CYCLIC OSCILLATIONS OF BASAL PLASMA GLUCOSE AND INSULIN CONCENTRATIONS IN HUMAN BEINGS

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Abstract In a study of whether oscillations in plasma glucose and insulin occur in human beings, plasma samples were taken at one-minute intervals from 10 normal subjects for periods lasting between one and two hours. In five subjects the basal plasma insulin concentrations cycled regularly, with a mean period of 13 minutes and mean amplitude of 1.6 mU per liter (11.5 pmol per liter). A concurrent plasma glucose cycle was demonstrated, with a mean amplitude (after averaging to minimize random error) of 0.05 mmol per liter (1 mg per deciliter). The average plasma glucose cycle was two minutes in advance of the plasma insulin. In the subjects with less regular plasma insulin cycles, a similar plasma glucose rise was demonstrated two minutes before the insulin rise. These phase relations are compatible with the presence of a negative-feedback loop between the liver and pancreatic beta cells that regulates both basal plasma insulin and glucose concentrations, although the cyclic beta-cell secretion could be independent of plasma glucose (N Engl J Med 301:1023-1027, 1979)

NORMAL and diabetic subjects each have characteristic basal plasma glucose and insulin concentrations,[1] but the control mechanisms involved have not been well delineated. Basal insulin secretion is dependent on the plasma glucose,[2-4] but may also be affected by the sympathetic nervous system[5,6] and plasma amino acids.[7,8] The basal glucose concentration is dependent on the hepatic glucose efflux, which is partly regulated by the insulin concentration in the portal vein.[9,10] Other influences include plasma glucagon[11,12] and glucose concentrations,[13,14] other hormonal and neurogenic stimuli, as well as peripheral glucose uptake.

In the basal state the negative-feedback loop between the liver and beta cells may be a predominant factor controlling both plasma glucose and insulin concentrations.[15] In such a system a delay between a change in input and attainment of the required output can produce an over-response leading to cyclical oscillations ("hunting"). Oscillations of plasma glucose and insulin concentrations have been seen in monkeys,[16] and we have investigated whether similar cyclical changes might be apparent in man.[17]

METHODS

Ten normal subjects — eight men and two women between the ages of 21 and 39 and of normal weight (<15 per cent above ideal) — were studied before rising after an overnight hospital stay. At 8 a.m. a 24-inch (610 mm) venous catheter (Bard I-Cath, Bard International Limited) was inserted under local anesthesia via a forearm vein into the region of the superior vena cava. The catheter was kept patent with 0.9 per cent saline, and 2.5-ml samples of blood were taken at one-minute intervals for up to two hours. The subjects were encouraged to sleep during the study period.

Plasma glucose was assayed in duplicate with a manual Boehringer (GOD-perid) kit. Plasma insulin[18] and C peptide[19] were measured by charcoal phase separation, and each subject's samples were set up in an assay with maximal precision at his plasma concentrations. The mean precision (±1 S.D.) of the assays, determined from all duplicates, was ±0.04 mmol per liter (0.7 mg per deciliter) for plasma glucose, ±0.6 mU per liter (4.3 pmol per liter) for insulin and ±0.02 pmol per liter for C peptide. The assay results of the minute samples were "smoothed" by a three-minute moving average,[20] the effect of which is to reduce the rapid fluctuations in the data owing to assay and experimental "noise" (Fig. 1). Rogue errors (>3 S.D. from the mean) were removed before smoothing. Regular periodicity of plasma insulin concentrations was sought by autocorrelation,[21] in which a copy of the original data is progressively moved to the right, and a correlation coefficient calculated at each interval. The correlation coefficients are plotted against the time-lapse intervals to produce a correlogram. The time of the first maximum positive correlation, after the initial trough, was used to define the period of oscillation, and the significance was determined by Fisher's Z-transformation. The autocorrelation period was then used as an initial estimate for fitting, by least-square analysis, a sine wave to the plasma insulin data, thus defining an origin for delineating the cyclical changes. The mean amplitude was estimated by averaging the plasma insulin changes between the troughs closest to the nadirs of the fitted curve and the peaks closest to the following apogees. The term "amplitude" has been used to denote the differences between troughs and peaks.

Each cycle was corrected for the slope between the origins of that cycle, and the data expressed as the difference from the mean of each cycle. Each cycle was then divided into 20 equally spaced divisions by linear interpolation between successive "smoothed" values. This standard array allowed cycles of a subject, and of subjects with different cycle lengths, to be averaged. The time delay between the average plasma glucose and insulin cycles, obtained by the standard array, of the 10 subjects was estimated formally by fitting

$$y = a \sin (\theta + b) + (\text{error})$$

separately for insulin and glucose by least-square analysis.

In the five subjects with less regular insulin oscillations, the individual plasma insulin cycles were also defined from the beginning of any positive deflection of the "smoothed" time series >1 standard deviation of the "unsmoothed" basal plasma insulin values. Statistical methods included analysis of variance by the F test.

RESULTS

In nine subjects the basal plasma insulin concentrations had significantly greater variation than would be expected from the precision of the assays (F test, $P<0.002$ in eight subjects). Autocorrelation of the plasma insulin time series demonstrated significantly regular oscillations in five subjects, with similar but not significant correlograms in the other subjects. Figures 2 and 3 show the plasma insulin changes in the two subjects with the most regular oscillations. Simultaneous plasma C-peptide concentrations were

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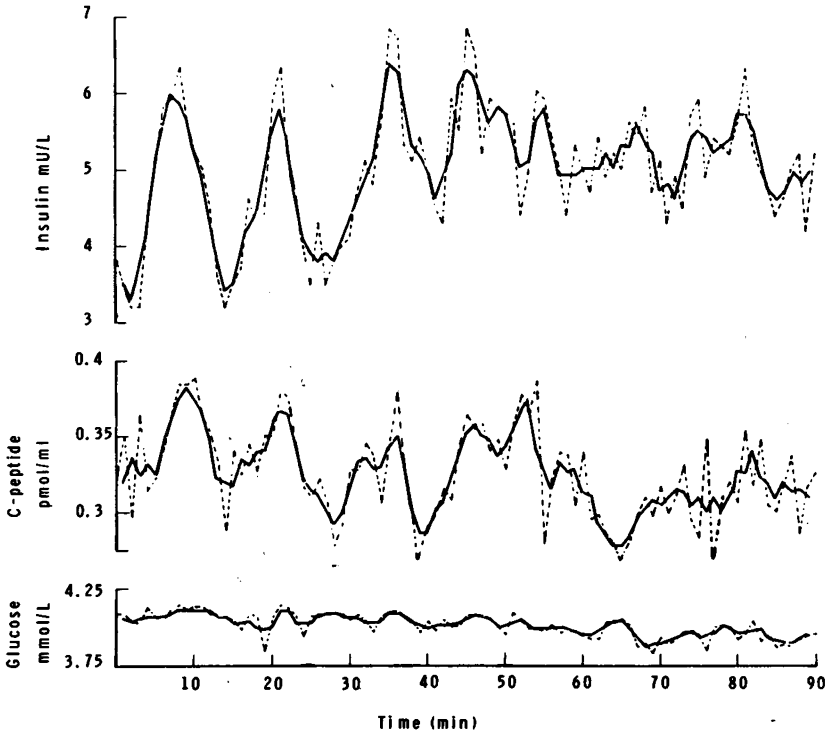

Figure 1.
A three-minute moving average (continuous line) of the fasting plasma insulin, C-peptide and glucose concentrations taken at one-minute intervals in Subject 1. The dashed line shows the "unsmoothed" data. Smoothing reduces the rapid fluctuations, which are probably due to "noise," and also blunts the amplitude. The simultaneous insulin and C-peptide cycles disappear after 50 minutes.
To convert glucose values from millimoles per liter to milligrams per deciliter, multiply by 18; to convert insulin values to picomoles per liter, multiply by 7.17.

measured in four subjects and showed similar, but less marked, synchronous oscillations with significant correlations ($P<0.01$ in each subject) between the simultaneous plasma insulin and C-peptide values (Fig. 1–3). Each subject's plasma insulin and glucose results are summarized in the table. The periodicity of the autocorrelation-defined cycles ranged from 10 to 15 minutes (mean, 12). The mean amplitude was 1.4 mU per liter (10 pmol per liter), representing a mean ±13 per cent oscillation about the mean basal plasma insulin of 5.5 mU per liter (40 pmol per liter).

The two subjects with the most regular insulin cycles had approximately synchronous plasma glucose cycles detectable by eye, with a range of 0.2 mmol per liter (3.6 mg per deciliter) (Fig. 2 and 3). The standard array-averaging technique, using the origin and period of the fitted sine waves to the insulin data to define each insulin and glucose cycle, suggested glucose oscillations concordant with the insulin oscillations in seven of the 10 subjects. The plasma insulin and glucose oscillations of all the subjects, averaged by the standard array, had amplitudes of 0.8 mU per liter (5.7 pmol per liter) and 0.03 mmol per liter (0.5 mg per deciliter) respectively. The formally calculat-

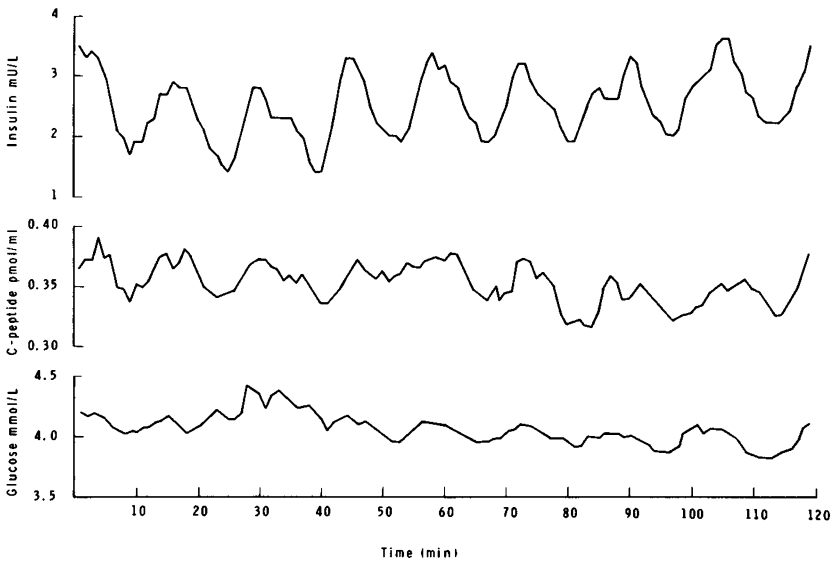

Figure 2. A Three-Minute Moving Average of Fasting Plasma Concentrations in Subject 8, Showing Regular Oscillations.

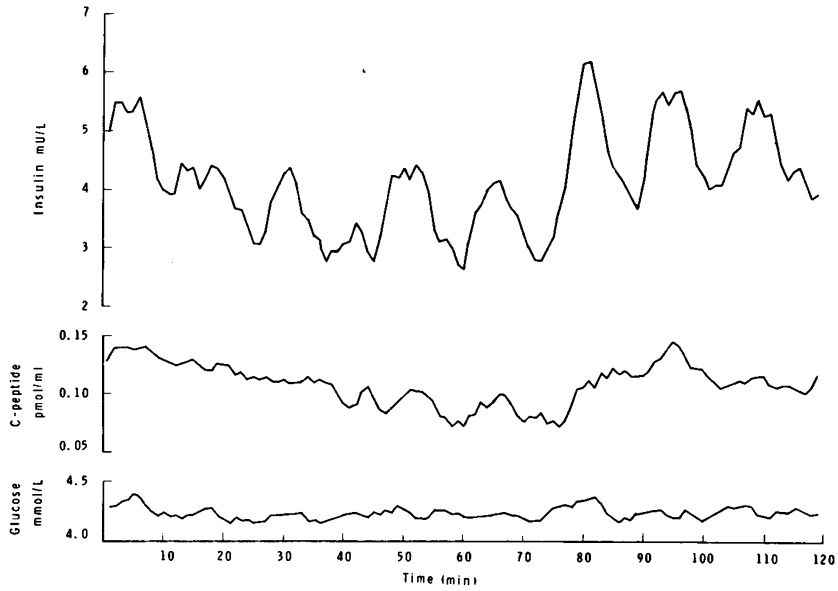

Figure 3. A Three-Minute Moving Average of Fasting Plasma Concentrations in Subject 5.

The timing of the regular oscillations was reset after the fourth insulin pulse, which was small.

ed delay between these two oscillations (1.08±0.17 minutes, ±S.E.M.) was significant ($P<0.001$).

The 10 subjects were divided into five with significantly regular plasma insulin cycles (autocorrelation period, $P<0.05$), and five with less regular oscillations in plasma insulin. In the five subjects with regular cycles, the standard array-averaging technique (using the origin and period of fitted sine waves to define individual plasma insulin cycles) demonstrated a plasma glucose cycle preceding the plasma insulin cycle by two minutes (Fig. 4). The average amplitude of the plasma glucose oscillation was 0.05 mmol per liter (1 mg per deciliter). The five subjects with the less regular cycles, on averaging by the same methods, showed a similar insulin cycle but no synchronous plasma glucose oscillation (Fig. 5). However, when these subjects' individual plasma insulin cycles were defined by the presence of a positive plasma insulin deflection, and their plasma insulin and glucose concentrations were averaged by the standard array, a plasma glucose oscillation preceding the plasma insulin deflection by two minutes was revealed, with a plasma glucose amplitude of ±0.04 mmol per liter (0.7 mg per deciliter) (Fig. 5).

Discussion

The demonstration of cyclic oscillations of the plasma insulin concentration in the basal state suggests that insulin secretion is episodic; this is confirmed by the concordant plasma C-peptide oscillations. Assay

Table 1. Summary of Plasma Glucose and Insulin Data.*

Subject No.	Duration of Study	Plasma Glucose	Plasma Insulin						
		Mean Basal Concentration		Analysis of Variance			Autocorrelation		
			Mean Basal Concentration	Variance of "Unsmoothed" Data	Assay Variance	P Value	Period Estimated from Correlogram	Correlation Coefficient at Period	P Value
	min	*mmol/liter*†	*mU/liter*‡				*min*		
1	90	4.02	5.0	0.70	0.16	<0.002	13	0.49	<0.001
2	60	4.07	6.2	2.07	0.74	<0.002	11	0.26	<0.05
3	90	3.91	5.1	0.57	0.23	<0.002	11	0.21	<0.05
4	120	4.08	7.2	1.48	0.62	<0.002	13	0.06	NS§
5	120	4.24	4.1	0.91	0.23	<0.002	14	0.53	<0.001
6	60	4.46	5.9	0.73	0.41	<0.05	11	−0.23	NS
7	60	4.14	8.6	0.50	0.34	NS	10	0.07	NS
8	120	3.99	2.5	0.38	0.10	<0.002	15	0.71	<0.001
9	60	4.11	7.2	0.86	0.34	<0.002	10	0.01	NS
10	60	3.97	2.9	0.32	0.11	<0.002	12	0.07	NS

*Analysis of variance shows the variations of plasma insulin concentrations to be greater than would be expected from assay precision. Five subjects had significantly regular plasma insulin cycles, as determined by autocorrelation.

†To convert from mU/liter to pmol/liter, multiply by 7.17.

‡To convert from mmol/liter to mg/dl, multiply by 18.

§Not significant.

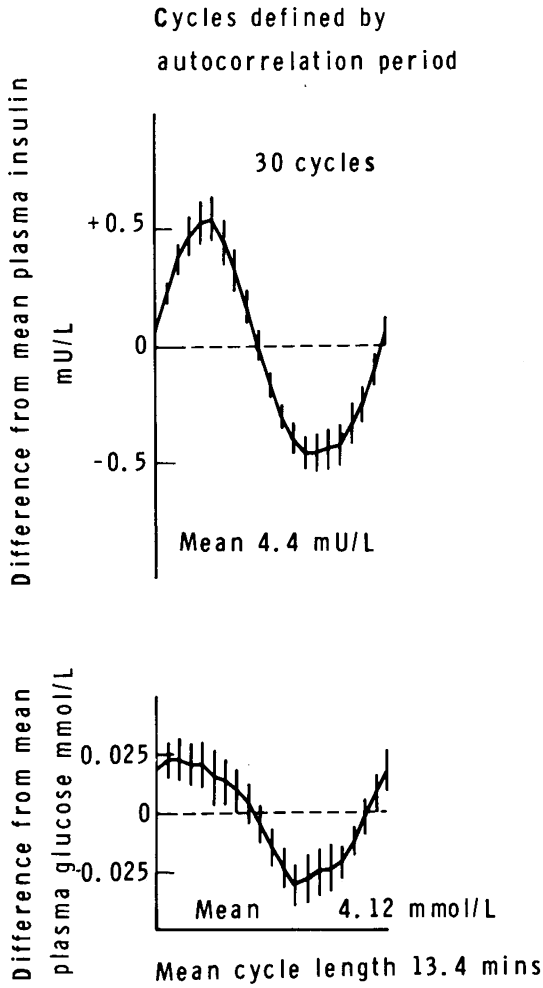

Figure 4. The Standard Array-Averaging Technique.
The curves show the mean ±S.E.M. plasma insulin and glucose changes in the five subjects with the more regular insulin oscillations. The plasma glucose oscillation precedes the insulin by two minutes. The mean plasma insulin amplitude was 1.0 mU per liter after averaging in the standard array, but 1.6 mU per liter when estimated by the differences between troughs and peaks of individual cycles.

of plasma insulin is the more appropriate measurement, since its half-life is shorter than that of C peptide and the fluctuations are thus easier to detect.[22]

Goodner et al.[16] have demonstrated cyclic changes in the basal concentrations of plasma insulin and glucose in the rhesus monkey that have a mean cycle length of nine minutes. They were, however, unable to confirm regular oscillations in man. This failure may have been due to their less frequent sampling interval of two minutes and because they did not attempt to reduce experimental and assay "noise" by averaging. Plasma glucose oscillations are less easy to detect than insulin oscillations because the small plasma glucose changes tend to be lost among random assay and experimental error. However, a glucose oscillation in advance of the plasma insulin can be demonstrated once random noise is minimized by averaging. We have used two independent techniques to improve the signal-to-noise ratio.[23] The assay results were initially smoothed with use of a three-minute moving average, which improves the precision of each point at the expense of reducing the amplitude of any oscillations present. The second technique averaged many cycles in a standard array. This method is similar to the detection of evoked potentials,[24] except that instead of using an external stimulus we have used the changes in plasma insulin as an endogenous stimulus and have averaged the associated changes in plasma glucose. Any plasma glucose changes detected are those related to the plasma insulin, since any random fluctuations or asynchronous hormonal, metabolic or neurogenic inputs are suppressed or "averaged out" by this technique. The use of endogenous events to time hormonal interactions has previously been used to study the changes associated with luteinizing hormone secretion at ovulation.[25] The best-fit sine-wave technique is a useful objective method of delineating

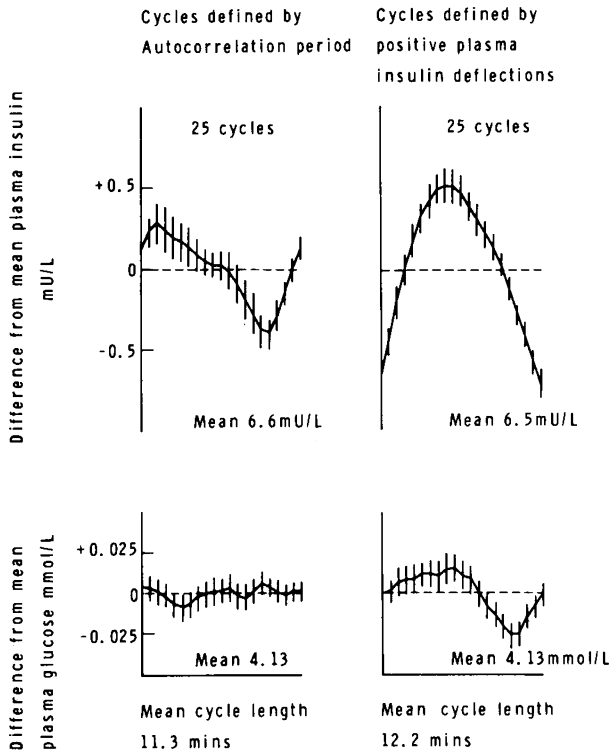

Figure 5. Mean Plasma Insulin and Glucose Changes in the Five Subjects with the Less Regular Insulin Oscillations.
A plasma glucose rise two minutes before the plasma insulin change is apparent when the cycles are defined by the individual plasma insulin deflections.

cycles, but is not suitable for subjects who oscillate irregularly. Sine waves are not an apposite description since, even in subjects with regular cycles, there is an abrupt rise in plasma insulin concentrations. Thus the formally calculated delay between sine waves fitted to the average plasma glucose and insulin cycles (1.08 minutes) was less than that obtained by inspection of Figures 4 and 5 (two minutes). Amplitudes have been expressed as the differences between troughs and peaks; their magnitude may be better estimated by direct measurement than by the standard array averaging technique, which reduces the mean amplitude because of variation between individual cycles.

The demonstration of cyclical changes in plasma insulin concentration, preceded by similar changes in plasma glucose, is in accord with the hypothesis that both are controlled in a simple negative-feedback loop that "hunts." Alternatively, the beta cells could have been stimulated by a cyclic, glucose-independent input, such as an intra-islet or autonomic nervous system "pacemaker." Recent studies[26] using in vitro perfusion of dog pancreas at constant glucose concentrations have shown similar oscillations in insulin secretion, with the demonstration of a 10-minute cycle rather than the in vivo human 13-minute cycle. Thus intrinsic cyclic secretion by beta cells might simply coincide with induced changes in plasma glucose rather than being caused by them. However, one would not expect such a close temporal relation between the rise in plasma glucose and insulin concentrations in the subjects with irregular cycles, unless the plasma glucose modulates the "pacemaker." The small amplitude of the plasma glucose oscillations would then suggest that the beta cells or "pacemaker" are remarkably sensitive to changes in plasma glucose. Another explanation might be that cyclic changes in plasma glucose are synchronous with, but not induced by, the plasma insulin fluctuations; however, hepatic, rather than peripheral, glucose flux is sensitive to small changes in plasma insulin[9,10] and could be responsible for the glucose changes. The irregularity of the cycles in some subjects may be due to factors other than the involvement of plasma glucose and insulin. Thus, in Figure 3, after three cycles a smaller-than-usual insulin response was followed by an early, larger response that reset the timing of the subsequent cycles. This episode may be an example of adventitious stimuli producing irregular cycles in other subjects.

Control systems are often investigated by means of applying external perturbations. The examination of data, averaged in relation to an endogenous event, provides an additional means of analysis. The use of such techniques to demonstrate dynamic relations between variables may help to elucidate control mechanisms in health and disease.

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