Glucose Entrainment of High-Frequency Plasma Insulin Oscillations in Control and Type 2 Diabetic Subjects

Catherine S. Mao, Nancy Berman, Karen Roberts, and Eli Ipp

Regular high-frequency oscillations of insulin secretion are characteristic of normal β-cell function. These oscillations are easily entrainable to an exogenous rhythm by small changes in glucose concentration in vitro. We tested whether high-frequency insulin oscillations in vivo would also be entrainable by glucose and whether a lack of entrainment would characterize the diabetic β-cell. We tested 13 control subjects and 11 patients with type 2 diabetes. Subjects underwent serial blood sampling at 1-min intervals for 60–120 min in the basal state or with small (15 mg/kg) boluses of glucose injected intravenously at exact 29-min intervals. Time series analysis was carried out using spectral analysis. Oscillations of basal plasma glucose concentrations were observed in both control and type 2 diabetic subjects, with a mean period of 11.3 ± 3.1 and 11.6 ± 2.0 min, respectively. These oscillations were entrained to mean periods of 15.0 ± 0.6 and 14.2 ± 0.9 min, respectively, by exogenous glucose. Regular high-frequency insulin oscillations were observed in control subjects; the mean period of basal plasma insulin oscillations was 10.7 ± 1.2 min and was entrained to exogenously injected glucose, with a period of 15.2 ± 0.1 min. In contrast, in the type 2 diabetic subjects, spontaneous insulin oscillations were unchanged by the glucose rhythm; the mean periods were 10.0 ± 1.0 min during the basal period, and 10.1 ± 0.0 min during glucose injections. These results demonstrate that spontaneous high-frequency insulin oscillations can be successfully entrained by glucose in control subjects. However, these oscillations in type 2 diabetic subjects are not similarly entrained. We conclude that loss of entrainment of spontaneous high-frequency insulin oscillations in type 2 diabetes is a highly sensitive manifestation of β-cell secretory dysfunction. *Diabetes* 48:714–721, 1999

Pulsatile rather than continuous secretion is common in many endocrine systems. Although the mechanisms for this process are not well understood, oscillatory secretion is believed to enhance the sensitivity of control systems (1). High-frequency oscillations of fasting plasma insulin concentrations are an example of a hormonal rhythm with a regular pattern. Insulin has been shown to oscillate with a period of 8–14 min in vivo. These high-frequency oscillations were demonstrated in nondiabetic humans, dogs, rats, and monkeys (2–10). Abnormalities in the normal oscillatory patterns have also been described in disease states. These have taken a number of different forms, including loss of regularity (11), a decrease in amplitude (12), or an alteration in frequency (13,14) of the high-frequency insulin oscillations. These abnormalities have been observed in patients with type 1 and/or type 2 diabetes, patients with insulinomas (15), and patients who have had pancreatic transplant (16), vagotomy, or Whipple's procedure (14).

It has also been suggested that a loss of regularity in high-frequency plasma insulin oscillations might represent an early secretory defect that can be demonstrated before the development of diabetes. Subjects predisposed to type 1 diabetes (17) or type 2 diabetes (18,19) were reported to have lost the regular pattern of high-frequency insulin oscillations seen in control subjects. The present study was designed to find a more sensitive methodology that might detect β-cell dysfunction before the loss of regularity in basal insulin oscillations in humans. We tested whether high-frequency insulin oscillatory activity is entrainable by glucose. In vitro studies using isolated rat islets have demonstrated spontaneous high-frequency insulin oscillations that were easily entrained to the rhythm of glucose pulses delivered at a different frequency (20). Also, low-frequency insulin oscillations are entrainable in normal subjects but not in diabetic subjects (21). However, high-frequency oscillations have not yet been shown to be entrainable in vivo. Using an approach designed specifically to entrain high-frequency pulses, we tested the susceptibility to entrainment of spontaneous high-frequency insulin oscillations by exogenous glucose in vivo in nondiabetic and type 2 diabetic subjects.

RESEARCH DESIGN AND METHODS

Subjects. We studied 13 control subjects and 11 type 2 diabetic subjects. The control group consisted of four women and nine men aged (mean ± SD) 37.0 ± 12.5 years (range 23–63) whose BMI (calculated as weight in kilograms divided by the square of height in meters) was 25.5 ± 4.9 (20.1–39.1). The type 2 diabetic subjects consisted of seven women and four men with a diabetes duration of 1–15 years, a mean age of 43.5 ± 14.9 years (25–68), and a mean BMI of 32.9 ± 5.3 (23.2–41.0). All studies were performed at the General Clinical Research Center at Harbor-UCLA Medical Center. All patients gave their informed consent to participate in these studies, which were approved by the institutional review board.

Procedures. Control and diabetic subjects were studied after an overnight fast. Subjects with type 2 diabetes were treated with either diet alone or oral hypoglycemic agents. Type 2 diabetic subjects treated with oral hypoglycemic agents stopped using these medications for 7 days before being tested. All subjects were instructed to maintain a diet containing at least 200 g/day of carbohydrates for the 3 days preceding the study. All studies were initiated between 9:00 and 10:00 A.M. Subjects assumed a resting recumbent position and remained in that position throughout the test period. Blood was sampled through a 23-gauge needle that was inserted retrograde in a dorsal hand vein, heated for arterialization (22). For the

From the Departments of Medicine (K.R., E.I.) and Pediatrics (C.S.M., N.B.), Harbor-UCLA Medical Center, Torrance, California.

Address correspondence and reprint requests to Eli Ipp, MD, Harbor-UCLA Medical Center, 1000 W. Carson St., Torrance, CA 90509-2910. E-mail: ipp@harbor2.humc.edu.

Received for publication 9 February 1998 and accepted in revised form 4 December 1998.

entrainment studies, subjects had cannulation of a deep vein in the contralateral arm for glucose injections. After a 15- to 30-min rest period, subjects underwent arterialized venous blood sampling at 1-min intervals for up to 180 min. Samples were placed on ice immediately, centrifuged at –4°C within 1 h and stored at –20°C immediately after separation.

Protocols. Two basic protocols were used: a basal study and an entrainment study. For the basal study, subjects were studied after an overnight fast, and serial sampling was performed as described above. In addition, subjects received a bolus of normal saline at 29-min intervals during an early set of basal studies, as a control for the entrainment protocol. Three control subjects and two type 2 diabetic subjects participated in this control protocol. Insulin oscillations observed during this protocol were compared with the spontaneous insulin oscillations from a group of non–saline-injected subjects during the basal studies. After it had been established that the normal saline injections had no effect on spontaneous insulin oscillations, saline injections during the basal studies were discontinued, and the results of all basal studies were combined for presentation purposes.

For the entrainment study, small boluses of glucose were injected intravenously at exact 29-min intervals during serial sampling. This frequency of injections was calculated to allow plasma glucose concentrations to return to baseline before each subsequent injection so that overall glucose concentrations would remain stable and an upward drift would be prevented. Glucose injections consisted of a 25% dextrose solution given over a period of 30 sec and flushed with 5 ml normal saline. As in the basal studies, serial sampling at 1-min intervals was performed.

Three different approaches were used in the entrainment studies, and the results were later combined. The studies varied as follows.

Dose-response entrainment study. Initially, a 120-min entrainment study was performed. The entrainment study consisted of four bolus injections of glucose. The dose of the boluses was determined in the initial dose-response studies, using a range of doses from 10 to 40 mg/kg. An optimal dose was chosen thereafter to cause the smallest possible perturbation in insulin concentrations in response to the glucose bolus. Dose-response studies were carried out in both control (n = 3) and diabetic (n = 4) subjects. Once the dose-response studies were completed, the protocol was changed to deliver a constant dose of glucose (15 mg/Kg) in sequential bolus injections. Because of the long duration of this study, another (120-min) entrainment study using 1-min sampling could not be carried out consecutively. Also, because of the large amount of blood required, 120-min basal and entrainment studies were usually performed in different subjects.

Combined basal and entrainment study. The duration of the combined basal and entrainment study was 180 min. This study enabled comparison of oscillations in the basal state (60 min) and during entrainment (120 min) within an individual subject in consecutive fashion, yet it did not exceed blood volume restrictions. The entrainment part of the study consisted of four glucose bolus injections in 120 min. The combined study was performed in eight control subjects and four diabetic subjects.

Abbreviated study. Finally, in a few additional subjects, an attempt was made to determine whether shortening the duration of the experiments to a total of 120 min for a combined basal (60 min) and entrainment (60 min) study, would provide the same results as a full 180-min study. The entrainment part of the study consisted of two glucose bolus injections in 60 min. This abbreviated study was a first attempt to find more clinically relevant methods of evaluating entrainment. It made the study much less laborious and therefore easier on subjects and personnel. Three normal subjects participated in a 120-min combined basal and entrainment study.

Analysis. All samples from an individual study were measured in duplicate in the same assay. Plasma glucose was measured using the hexokinase method with an autoanalyzer (Abbott, Abbott Park, IL) (23). Intra-assay coefficient of variation was 1–2%. Plasma insulin was measured by sensitive radioimmunoassay, which is a modification of a previously described assay (24). The intra-assay coefficient of variation for the insulin assay was 5–6% with a lower limit of sensitivity of 1 μU/ml.

Statistical analysis. The time series results for individual data sets were smoothed using a 3-point moving average to reduce rapid fluctuations in the data due to assay or experimental noise. Time series analysis was performed using spectral analysis with SAS software (SAS Institute, Cary, NC). Any linear time trends and low-frequency oscillations in plasma glucose and insulin were eliminated using regression analysis (detrending) to filter out peaks with periods >20 min. Spectral analysis results were presented as the spectral power of the dominant peak expressed as the percentage of total power in the time series. Differences between groups were evaluated using Student's t test. Fisher's test was used to determine whether the dominant peaks were significantly different from noise (25). Data are presented as means ± SD.

RESULTS

Subject characterization. Table 1 shows the demographic information and fasting plasma glucose and insulin for each subject. The mean fasting plasma glucose was 88 ± 6.8 mg/dl for the control subjects and 198 ± 48.2 mg/dl for the diabetic subjects (P < 0.00002). The mean fasting plasma insulin was 7.1 ± 5.5 μU/ml for the control subjects and 29.5 ± 18.5 μU/ml for the diabetic subjects (P = 0.013).

Basal studies. During the basal studies, oscillations of both plasma glucose and plasma insulin concentrations were observed in control and type 2 diabetic subjects (Fig. 1 and Table 2). Figure 1 shows the time series of the 3-point moving average of basal plasma glucose and insulin concentrations for a representative control subject and a representative type 2 diabetic subject in 120-min studies. The mean period of basal plasma glucose oscillations was 11.3 ± 3.1 min in control subjects and 11.6 ± 2.0 min in type 2 diabetic subjects as determined by spectral analysis (P < 0.05 in all basal studies). The mean period of basal plasma insulin oscillations was 10.7 ± 1.2 min in the control subjects and 10.0 ± 1.0 min in the type 2 diabetic subjects as determined by spectral analysis (P < 0.05 in all basal studies). The results of spectral analysis using 3-point moving averages of plasma glucose and insulin concentrations in basal studies for all subjects are also summarized in Table 2. Every subject showed at least one peak on spectral analysis that was significantly different from noise (P < 0.05). Percentage of total power ranged from 12.7 to 74.4%. Results of the 60-min basal studies were not different from the 120-min basal studies. Data from all basal studies are therefore combined in Table 2.

The control subjects in whom saline, instead of glucose, was injected as a bolus during the basal protocol showed no differences in glucose or insulin oscillations compared with subjects who were not injected with saline. The mean period of insulin oscillations in saline-injected control subjects (n = 3) versus that in non–saline-injected subjects (n = 7) was 10.8 ± 1.7 vs. 10.7 ± 0.9 min, respectively. The mean period was 9.7 ± 0.6 min in saline-injected diabetic subjects (n = 2) versus 10.1 ± 1.2 min in non–saline-injected subjects (n = 7). After an early preliminary analysis revealed no significant difference, saline injections were discontinued.

Entrainment studies

Glucose boluses. In response to sequential glucose bolus injections, corresponding peaks occurred in plasma glucose concentrations (Figs. 2 and 3). The change in plasma glucose concentrations in response to the exogenously administered glucose boluses was 24.5 ± 9.9% in control subjects and 21.3 ± 13.7% in type 2 diabetic subjects. These percentages corresponded to a mean change in glucose concentration of 21.8 ± 9.0 mg/dl in control subjects and 37.0 ± 2.3 mg/dl in type 2 diabetic subjects. As predicted, plasma glucose concentrations fell back to baseline before administration of the next glucose bolus, so that mean plasma glucose and insulin levels at the end of the study were similar to starting levels (Table 3).

Glucose oscillations. Oscillations of plasma glucose concentrations were entrained by exogenous glucose in both control and diabetic subjects. Figures 2 and 3 show time series of the 3-point moving average of the plasma glucose response to serial incremental (Fig. 2*A* and *B*) or fixed-dose (Fig. 3*A* and *B*) glucose boluses for representative control and diabetic subjects. Smaller secondary peaks follow the peaks from the glucose boluses ~15 min after the injection in both the normal (Figs. 2*A* and 3*A*) and diabetic (Figs. 2*B* and 3*B*) subjects. The mean entrainment period (Table 3) was found to be 15.0 ± 0.6 min in control subjects (P < 0.003 vs. basal study) and 14.2 ± 0.9 min (P < 0.005 vs. basal study) in type 2

TABLE 1
Subject characteristics

Subjects	Sex	Age (years)	BMI	Fasting plasma Glucose (mg/dl)	Fasting plasma Insulin (μU/ml)
Control					
1	F	25	21.54	79	2.7
2	M	41	25.07	88	5.1
3	M	23	27.20	106	21.5
4	M	33	24.10	85	5.8
5	M	30	24.67	97	13.6
6	M	28	23.37	86	6.1
7	F	26	20.06	88	11
8	M	29	24.20	87	5.1
9	M	54	24.74	89	2.9
10	M	42	22.53	89	5.7
11	F	63	23.19	89	4.1
12	F	36	39.10	83	7.3
13	M	51	31.28	82	1.2
Mean ± SD	—	37.9 ± 12.5	25.5 ± 4.9	88 ± 6.8	7.1 ± 5.5
Diabetic					
14	F	66	27.96	178	24.1
15	M	61	28.38	153	16.2
16	M	32	35.53	213	49.1
17	F	36	32.02	155	13.9
18	M	68	23.20	213	10.9
19	F	41	40.00	148	69
20	F	36	30.00	187	27.8
21	F	46	35.80	218	20.6
22	F	25	41.05	308	11.6
23	M	31	33.42	243	44
24	F	36	34.84	161	37.2
Mean ± SD	—	43.5 ± 14.9	32.9 ± 5.3	198 ± 48.2	29.5 ± 18.5

TABLE 2
Results of spectral analysis in control and type 2 diabetic subjects in the basal state

Subject	Basal glucose Period (min)	Basal glucose Power (%)	Basal insulin Period (min)	Basal insulin Power (%)
Control				
2	10.2	32.8	10.2	41.9
3	10.2	25.9	8.7	35.6
4	10.2	24.3	12.2	35.7
5	6.1	19.3	12.2	34.13
6	10.1	25.1	12.1	23.2
7	17.3	25.1	12.1	22.4
8	8.1	15.2	10.1	38.4
10	12.2	25.3	10.2	74.4
11	15.3	41.2	10.2	58.7
12	12.2	24.0	10.2	46.0
13	12.2	47.3	10.2	37.3
Mean ± SD	11.3 ± 3.1	27.8 ± 9.3	10.7 ± 1.2	40.7 ± 14.0
Diabetic				
14	15.1	12.7	10.1	46.9
15	13.4	28.0	9.3	28.6
18	11.6	39.9	10.1	33.2
19	10.1	21.4	10.1	51.0
20	8.4	14.8	8.4	28.6
21	11.2	23.6	9.2	38.3
22	10.2	29.4	10.2	60.1
23	12.2	40.6	12.2	37.7
24	12.2	27.8	10.2	58.7
Mean ± SD	11.6 ± 2.0	26.5 ± 9.7	10.0 ± 1.0	42.6 ± 12.1

diabetic subjects as determined by spectral analysis. The mean period for glucose oscillations (Table 3) determined by spectral analysis corresponds with peaks observed in the time series data (e.g., Figs. 2*A* and *B* and 3*A* and *B*).

Insulin oscillations. Plasma insulin concentrations in control subjects responded with a rapid increase that followed each glucose bolus, resulting in a series of insulin peaks that fell rapidly to baseline (Figs. 2*C* and 3*C*). A smaller secondary peak of insulin followed, also about halfway between the larger glucose-induced peaks. Figures 2*C* and 3*C* show time series of the 3-point moving average of the plasma insulin response to serial incremental glucose boluses (Fig. 2) or fixed-dose glucose boluses (Fig. 3) for representative control subjects. Both protocols were associated with an obvious change in the period of spontaneous insulin oscillations in control subjects. High-frequency insulin oscillations were entrained to the exogenously injected glucose, with a mean period of 15.2 ± 0.1 min ($P < 0.00001$ versus the basal studies when all data were pooled [Table 3]). In contrast, glucose boluses did not result in any discernible acute insulin secretory response in the type 2 diabetic subjects. Furthermore, spontaneous insulin oscillations were unchanged by exogenous glucose (Figs. 2*D* and 3*D*). The mean insulin period in the type 2 diabetic subjects was 10.1 ± 0.0 min with exogenous glucose ($P = 0.8$ versus the basal studies [Table 3]).

We also evaluated whether the protocol variations influenced the results in any way. We tested whether the dose-response characteristics of glucose injection in the earlier studies or shortening of the study duration might have influenced entrainment of insulin oscillations. There was no significant difference in the results of studies in which glucose was injected in dose-response fashion compared with fixed-dose boluses. Also, when the protocol was shortened to a 60-min entrainment period and fixed-dose boluses were used, data were not significantly different from those of the longer, 120-min entrainment protocol. In the entrainment studies in control subjects, the mean insulin periods were 15.1 ± 0, 15.2 ± 0.1, and 15.3 ± 0 min in the dose-response 120-min ($n = 3$), the fixed-dose 120-min ($n = 8$), and the fixed-dose 60-min ($n = 3$) protocols, respectively. In entrainment studies in type 2 diabetic subjects, the mean insulin periods were 10.1 min in both dose-response ($n = 4$) and fixed-dose ($n = 4$) 120-min protocols. An example of the similarity is provided by comparing the time series illustrated in Figs. 2 and 3, which demonstrate the results of dose-response (Fig. 2) and fixed-dose (Fig. 3) boluses in control and type 2 diabetic subjects. Because there were no significant differences in glucose or insulin oscillations using the different protocols, data were combined for presentation in Table 3.

Spectral analysis. The results of spectral analysis performed using 3-point moving averages of plasma glucose and insulin concentrations in entrainment studies for all subjects are also summarized in Table 3. In every subject, the predominant peak shown in the table was significantly different from noise ($P < 0.05$) in all entrainment studies. Percentage of total power ranged from 17.0 to 67.8%. Figures 4 and 5 illustrate the power spectra of insulin concentrations for representative examples of the different protocols used in this study. In

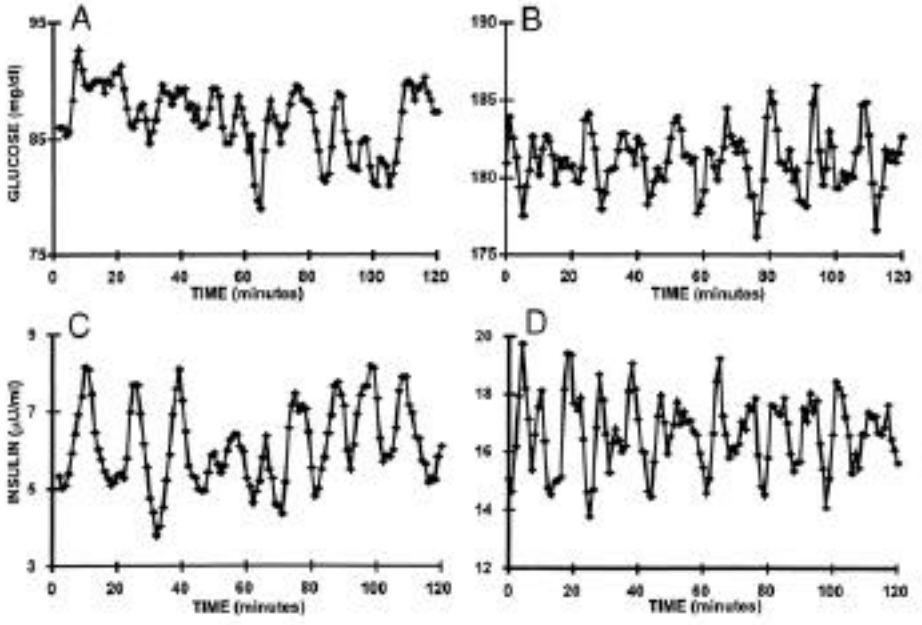

FIG. 1. Basal study results shown as the 3-min moving average of basal plasma glucose and insulin concentrations in a control (*A* and *C*) and a diabetic (*B* and *D*) subject. The data are detrended. Note the differences in scale between the data of the two subjects.

Fig. 4 (normal subjects), a predominant peak at ~10 min was observed in the basal study, but in entrainment studies, the predominant spectral peak was at 15 min, irrespective of duration or consistency of glucose dosing. However, in the diabetic subjects (Fig. 5), there was no change in the pattern of spectral analysis of insulin oscillations when glucose was injected. The predominant peak remained stable at ~10 min, irrespective of whether a basal or an entrainment study is demonstrated or whether the dose or duration of the study is varied.

DISCUSSION

This study was initiated to determine whether abnormalities in high-frequency insulin oscillations could be used as a sensitive measure of abnormal β-cell function. Previous studies had demonstrated a lack of regularity in high-frequency insulin oscillations in subjects with type 2 diabetes, in subjects with early type 1 diabetes, or in relatives of people with type 2 diabetes (11,17–19). Group differences were clearly defined in those reports; however, overlap existed in the degree of regularity of oscillations of individuals when subjects with diabetes were compared with normal volunteers (11,17–19). Some normal subjects had irregularities observed in their oscillations, and some diabetic patients or subjects at risk for diabetes demonstrated regularity of insulin oscillations, as previously emphasized (17). Thus, basal plasma insulin oscillations do not appear to be a good test of individual risk for disease in any one subject because of the likelihood of low sensitivity and specificity, although these values were not previously calculated.

We therefore considered the possibility that entrainment of high-frequency insulin oscillations might be a better approach

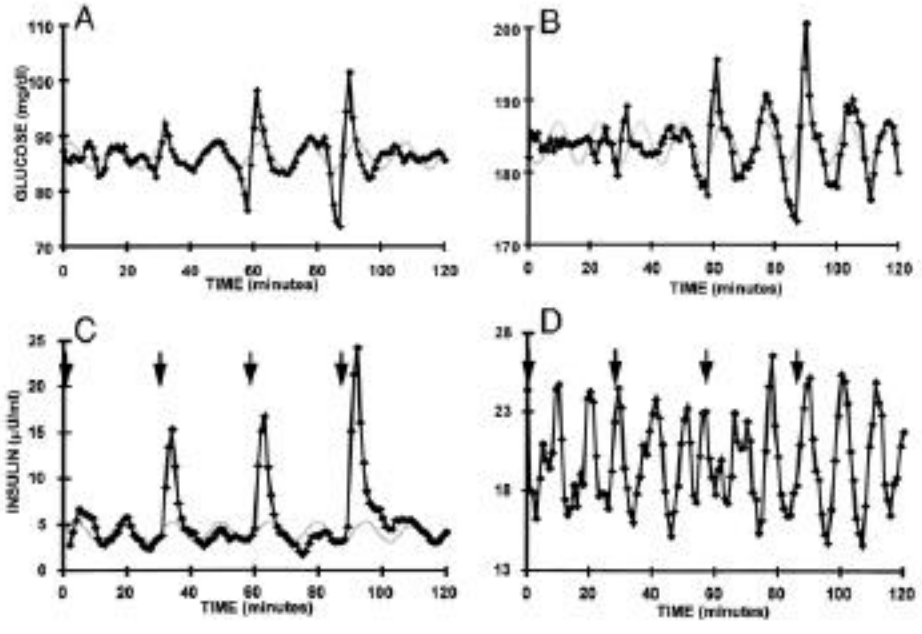

FIG. 2. Entrainment study results shown as the 3-min moving average of plasma glucose and insulin concentrations in representative control (*A* and *C*) and diabetic (*B* and *D*) subjects injected with serial incremental intravenous glucose boluses (*arrows*) at 29-min intervals. The data are detrended and presented with a fitted sine wave, using the frequency and amplitude of the dominant period, as determined by spectral analysis. Note the differences in scale between the data of the control and diabetic subjects.

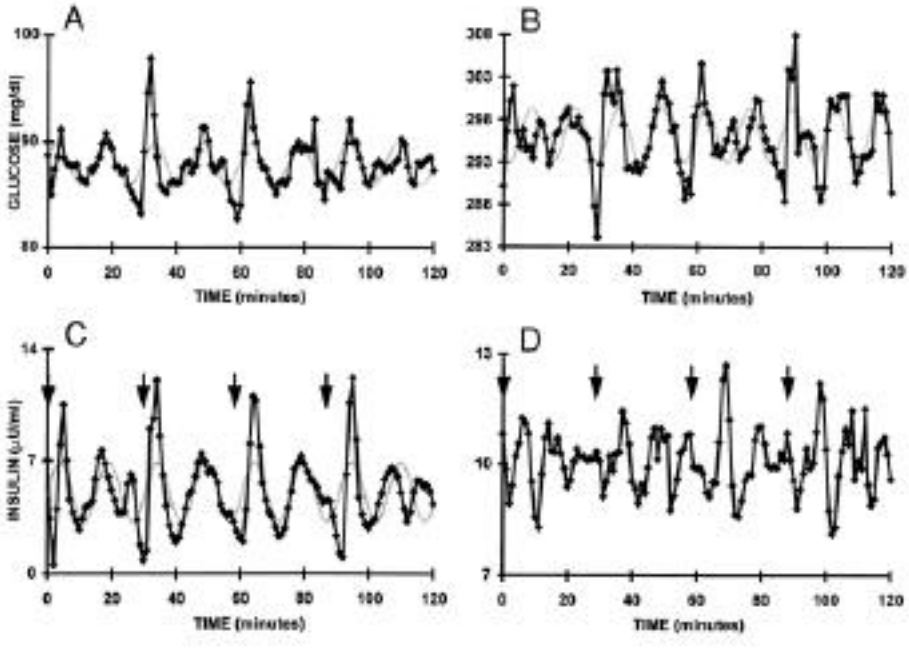

FIG. 3. Entrainment study results shown as the 3-min moving average of plasma glucose and insulin concentrations in representative control (*A* and *C*) and diabetic subjects (*B* and *D*) injected with fixed-dose intravenous glucose boluses (15 mg/kg) (*arrows*) at 29-min intervals. The data are detrended and presented with a fitted sine wave, using the frequency and amplitude of the dominant period, as determined by spectral analysis. Note the differences in scale between the data of the control and diabetic subjects.

to determining abnormal β-cell function. This premise was based on the findings in vitro that the normal β-cell appears to be very sensitive to entrainment by glucose. In studies of isolated rat islets, a shift of the predominant period of insulin oscillations was observed when islets were exposed to pulsatile glucose delivery at a different frequency, even though the amplitude of glucose oscillations was quite small (20). The present study confirms the hypothesis that high-frequency insulin oscillations can be entrained to exogenous glucose in normal subjects.

The period of basal insulin oscillations in control subjects studied without any exogenous glucose demonstrated variability among subjects, although it remained within a fairly tight range of 8–12 min. In contrast, in response to glucose, the period of the insulin oscillations was entrained to a single period. The lack of variation in the period of the glucose-

TABLE 3
Results of spectral analysis in control and type 2 diabetic subjects in the entrainment studies

	Bolus glucose		Bolus insulin		Glucose (mg/dl)*		Insulin (μU/ml)*	
Subjects	Period (min)	Power (%)	Period (min)	Power (%)	Start	End	Start	End
Control								
1	15.1	22.9	15.1	25.9	79	87	2.7	4.5
2	15.3	44.3	15.3	34.7	88	81	5.1	2.2
3	15.1	24.8	15.1	27.0	106	98	21.5	15.6
4	15.3	54.3	15.3	37.4	88	84	4.5	6.7
5	15.3	35.1	15.3	29.2	96	100	13.0	8
9	15.1	31.1	15.1	26.7	89	97	2.9	3.5
10	15.1	37.5	15.1	33.1	85	85	3.5	2.5
11	13.1	17.0	15.3	31.4	88	86	3.8	1.8
12	15.1	20.9	15.1	23.8	82	81	3.8	5.2
13	15.1	23.1	15.1	24.3	82	78	1.1	1.0
Mean ± SD	15.0 ± 0.6	31.1 ± 11.8	15.2 ± 0.1	29.4 ± 4.6	88.3 ± 7.8	87.7 ± 7.8	6.2 ± 6.3	5.1 ± 4.3
Diabetic								
14	15.1	29.2	10.1	33.0	178	153	24.1	24.6
15	13.4	21.1	10.1	23.5	153	147	16.2	10.3
16	15.1	17.0	10.1	39.8	213	214	49.1	37.6
17	13.4	24.9	10.1	32.0	155	160	13.9	15.9
22	13.4	26.6	10.1	37.3	307	275	7.7	13.8
23	13.4	26.3	10.1	67.8	202	167	38.8	39.8
24	15.1	19.4	10.1	27.9	143	135	28.8	23
Mean ± SD	14.2 ± 0.9	23.6 ± 4.5	10.1 ± 0.0	37.3 ± 4.5	193.0 ± 56.6	178.7 ± 49.3	25.5 ± 14.6	23.7 ± 11.5

*Plasma concentrations at the start and end of the entrainment experiments. The data demonstrate that repeated glucose boluses do not induce an overall trend in glucose or insulin concentrations.

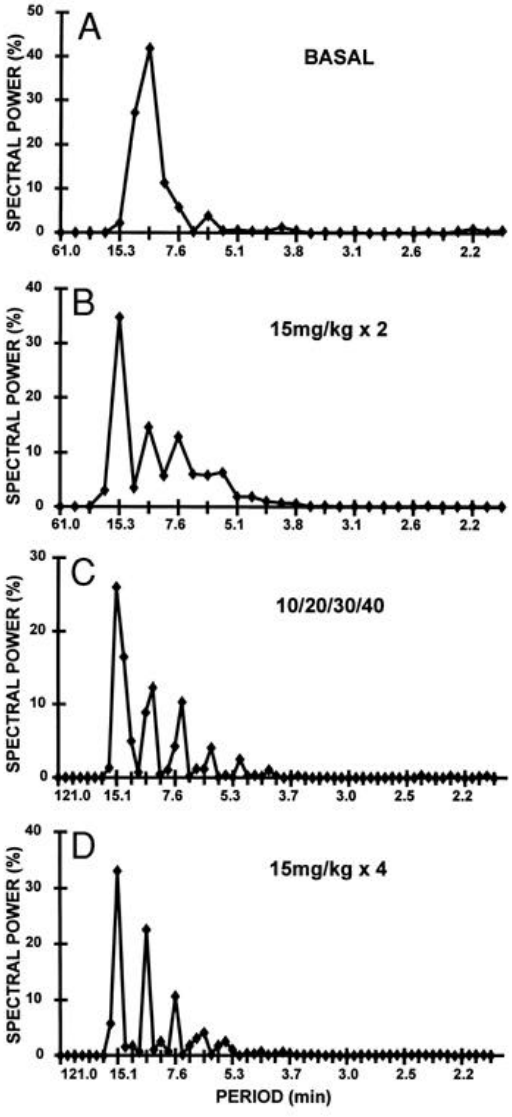

FIG. 4. Results of spectral analysis of plasma insulin oscillations in normal subjects, presented as percentage of total power. *A*: Spectrum during a basal study. *B*: Spectrum of the same control subject as in *A* during a 60-min entrainment study (time series not shown). *C*: Spectrum of a control subject during an entrainment study using dose-response bolus injections (corresponds to the insulin time series of the normal control subject of Fig. 2*C*). *D*: Spectrum of a control subject during an entrainment study using repeated fixed-dose bolus injections (corresponds to the insulin time series of the normal control subject of Fig. 3*C*). Note the consistent shift in the dominant period in *B*–*D*, compared with *A*. Note that scales on the abscissas of *C* and *D* differ from those of *A* and *B* and represent a different duration of the time series (60 min for *A* and *B* and 120 min for *C* and *D*; see METHODS for explanation).

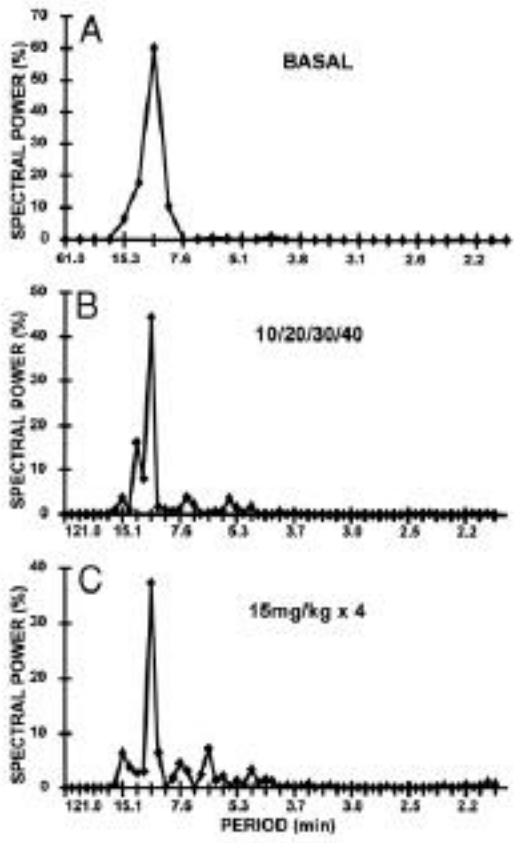

FIG. 5. Results of spectral analysis of plasma insulin oscillations in diabetic subjects, presented as percentage of total power. *A*: Spectrum of a diabetic subject during the basal study (corresponds to the spectral analysis of the diabetic subject in 5*C*). *B*: Spectrum of a diabetic subject during the entrainment study using dose-response bolus injections (corresponds to the insulin time series of diabetic subject in Fig. 2*D*). *C*: Spectrum of a diabetic subject during an entrainment study using repeated fixed-dose bolus injections (corresponds to the insulin time series of diabetic subject in Fig. 3*D*). Note the lack of any shift in the dominant period in *B* and *C* (entrainment study) compared with *A* (basal study). Note that scales on the abscissas of *A* differ from those of *B* and *C* and represent a different duration of the time series (60 min for *A* and 120 min for *B* and *C*; see METHODS for explanation).

injected group indicates an external source that successfully entrained the oscillations to a given frequency, in this case a harmonic of the frequency of the glucose boluses, i.e., ~15 min. The results of this study demonstrate that a relatively simple methodology results in entrainment of normal high-frequency insulin oscillations to the newly induced glucose oscillatory rhythm in control subjects. This response of insulin oscillations to entrainment by glucose in control subjects indicates that a complete resetting of the endogenous oscillatory process has taken place. The period of the insulin oscillations is quite different from the basal period and follows closely the alterations in the glucose oscillatory pattern. These alterations include a shift in phase initiated by the first glucose bolus and a subsequent pattern of large and small alternating peaks of plasma insulin that appear tightly regulated by the alterations in glucose.

The mechanisms responsible for high-frequency insulin oscillations or their entrainment by glucose are not understood. In vitro studies of these spontaneous insulin oscillations in isolated islets suggest that oscillations of glycolysis may be the underlying pulse generator (26,27), in which case a sudden, rapid increase of glucose delivered to the β-cell (after bolus injection) may override the ongoing oscillatory activity in the glycolytic pathway and reset the phase and frequency of the oscillations. In diabetes, there may be a failure of glycolytic oscillations to be entrained by glucose. It could also be argued that entrainment in the context of this experimental model is related to the magnitude of insulin secreted per pulse in response to glucose (9,10). Because dilution and hepatic extraction reduce the insulin concentrations in the peripheral circulation, failure of entrainment could then be a failure of the pulse mass or the amplitude

of insulin release to be reflected in the peripheral circulation in type 2 diabetic patients, whose insulin response to glucose is known to be impaired. However, the entrainment failure that is observed in these studies is not simply a function of the failure to increase the magnitude of insulin secretion in response to glucose. The fact that there was no observable response in frequency or phase of oscillations in the type 2 diabetic subjects suggests a complete failure to respond to all the elements of entrainment.

The lack of entrainment of high-frequency insulin oscillations to the exogenous glucose stimulus was seen universally in every type 2 diabetic subject tested, despite plasma glucose responses that were similar to those of control subjects. The lack of a shift in phase or period of the insulin oscillations indicates failure of glucose recognition by the β-cell of the process(es) regulating both of these oscillation parameters. These findings are supported by previous studies of insulin oscillations that occur in the low-frequency range, which also showed that entrainment was abnormal in diabetic subjects (21,28). The mechanisms for low-frequency oscillations (29) are different from the high-frequency type (26,27) evaluated in this study, so that the impact of glucose entrainment may not necessarily be the same. Nevertheless, entrainment failure has now been observed in both high- and low-frequency insulin oscillations, which suggests that failure to entrain may be a fundamental defect of the β-cell in type 2 diabetes. However, we cannot yet rule out the possibility that lack of entrainment could be due to β-cell dysfunction secondary to chronically elevated plasma glucose concentrations, i.e., glucotoxicity (30), rather than to an inherent defect in β-cell function.

In our experience with type 2 diabetic subjects from southern California, we have seen significant regularity in basal high-frequency insulin oscillations in many subjects (31), in contrast to previous studies. In fact, the results of this study demonstrate that plasma insulin concentrations in type 2 diabetic subjects oscillate with a sustained regular period of 8–13 min per cycle and at a frequency and spectral power that are similar to those in control subjects. This finding differs from the previously published results of Lang et al. (11) in subjects with diet-controlled diabetes who were found to have brief irregular oscillations in basal plasma insulin concentrations compared with regular oscillations in control subjects. The reason for this difference is unknown but may be explained by differences in the study population or by the heterogeneity inherent in type 2 diabetes. Our type 2 diabetic subjects differed from those studied by Lang et al. (11) in that they were overweight (the mean BMI was 32.9 versus a mean percentage body weight of 106%), they had higher mean fasting plasma insulin levels (29.5 vs. 9.1 μU/ml), and most were Hispanic.

In summary, the results of this study demonstrate that regular high-frequency insulin oscillations can be demonstrated in both control and type 2 diabetic subjects. Furthermore, exogenous glucose results in entrainment of these spontaneous high-frequency insulin oscillations in control subjects. However, high-frequency oscillations in type 2 diabetic subjects are not similarly entrained. This finding represents a distinct and striking difference from the responsiveness of the β-cell in normal subjects. We conclude that this simple approach to entrainment of insulin is effective in consistently distinguishing between the high-frequency insulin oscillations of control and diabetic subjects, and it may be a useful marker for β-cell dysfunction in vivo.

ACKNOWLEDGMENTS

These studies were supported in part by a grant from the National Institutes of Health to support the General Clinical Research Center at Harbor-UCLA Medical Center (MO1-RR-00425) and the Endocrine Fellows Foundation.

The authors are indebted to the nurses, dietary staff, and core laboratory technicians of the Clinical Study Center at Harbor-UCLA Medical Center for their excellent assistance in the performance of these studies.

REFERENCES

1. Weigle DS: Pulsatile secretion of fuel-regulatory hormones. *Diabetes* 36:764–775, 1987
2. Hansen BC, Jen KC, Pek SB, Wolfe RA: Rapid oscillations in plasma insulin, glucagon, and glucose in obese and normal weight humans. *J Clin Endocrinol Metab* 54:785–792, 1982
3. 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
4. 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
5. Chou HF, McGivern R, Berman N, Ipp E: Oscillation in circulatory plasma insulin concentration in the rat. *Life Sci* 48:1463–1469, 1991
6. 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
7. Goodner CJ, Hom FG, Koerker DJ: Hepatic glucose production oscillates in synchrony with the islet secretory cycle in fasting Rhesus monkeys. *Science* 215:1257–1259, 1982
8. Jaspan JB, Lever E, Polonsky KS, Van Cauter E: In vivo pulsatility of pancreatic islet peptides. *Am J Physiol* 251:E215–E226, 1986
9. 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
10. Porksen N, Nyholm B, Veldhuis J, Butler P, Schmitz O: In humans at least 75% of insulin secretion arises from punctuated insulin secretory bursts. *Am J Physiol* 273:E908–E914, 1997
11. 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
12. Goodner CJ, Koerker DJ, Weigle DS, McCulloch DK: Decreased insulin- and glucagon-pulse amplitude accompanying β-cell deficiency induced by streptozocin in baboons. *Diabetes* 38:925–931, 1989
13. 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
14. Matthews DR, Lang DA, Burnett MA, Turner RC: Control of pulsatile insulin secretion in man. *Diabetologia* 24:231–237, 1983
15. Berman N, Genter P, Chou HF, Cortez C, Bowsher R, Ipp E: Erratic oscillatory characteristics of plasma insulin concentrations in patients with insulinoma: mechanism for unpredictable hypoglycemia. *J Clin Endocrinol Metab* 82:2899–2903, 1997
16. O'Meara NM, Struris J, Blackman JD, Byrne MM, Jaspan JB, Roland DC, Thistlethwaite JR, Polonsky KS: Oscillatory insulin secretion after pancreas transplant. *Diabetes* 42:855–861, 1993
17. Bingley PJ, Matthews DR, Williams AJK, Bottazzo GF, Gale EAM: Loss of regular oscillatory insulin secretion in islet cell antibody positive non-diabetic subjects. *Diabetologia* 35:32–38, 1992
18. 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
19. Schmitz O, Porksen N, Nyholm B, Skjaerbaek C, Butler P, Veldhuis J, Pincus SM: Disorderly and nonstationary insulin secretion in relatives of patients with NIDDM. *Am J Physiol* 272:E218–E226, 1997
20. Chou HF, Ipp E: Pulsatile insulin secretion in isolated rat islets. *Diabetes* 39:112–117, 1990
21. 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
22. Ipp E, Forster B: Sparing of cognitive function in mild hypoglycemia: dissociation from the neuroendocrine response. *J Clin Endocrinol Metab* 65:806–810, 1987

23. Genter PM, Ipp E: Accuracy of plasma glucose measurements in the hypoglycemic range. *Diabetes Care* 17:595–598, 1994
24. Yalow RS, Berson SA: Immunoassay of endogenous plasma insulin in man. *J Clin Invest* 39:1157–1175, 1960
25. Shimshoni M: On Fisher's test of significance in harmonic analyses. *Geophys J R Astr Soc* 23:373–377, 1971
26. 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
27. Tornheim K: Are metabolic oscillations responsible for normal oscillatory insulin secretion? *Diabetes* 46:1375–1380, 1997
28. Byrne MM, Struris J, Clement K, Vionnet N, Pueyo ME, Stoffel M, Takeda J, Passa P, Cohen D, Bell GI, Velho G, Froguel P, Polonsky KS: Insulin secretory abnormalities in subjects with hyperglycemia due to glucokinase mutations. *J Clin Invest* 93:1120–1130, 1994
29. Sturis J, Polonsky KS, Mosekilde E, Van Cauter E: Computer model for mechanisms underlying ultradian oscillations of insulin and glucose. *Am J Physiol* 260:E801–E809, 1991
30. Leahy JL, Bonner-Weir S, Weir GC: Beta-cell dysfunction induced by chronic hyperglycemia: current ideas on mechanism of impaired glucose-induced insulin secretion. *Diabetes Care* 15:442–455, 1992
31. Mao CS, Berman N, Roberts K, Ipp E: High frequency plasma insulin oscillations are entrainable in normal subjects but not in NIDDM patients (Abstract). *J Invest Med* 45:166A, 1997