, 2, 3, etc., up to 15 min were calculated. Similarly, in the cross-correlation analyses, the coefficients of cross-correlation between the glucose and insulin series at lag times of 0 (i.e., simultaneous values of glucose and insulin),  $\pm 1$  (i.e., glucose leading insulin by 1 min or vice versa),  $\pm 2$ ,  $\pm 3$ , etc., up to  $\pm 15$  min were calculated. The largest coefficient of cross-correlation and the lag time at which it occurred were assessed in each case.

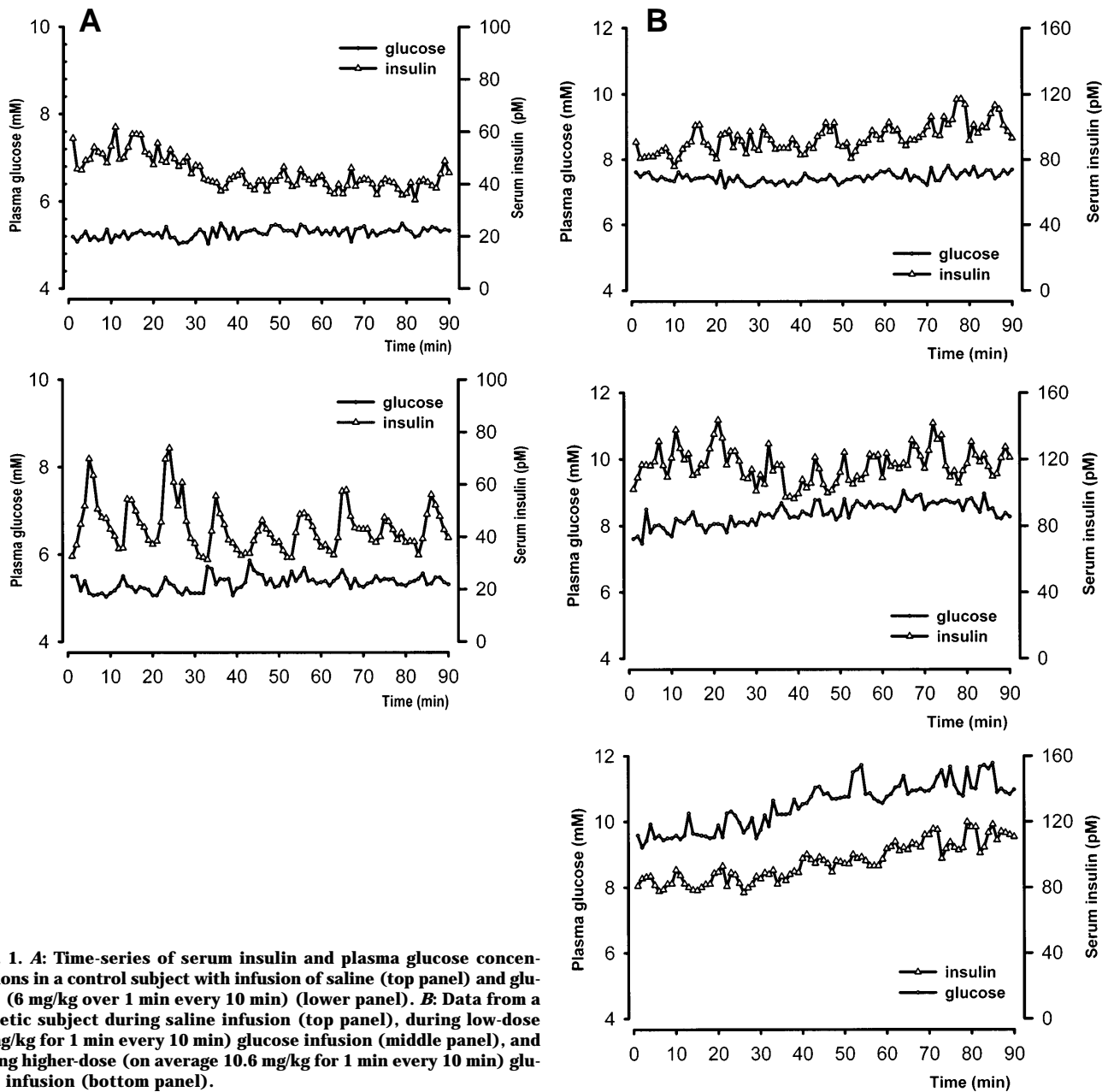
**Quantification of irregularity.** The regularity of serum insulin concentration time-series was assessed by application of approximate entropy (ApEn), which is a model-independent and scale-invariant statistic (20–22). ApEn assigns a single non-negative number to a time-series, in which larger absolute values correspond to greater apparent process randomness and smaller values correspond to more instance of recognizable patterns or consistent features in the data. Briefly, ApEn measures the logarithmic likelihood that runs of patterns that are close (within  $r$ ) for  $m$  contiguous observations remain close (within the same tolerance width  $r$ ) on the next incremental comparison; the precise mathematical definition is given (22). For this study, we calculated approximate entropy values for all data sets, with  $m = 1$  and  $r = 20\%$  as well as  $r = 100\%$  of the SD, in the individual subject time-series. The choice of  $r$  value of 0.2 SD is a more microscopic parameter, whereas  $r = 1.0$  SD provides a coarser-scale (macroscopic) assessment of subpattern persistence within time-series.

**Ten-minute periodicity means.** The plasma glucose and serum insulin concentration changes occurring as a consequence of glucose infusion every 10 min were assessed by calculating average concentrations for all 9 cycles in data from glucose pulse induction every 10 min for 90 min; i.e., concentrations at

TABLE 1  
Clinical characteristics of the subjects

	Control subjects	Type 2 diabetic patients
Sex (F/M)	7/3	4/6
Age (years)	48 $\pm$ 2	54 $\pm$ 3
BMI (kg/m <sup>2</sup> )	25.3 $\pm$ 1.1	28.2 $\pm$ 1.3
Fasting plasma glucose concentration (mmol/l)	4.7 $\pm$ 0.1	9.3 $\pm$ 1.0
HbA <sub>1c</sub> (%)	—	7.9 $\pm$ 0.5
Serum insulin concentration (pmol/l)	36 $\pm$ 6	55 $\pm$ 13
C-peptide concentration (mmol/l)	529 $\pm$ 58	722 $\pm$ 102
Fasting serum FFA concentration (mmol/l)	0.51 $\pm$ 0.03	0.68 $\pm$ 0.08

Data are  $n$  or means  $\pm$  SE.



**FIG. 1. A:** Time-series of serum insulin and plasma glucose concentrations in a control subject with infusion of saline (top panel) and glucose (6 mg/kg over 1 min every 10 min) (lower panel). **B:** Data from a diabetic subject during saline infusion (top panel), during low-dose (6 mg/kg for 1 min every 10 min) glucose infusion (middle panel), and during higher-dose (on average 10.6 mg/kg for 1 min every 10 min) glucose infusion (bottom panel).

times 1, 11, 21, 31, 41, etc., and times 2, 12, 22, 32, and 42 and so forth to 10, 20, 30, 40, and 50 to yield a mean 10-min periodicity. The mean concentration change of the oscillation was calculated in each data set by subtracting the lowest concentration from the highest concentration. Absolute excursions were calculated as the maximum value minus the minimum value for the 10-min periodicity means, and the relative amplitude was estimated as maximum excursion minus lowest value and divided by the maximum excursion for the 10-min periodicity means.

**Statistics.** The statistics dealing with data analysis are described above. All data in text and figures are given as means  $\pm$  SE, and a significance level of 5% was used if not otherwise stated. Student's 2-tailed paired and unpaired *t* tests were used to evaluate statistical significance. Statistical comparisons were performed using the statistical software package SPSS version 9.0 (SPSS).

## RESULTS

**Serum insulin and plasma glucose concentrations.** As expected, fasting plasma glucose concentrations were markedly lower in the control subjects versus the diabetic individuals ( $P < 0.01$ ), whereas serum insulin tended to be

increased only in the latter group (Table 1). Inspection of glucose concentration time-series (Fig. 1) revealed obvious but small glucose concentration changes during glucose entrainment versus more random glucose concentration changes during saline infusion. Absolute glucose excursions calculated as a 10-min periodicity mean observed during glucose entrainment (protocol 2) in control subjects ( $0.23 \pm 0.1$  mmol/l) and diabetic subjects ( $0.29 \pm 0.1$  mmol/l) were identical. During protocol 3, absolute glucose excursions were significantly augmented in the diabetic subjects ( $0.40 \pm 0.1$  mmol/l;  $P < 0.05$ ) compared with the control group. The relative glucose excursions were  $4.9 \pm 0.6\%$  (control),  $3.4 \pm 0.5\%$  (diabetic, protocol 2), and  $4.8 \pm 0.7\%$  (diabetic, protocol 3) (Fig. 2). In contrast to the rather small excursions in plasma glucose concentrations caused by glucose entrainment, changes in serum insulin concentration were much more pronounced in the healthy control subjects than in the

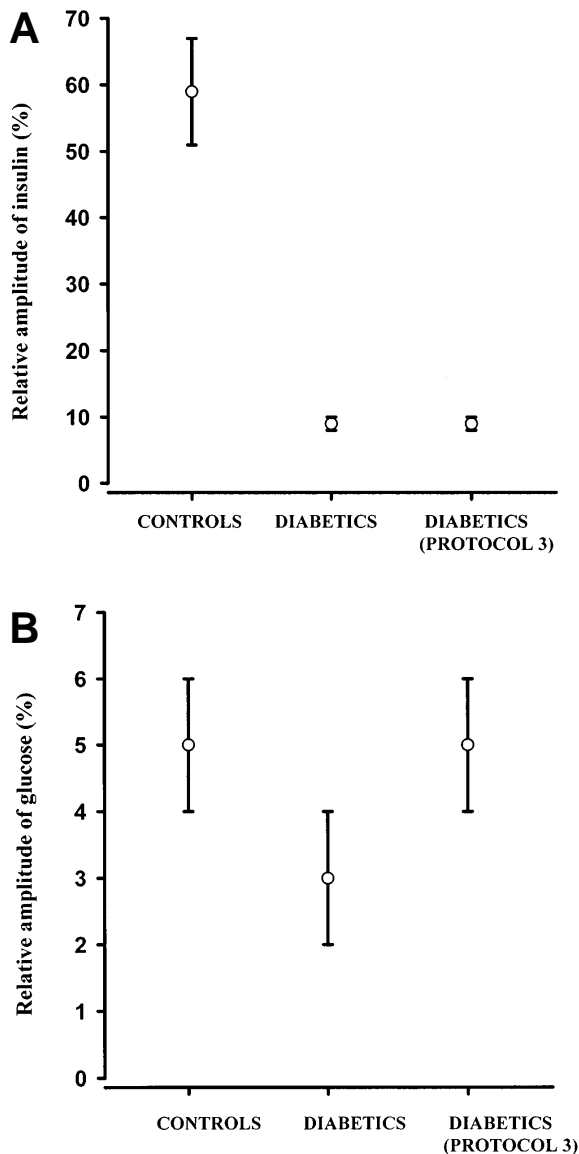


FIG. 2. The relative amplitude ( $\pm$  SE) of the 10-min periodicity for insulin (A) and glucose excursions (B) during glucose entrainment in control subjects and type 2 diabetic subjects. Protocol 3 indicates higher dose glucose infusion.

diabetic individuals (Fig. 1). Average serum insulin concentrations oscillated during glucose entrainment by  $19 \pm 3$  pmol/l ( $59 \pm 8\%$ ) in the healthy subjects, which was considerably augmented ( $P < 0.001$ ) compared with the oscillations in the diabetic subjects ( $4 \pm 1$  pmol/l [ $9 \pm 1\%$ ] and  $5 \pm 1$  pmol/l [ $9 \pm 1\%$ ] for protocol 2 and 3, respectively) (Fig. 2).

**Time-series analysis.** During saline administration (protocol 1), insulin oscillatory peaks at 10-min periodicity were comparable in the 2 groups irrespective of the time-series analysis used. Because data analysis assumes this periodicity, this comparison does not allow for any qualitative comparison of data pattern. Similarly, no significant differences in spectral density or autocorrelation analysis were detected using observed peaks. However, during glucose entrainment (protocol 2, 6 mg/kg), significant insulin peaks became manifest at 10 min periodicity in the control subjects using spectral analysis (normalized spectral density  $17.2 \pm 0.93$ , range

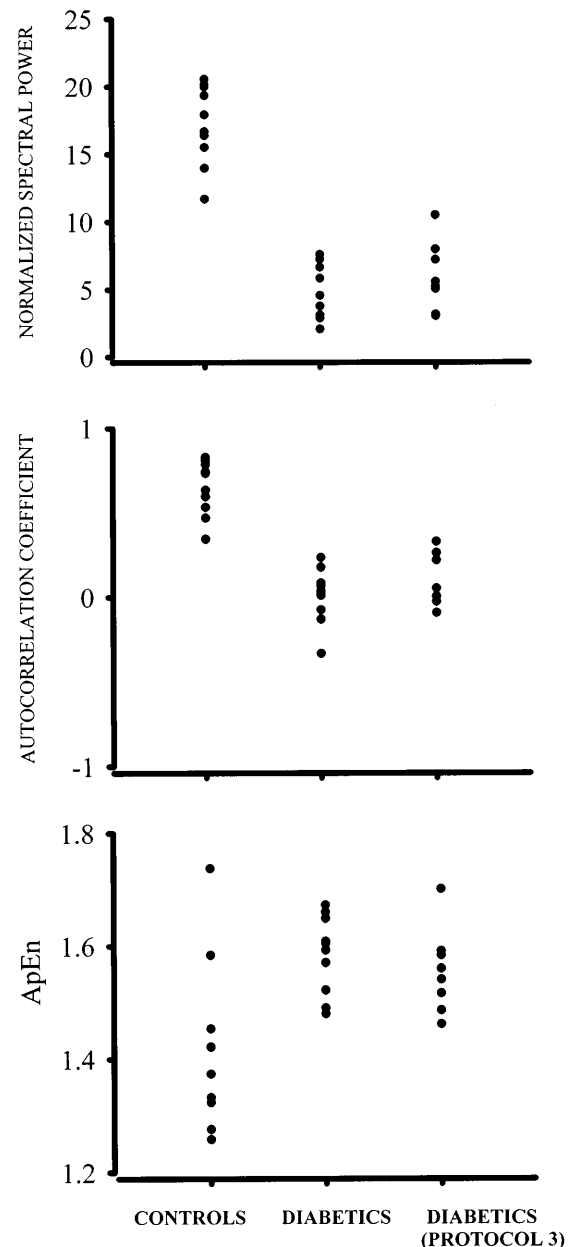


FIG. 3. Normalized spectral power, autocorrelation coefficient, and ApEn of serum insulin concentration time-series during glucose entrainment in control and diabetic subjects. There is complete separation of normal and diabetic data using spectral and autocorrelation analysis during both protocols. ApEn showed a statistically significant difference between the 2 groups ( $P < 0.01$ ), albeit with some overlap.

11.7–20.6% total power). This result was almost 4-fold higher ( $P < 0.001$ ) than the normalized spectral density present in type 2 diabetic subjects ( $4.76 \pm 0.62$ , range 2.08–7.6% total power). Of note, the glucose pulse entrainment resulted in complete separation between the 2 groups (Fig. 3). A similar pattern was observed when analyzing the glucose pulse-entrained serum insulin concentration time-series at a lag time of 10 min by autocorrelation analysis. The glucose-pulsed infusions induced large and synchronized insulin oscillations in the control subjects (autocorrelation coefficient  $0.65 \pm 0.05$ ; range 0.35–0.83), whereas the same regimen

failed to prompt oscillations in the type 2 diabetic subjects (autocorrelation coefficient  $0.01 \pm 0.05$ ; range  $-0.33$  to  $0.24$ ). Similar to spectral analysis, autocorrelation analysis led to a separation of the 2 groups without any overlap ( $P < 0.001$ ) (Fig. 3). Finally, glucose entrainment resulted in a higher degree of orderliness of the insulin concentration time-series in the control subjects than in the type 2 diabetic subjects, as assessed by ApEn ( $1.42 \pm 0.05$  [range  $1.26$ – $1.74$ ] vs.  $1.59 \pm 0.02$  [ $1.48$ – $1.67$ ],  $P < 0.005$ ) (Fig. 3). However, ApEn did not allow a complete separation of the 2 groups. Similar data were obtained using a coarser-scale assessment of subpattern persistence within the serum insulin time-series ( $r = 1.0$  SD) (data not shown).

There was no relationship among autocorrelation coefficients, spectral density peaks, and ApEn versus glycemic control in type 2 diabetic subjects, as assessed by fasting plasma glucose concentration or HbA<sub>1c</sub> ( $r < 0.51$  and  $P > 0.13$  in all calculations). Similarly, no relationship was found between the results of time-series analysis versus BMI ( $r < 0.47$  and  $P > 0.17$  in all calculations).

Cross-correlation analysis between plasma glucose and serum insulin concentrations during glucose entrainment showed a higher coefficient at a lag time of 10 min in the control group ( $0.47 \pm 0.06$ ) compared with that in the diabetic subjects ( $0.08 \pm 0.03$ ;  $P < 0.001$ ). In contrast, no difference was observed between the 2 groups during saline administration.

Entrainment with a larger glucose pulse (protocol 3, on average  $10.6$  mg/kg) in 8 of the diabetic subjects lead to an expected, almost identical, relative fractional plasma glucose excursion in type 2 diabetic subjects and control subjects ( $4.9$  vs.  $4.8\%$ ). Nevertheless, spectral density, autocorrelation coefficients, or regularity, as assessed by ApEn, were not altered significantly—the 2 former techniques again discriminating completely between the 2 groups (Fig. 3).

## DISCUSSION

In the present study, we examined high-frequency pulsatile insulin secretory patterns under basal conditions and during punctuated minimal glucose infusions at 10-min intervals in healthy and type 2 diabetic individuals. To compare the release patterns in diabetic and nondiabetic subjects, serum insulin concentration time-series were analyzed by complementary algorithms to quantify data regularity (spectral analysis, autocorrelation analysis, and ApEn). Insulin release during basal and glucose infusion conditions was compared to evaluate the possible merit of glucose pulse entrainment to detect secretory dysfunction in diabetes. The predictive value of insulin concentration time-series analysis to discriminate  $\beta$ -cell pathophysiology in type 2 diabetes was markedly improved by application of minimal glucose infusions. Indeed, glucose drive allowed complete separation of insulin secretory patterns in healthy and diabetic subjects, based on defined thresholds in spectral density and autocorrelation coefficients.

Entrainment of ultradian insulin oscillations by slow oscillatory infusions of exogenous glucose is now well established. A number of studies have demonstrated increasing failure of glucose entrainment in individuals with impaired glucose tolerance and type 2 diabetes (14,23,24). Conversely, a substantial improvement in  $\beta$ -cell responsiveness to oscillatory glucose infusion after in vivo antidiabetic treatment with troglitazone (25) and glucagon-like peptide 1 has been

reported (26). In contrast, literature on glucose entrainment of high-frequency insulin pulsatility in humans is limited. In a recent study, we evaluated the present model in healthy humans (17), but another study has lately examined glucose entrainment of rapid insulin pulsatility in type 2 diabetes (27). In line with our observations, the latter report demonstrated a diminished ability of glucose to entrain high-frequency insulin secretion in type 2 diabetes. However, clear differences emerge between the study of Mao et al. (27) and the current analysis in terms of design and results. First, in the former study,  $15$  mg/kg glucose was injected every 29 min, whereas we sought to mimic physiological glucose excursions more closely by injecting smaller amounts of glucose ( $6$  mg/kg) even more frequently (every 10 min). Second, unlike the study by Mao et al., our design completely separated control and diabetic subjects, not only by spectral analysis, as used in both protocols, but also by autocorrelation analysis.

It could be argued that the failure of type 2 diabetic individuals to respond normally to glucose oscillations is due to their increased glycemic level, which thus imposes potential differences on the enzyme kinetics crucial for the insulin release mechanism or alternatively imposes glucose toxicity. This issue can of course only be addressed in studies with type 2 diabetic individuals, in whom plasma glucose is (near)-normalized, but failure to demonstrate any relationship between glycemic level and abnormal insulin pulsatility makes this assumption less likely.

Although the mechanisms behind the development of type 2 diabetes are heterogeneous and complex and so far only understood rudimentarily, both insulin resistance and deranged insulin release are defects present in healthy first-degree relatives of type 2 diabetic individuals (28)—a group at a considerable risk of developing type 2 diabetes. Whereas many studies have demonstrated impaired insulin-stimulated glucose uptake in potentially prediabetic individuals, only fewer have defined abnormalities in insulin secretion per se (29–35). Studies revealing disturbed high-frequency pulsatile secretory patterns in first-degree relatives of type 2 diabetic individuals (33,34) suggest that an impairment of insulin pulsatility may be an early marker of  $\beta$ -cell dysfunction. However, a substantial overlap indicated that even rather complicated data analysis of frequently sampled insulin concentration time-series did not allow good prediction of diabetic versus nondiabetic insulin release patterns. The important aspect of the current study is the ability to separate the secretory patterns vividly by applying a punctuated minimal glucose stimulus every 10 min to entrain the coordinate release of insulin. This observation further implies that such methods of glucose pulse induction may be advantageous in studies in prediabetes and in mechanistic studies of metabolic and inherited abnormalities that cause  $\beta$ -cell dysfunction.

Although minimal glucose infusion may be informative as a tool in the evaluation of some aspects of  $\beta$ -cell performance, the observed defects in  $\beta$ -cell function might be linked to one or more distinct insulin secretory abnormalities. First-phase (and pulsatile) insulin secretion likely represent the amount of insulin that can be immediately released upon a sudden maximal glucose challenge, which presumably relates to granular stores of insulin residing at the  $\beta$ -cell membrane. In contrast, postprandial insulin concentration excursions result from the ability to mobilize and release

stored insulin upon stimulation by absorbed metabolites and by the entero-hormonal secretagogues/inhibitors released (such as glucagon-like peptide 1, gastric inhibitory polypeptide, somatostatin, and glucagon). Pulsatile insulin secretion reflects the oscillatory pattern of insulin release that may be controlled by neuronal factors (11,36) but, as demonstrated herein, may also be controlled by minimal glucose oscillations. Impaired insulin oscillations could mirror defects at the  $\beta$ -cell level, defects in coordinating the release, or impaired circulating glucose oscillations secondary to impaired insulin action. Based on the present data, it is unlikely that impaired glucose oscillations due to insulin oscillations caused defects in insulin pulsatility; similar glucose-enforced oscillations in diabetic and healthy subjects (both as absolute and relative changes) failed to entrain oscillatory insulin release in diabetic subjects only. Therefore, the insulin secretory defect in diabetes demonstrated here seems to correspond to an inability to sense and/or respond to small glucose concentration changes in diabetes. The defect may reside in one or more of the numerous biochemical steps involved in stimulus-secretion coupling. It therefore seems plausible that the minimal glucose infusion method may be able to monitor consequences of defects in  $\beta$ -cell glucose uptake, in glucose metabolism, and in ATP-dependent membrane depolarization with subsequent calcium mobilization.

In conclusion, a pronounced failure to respond adequately with regular oscillatory insulin secretion to recurrent high-frequency and (near)-physiological glucose excursions is a manifest feature of  $\beta$ -cell dysfunction in type 2 diabetes. Whether this abnormality is also present in the prediabetic state and thus could serve to predict type 2 diabetes remains to be clarified.

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