Brief, Irregular Oscillations of Basal Plasma Insulin and Glucose Concentrations in Diabetic Man

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SUMMARY

The basal plasma insulin and glucose concentrations of 12 diet-treated maturity-onset diabetics were measured at minute intervals for 2 h. Brief, irregular oscillations (mean period 8.8 min) in plasma insulin were superimposed on longer term fluctuations (> 30 min). Time series analysis demonstrated a synchronous plasma glucose oscillation (mean amplitude 0.03 mmol/L) associated with short insulin cycles. The glucose changes seen in diabetic subjects were similar to the short plasma insulin cycles (< 10 min) observed in normal subjects. In contrast, the longer plasma insulin cycles (> 10 min) of normal subjects were associated with a plasma glucose oscillation that rose before the end of the cycle. The demonstration of insulin oscillations independent of preceding plasma glucose changes in both normal and diabetic subjects suggests a pancreatic oscillating mechanism or "pacemaker." The associated glucose changes may reflect the entrainment, by the insulin cycles, of glucose production or utilization. DIABETES *30*:435–439, May 1981.

The basal concentrations of plasma insulin and glucose oscillate about a mean value in both monkeys,[1] and normal human beings.[2] In both monkeys[3] and man,[2] the plasma insulin changes are accompanied by parallel changes in C-peptide, and therefore probably represent fluctuations in the rate of insulin release. In both species, the plasma glucose concentration appears to rise 1–2 min before a rise in the plasma insulin concentration. These phase relationships are compatible with the basal plasma insulin and glucose being regulated in part by a limit cycle in the feedback loop between the pancreas and the liver. However, they are also in accord with the hypothesis that the plasma glucose is "entrained"[4] to an oscillating insulin/glucagon signal from the islets. Recent studies[5] of isolated canine pancreas, performed at a constant glucose concentration, have demonstrated cyclical secretion of insulin, glucagon, and somatostatin with periods similar to those described in vivo (13 and 9 min for insulin cycles in man[2] and monkey,[1] respectively). This suggests that the pancreas secretes peptides rhythmically in response to an intrapancreatic oscillating mechanism or "pacemaker."

Normal-weight maturity-onset diabetics have raised basal plasma glucose concentrations accompanied by near-normal fasting plasma insulin concentrations.[6] Each patient appears to have a characteristic "set" plasma insulin and plasma glucose concentration[7] that remains stable overnight. The present study investigated whether the cyclical oscillations in the fasting plasma glucose and insulin concentrations seen in normal subjects are found in patients with mild maturity-onset diabetes. A control group of normal subjects was studied, and included a subgroup in whom mild hyperglycemia was induced by a glucose infusion.

METHODS

Twelve maturity-onset diabetics, 10 males and 2 females, were studied before rising after an overnight fast. All had originally presented symptomatic glycosuria with fasting plasma glucose concentrations > 6 mmol/L. All were treated by diet alone. Six continued to have basal hyperglycemia (> 6 mmol/L), and six had normal fasting plasma glucose concentrations. Their mean age was 60.3 yr (range 40–67 yr) and their mean percentage ideal body wt was 106% (range 93–128%). At 0800 h, a 24-in i.v. cannula was inserted under local anesthetic, via a cubital fossa vein, into the region of the subclavian vein or superior vena cava and kept patent with 0.154 M saline. At 0900 h, 2.5-ml samples of blood were taken at minute intervals for 2 h, during which time the subjects were encouraged to sleep. At the end of the study, a 30-min i.v. glucose tolerance test was performed in the six normoglycemic diabetics. Dextrose (0.2 g/kg ideal body wt) was given over 2 min and followed by minute samples for 7 min. The first phase insulin response

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was calculated from the incremental area under the plasma insulin curve for the first 10 min.[8]

Plasma glucose was assayed using a manual glucose oxidase kit (Boehringer GOD-perid) and plasma insulin by a charcoal-phase separation radiommunoassay[9] designed to be maximally sensitive over the range of each subject's plasma insulin concentration. The mean precision (± SD) of the plasma glucose assay was 0.04 mmol/L[2] and of the plasma insulin assay, 0.8 mU/L for values < 20 mU/L.

The assay results were "smoothed" using a 3-min moving average that reduced the rapid fluctuations in the data due to assay or experimental error ("noise") at the expense of reducing the amplitude of any oscillations present.[10] Autocorrelation was used to identify significant, regular short-term oscillations in the plasma insulin data. This technique correlates the original data with a "copy" of the data that is progressively moved to the right. Thus, for a lag of 0, the correlation is one and for a lag of 1 min, there is generally a highly significant correlation coefficient, because of the time series nature of the data. If there is a significant period, the correlation coefficients become negative when the "copy" is moved one-half of a cycle to the right (180° out of phase) and strongly positive when there is one cycle delay from the original (360° out of phase). Thus, the lag interval of the first positive peak after a negative trough is an estimate of the period of oscillations present and the value of the correlation coefficient determines its significance.[11] However, long-term linear or nonlinear trends in the data inhibit the rapid fall in the correlation coefficient because of longer-term correlations. Long-term trends were considered to be present if the correlation coefficient of the correlogram of the whole data set (120 values) failed to become negative[10] before the 10-min lag interval. The prevalence of long-term trends was assessed by comparing 6 normal subjects who had been studied for 2 h (three included from a previous study) with the 12 diabetics. The proportions of those with and without long-term trends were compared by the Chi-squared distribution with Yates' correction.

The long-term trends in the plasma insulin data of both diabetics and normal subjects were removed ("detrended") so that short-term oscillations (< 20 min) could be identified. The plasma insulin data were divided into linear segments (> 25 min long). The intersecting time points were then selected by maximizing the sum of the absolute value of the correlation coefficients of adjacent segments. In those subjects whose data contained simple linear trends, or no long-term changes, the whole data set was considered as one segment. The data in each segment were corrected for the slope of the segment and expressed as the difference from the segment's mean. Each individual "detrended" segment was examined for regular short-term oscillations using autocorrelation. The lag interval considered was restricted to < one-third of the number of data points in each segment.[10]

In order to identify changes in plasma glucose associated with short-term insulin oscillations, individual plasma insulin cycles were defined, in both diabetic and normal subjects, from the beginning of any positive deflection in the "detrended" plasma insulin data that was ≥ 1 standard deviation of the subjects' "detrended" data. The plasma insulin cycle origins were then used to divide the plasma glucose data. The plasma insulin and glucose cycles were then averaged by the standard array averaging technique[2] so that cycles of unequal length could be averaged, both within and between subjects. The plasma insulin and glucose data of each cycle was first expressed as the difference from the cycle's mean after correcting for the slope between the beginning and end of each cycle. Each cycle was then divided into 20 equally spaced divisions by linear interpolation between successive values.

The amplitudes of individual plasma insulin and glucose cycles were calculated as the difference between the lowest and highest values in the standard array. The amplitudes of the average oscillations were similarly expressed after averaging in the standard array.

Twenty-eight normal subjects, aged 19–27 yr, underwent similar studies, and 5 normal subjects, after 60 min, had glucose infused at a rate of 1.5 mg/kg/min for another 90 min via a cannula previously inserted in the opposite forearm. The cycle definition from the basal period was used to define the cycles in the post-infusion period after correction for long-term trends. Statistics used included Student's unpaired *t* test.

RESULTS

The mean fasting plasma glucose and plasma insulin concentrations of the 12 maturity-onset diabetics were 7.0 mmol/L (range 3.5–16.9 mmol/L) and 9.1 mU/L (range 1–42 mU/L), respectively. The mean fasting plasma glucose and plasma insulin concentrations of the 28 normal control subjects were 4.0 mmol/L (range 3.3–4.9 mmol/L) and 6.5 mU/L (range 1.9–15.2 mU/L), respectively. Six of these diabetics were normoglycemic at the time of the study (mean fasting plasma glucose concentration < 6 mmol/L) and six were hyperglycemic (> 6 mmol/L). All six normoglycemic subjects had abnormal first-phase plasma insulin response to i.v. glucose [mean first-phase response 16.9 mU/L•min (normal range 32–408 mU/L•min)]. Inspection of the plasma insulin data of the maturity-onset diabetics suggested that there were short, irregular oscillations superimposed upon long-term trends (Figures 1a–c). A significantly greater number of diabetics (11/12) had long-term trends than a group of normal subjects (2/6) who had also been studied for 2 h ($X^2 = 10.0$, $P < 0.01$). Persistently regular cycles, similar to those found in some normal subjects,[2] were not seen in any diabetic subject. When individual "detrended" segments were examined by autocorrelation, a significantly regular short-term plasma insulin oscillation was found in 6 out of 33 segments from the 12 diabetics, and 7 out of 36 segments in the normal subjects.

The plasma insulin cycles, defined as positive deflections in the "detrended" plasma insulin data, were significantly shorter in the diabetic subjects (8.8 ± 3.8 min) than in the normal subjects (10.7 ± 3.9 min, $P < 0.01$). The mean amplitudes of the individual plasma insulin cycles were similar in both groups (1.4 ± 1.6 and 1.4 ± 0.8 mU/L, respectively). The standard array averaging technique demonstrated that in the normal subjects, there was an insulin-associated glucose oscillation (amplitude 0.02 mmol/L) that rose and fell before the plasma insulin cycle (N = 124 cycles). However, the plasma glucose changes associated with plasma insulin cycles of different periods were not the same (Figure 2). The 63 longer cycles (> 10 min) had an associated plasma glucose oscillation (amplitude 0.03

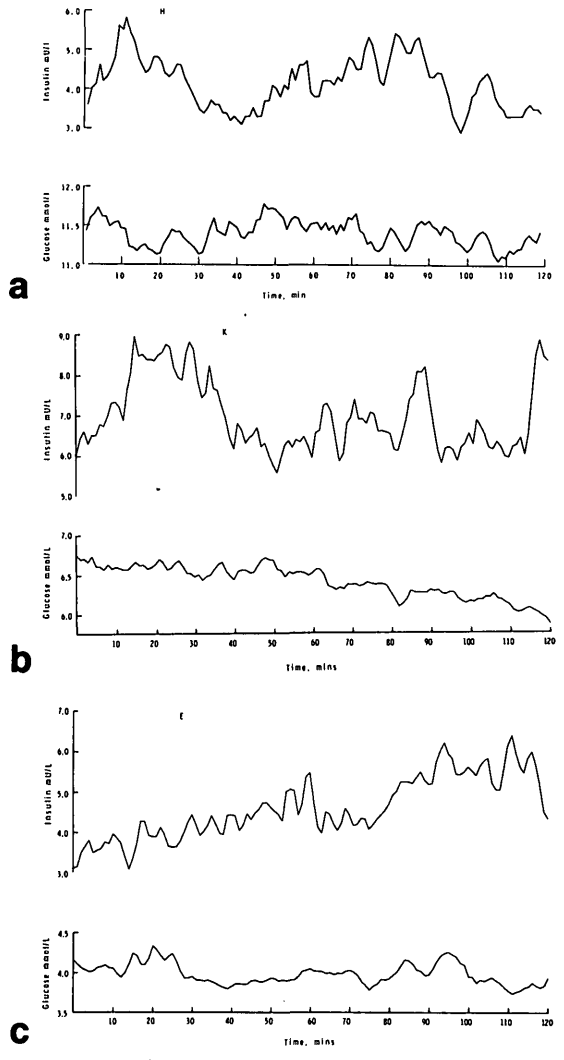


FIGURE 1. (a) A 3-min moving average of the fasting plasma insulin and glucose concentrations taken at 1-min intervals in a mild maturity-onset diabetic, showing short-term irregular oscillations in the plasma insulin concentration superimposed upon longer term (> 30 min) changes. (b) A 3-min moving average of the fasting plasma insulin and glucose concentration in a mild maturity-onset diabetic. (c) A 3-min moving average of the fasting plasma insulin and glucose concentrations in a mild maturity-onset diabetic whose mean plasma glucose concentration was less than 6.0 mmol/L.

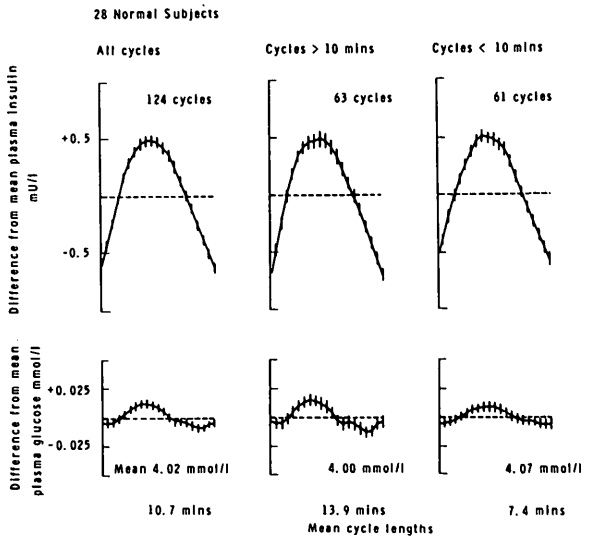


FIGURE 2. The standard array averaging technique. The curves represent the mean ± SEM plasma insulin and glucose concentrations of the cycles seen in the 28 normal subjects. The mean of all cycles is shown on the left. These cycles were also analyzed in two groups: those greater than 10 min (center) and those less than 10 min (right). The short cycles (< 10 min) had a synchronous mean plasma glucose oscillation, whereas the longer cycles (10–20 min) had a mean plasma glucose oscillation that preceded the insulin cycle by 1–2 min.

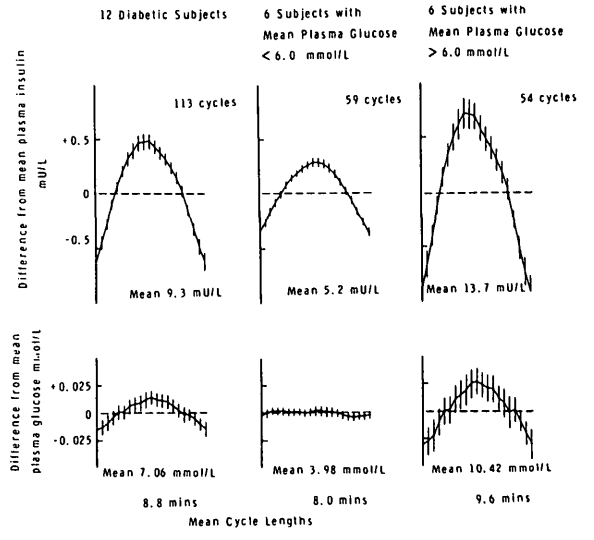
mmol/L) that rose before the end of the plasma insulin cycle. In contrast, the 61 shorter cycles (< 10 min) had a synchronous plasma glucose oscillation (amplitude 0.02 mmol/L).

A similar, synchronous insulin-associated glucose oscillation (amplitude 0.03 mmol/L) was seen when the plasma insulin cycles of the 12 diabetic subjects were used to average the plasma glucose data (Figure 3). There were significant correlations between the amplitudes of the individual plasma insulin and glucose cycles in both the normal and diabetic subjects ($P < 0.05$ and < 0.05, respectively).

The plasma insulin and glucose cycles of the hyperglycemic and normoglycemic diabetic subjects were different (Figure 3). The hyperglycemic diabetic subjects had longer ($P < 0.05$) and taller ($P < 0.01$) plasma insulin cycles with a synchronous, insulin-associated glucose oscillation (amplitude 0.06 mmol/L). In contrast, there was no insulin-associated plasma glucose oscillation seen in the normoglycemic diabetic subjects.

In the five normal subjects who had i.v. glucose infusions, the mean plasma glucose concentration rose from 4.14 mmol/L to 4.91 mmol/L and the mean plasma insulin concentration from 5.4 mU/L to 10.6 mU/L. In two of these subjects, there was a marked increase in the amplitude of the plasma insulin oscillations present, which then gradually decreased over the next 60 min (Figure 4). The mean plasma insulin cycle length during the glucose infusion was shorter (9.7 min, N = 42) than that observed in basal normal subjects (10.7 min, N = 124, $P < 0.05$).

FIGURE 3. Mean ± SEM plasma insulin and glucose concentrations in the mild maturity-onset diabetic subjects. When all 12 subjects were averaged, the average cycles were similar to the short cycles seen in normal subjects. However, the subjects whose mean plasma glucose concentration was > 6 mmol/L had larger plasma insulin and glucose oscillations, whereas those subjects who were normoglycemic at the time of the study did not have an insulin-associated glucose oscillation.



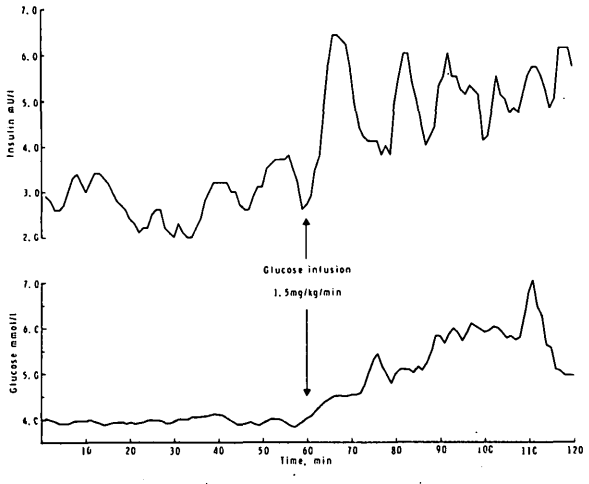


FIGURE 4. The 3-min moving average of the plasma insulin and glucose concentrations of a normal subject who had a glucose infusion after 60 min. The tall plasma insulin cycles seen after the beginning of the infusion gradually returned to normal amplitude.

DISCUSSION

In maturity-onset diabetics, treated by diet alone, the fasting plasma insulin concentration fluctuated with brief irregular oscillations (mean period 8.8 min) frequently superimposed upon longer-term changes. In contrast, in normal subjects the short-term changes (mean 10.5 min) were associated with a more stable long-term plasma insulin concentration. Thus, an isolated fasting plasma insulin estimation may not reflect the mean basal plasma insulin concentration in mild diabetics. The long-term changes are similar to the 1–2-h oscillations in plasma insulin and glucose concentrations, and peripheral glucose uptake induced by glucose infusions in dogs.[12,13] While the same mechanism might operate, the basal state is dissimilar to the hyperglycemia and hyperinsulinemia, associated with almost total inhibition of hepatic glucose production, in the infused dogs.

The short-term oscillations of the diabetics were more rapid than those of normal subjects, and were generally irregular. Although we have observed persistently regular and stable insulin cycles in some normal subjects,[2] this was not a feature of diabetic subjects.

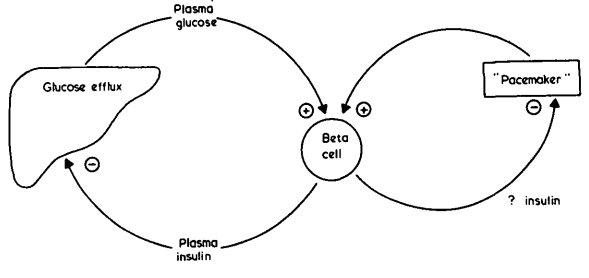
Irregular cycles pose problems of definition. If an amplitude criterion is chosen which is large, then neighboring cycles might not be distinguished, while the choice of a small amplitude of cycle will cause experimental "noise" to be included. We have used the definition of one standard deviation from the "detrended" data, and the similarities of the amplitudes and periods of the insulin cycles between normal and diabetic subjects suggest that similar phenomena were being examined. The association with a concordant cycle of an independent variable, plasma glucose, suggests that the cycles were physiologically meaningful.

The standard array averaging technique used the defined plasma insulin cycles to average the plasma glucose data in a manner analagous to the detection of visual-evoked potentials.[14] The rise of insulin was used as an endogenous event, and averaging then causes other differently timed influences on the blood glucose to be minimized. However, in contrast to visual-evoked potentials, the use of an endogenous rather than exogenous stimulus means that the associations between plasma insulin and plasma glucose changes may not necessarily be causal.

The present study confirms the observation that in normal subjects, the short-term plasma insulin oscillations are associated with a plasma glucose oscillation that precedes the plasma insulin oscillation.[2] However, plasma insulin cycles (< 10 min) were associated with a synchronous plasma glucose oscillation, whereas longer cycles (> 10 min) were associated with a plasma glucose oscillation which rose before the end of the cycle. The phase relationship, observed in the longer cycles, is in accord with a limit cycle of the feedback control system between insulin secretion and glucose production or utilization, with plasma glucose changes inducing the next insulin cycle. However, the demonstration of plasma insulin cycles in both normal and diabetic subjects, associated with synchronous plasma glucose changes, suggests that some other mechanism must be the primary stimulus to the beta cell. Nevertheless, a role for glucose is supported by the response to mild hyperglycemia seen in normal subjects, although this may be a "facilitative" or secondary effect.

The report of similar oscillations of insulin, glucagon, and somatostatin secretion by the excised canine pancreas[5] suggests that the oscillating stimulus or "pacemaker" is sited in the pancreas, rather than the central nervous system or the entero-insular axis. The source of stimuli might be from specialized cells (cf. the heart) or from an endogenous oscillating mechanism such as glycolysis[15] within one or more of the endocrine cell types. The numerous ganglia, and the abundant VIP neurons[16] that extend between and into islets, might coordinate individual islets, while each islet's response could be integrated by the intercellular connections between cell types.[17] Alternatively, an intrapancreatic "paracrine" feedback control system might be involved with inhibition of insulin secretion by insulin[18] or C-peptide,[19] or with more complex relationships between the A, B, and D cells.[20] If paracrine or plasma insulin concentrations normally inhibit the pacemaker, then the increased frequency of oscillations seen in the maturity-onset diabetics may be a response to impaired beta-cell secretion. Thus, the timing of the insulin secretory pulses might depend on the size of the previous pulse, and the phase relationships observed between the plasma insulin and glucose cycles could arise from the independent hepatic and "pacemaker" responses to insulin (Figure 5). "Entrainment"

FIGURE 5. Model of possible interaction between pacemaker and glucose control of beta-cells. The timing of the pacemaker stimulus may be dependent on the size of the previous insulin pulse, and the beta-cells may respond to a summation of glucose and "pacemaker" stimuli. The relationships between plasma glucose and insulin cycles might result from insulin separately inhibiting hepatic glucose efflux and a "pacemaker."



of hepatic glucose efflux by the intermittent pancreatic hormone secretion is supported by the significant correlation between the amplitudes of simultaneous plasma insulin and glucose cycles.

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