Postprandial oscillations of plasma glucose, insulin and C-peptide in man

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Summary. Postprandial plasma glucose, insulin and C-peptide profiles were studied in eight normal subjects, in the afternoon or in the evening. Two to five synchronous oscillations, with a mean period of 51 to 112 min were detected. The oscillations were highest after meals and were then damped, reverting to fasting levels after up to 340 min. Additional short-term oscillations, with periods of 20–30 min and 9–14 min, were observed. Cross-correlation studies of glu-

cose and insulin and of insulin and C-peptide revealed a high correlation in the frequency bands considered. The synchronous oscillations of insulin and C-peptide suggest cyclic variations in pancreatic secretion rather than cyclic changes in insulin degradation.

Key words: Plasma glucose, insulin, C-peptide, meals.

During the past few years, great attention has been paid to plasma glucose and insulin responses to various specific foods [1–4]. Most of these studies, in which blood was sampled at long-time intervals – 30 to 60 min – over 180 to 240 min, have shown glucose and insulin profiles as smoothed curves, and considered that, in normal subjects, fasting base-line was attained after such observation times. However, some authors have suggested that this smooth aspect is an artefact due to averaging since some subjects show secondary glucose and insulin peaks during the glucose tolerance test [5–7].

We recently described the existence of 53–113 min oscillations of plasma glucose, insulin and C-peptide levels in man during continuous enteral nutrition [8]. This study was designed to establish whether similar oscillations occur in the postprandial state. Frequent and integrated blood sampling was used to take into account the previously described 8–14 min oscillations [9–12], which can obscure the exact postprandial profiles by generating artefactual oscillatory periods. Since Ferrannini et al. [13] have reported that no more than 75% of ingested glucose appears in the plasma after 3.5 h, experiments were extended 8 to 12 h after meals.

Subjects and methods

Subjects and procedure

Eight healthy men, of normal body weight (90 to 110% of desirable body weight for men of medium frame, according to the Metropoli-

tan Life Insurance Co. Tables – 1959), aged 21–25 years, were studied. All subjects had normal oral glucose tolerance tests and were consuming a weight-maintaining diet, containing at least 200 g carbohydrate daily for 3 days before the study. None were taking any medication. Informed consent was obtained from all of them. The investigations were in accordance with the principles of the Declaration of Helsinki. The experiments were performed in a soundproof air-conditioned sleep room. The subjects were supine and were asked not to smoke. Two h before blood sampling began, a catheter was inserted, under local anaesthesia into an antecubital vein and kept patent with heparinised saline solution (500 UI heparin/ml of 0.9 NaCl/100 ml). Blood samples, pooled over 4 min, were collected continuously using a peristaltic pump. A maximum of 250 ml blood was removed during the experiments. Four subjects were studied in a first experimental series, from 11.30 to 20.30 hours. A standard meal (carrots: 150 g, mashed potatoes: 300 g, steak: 150 g, bread: 100 g, and an apple: 150 g; 900 kcal; carbohydrate: 45%, fat: 34%, protein: 21%) was eaten from 12.15 to 12.35 hours, after a 4-h fast. All subjects had a standard breakfast at 08.15 hours (400 kcal). Four other subjects took part in a second experimental series which, after a 6-h fast, lasted from 18.30 to 08.00 hours. The same standard meal was served at 19.15 hours.

Plasma sample measurements

Blood samples were immediately centrifuged at 4°C and the plasma stored at –20°C. Plasma insulin was assayed in duplicate with the double antibody radio-immunoassay of Hales and Randle [14]. The intra-assay coefficient of variation was 5.8%. The sensitivity of the assay was 2 mU/l. Plasma glucose was assayed in duplicate with a manual glucose oxydase method (Orimbio kit, Paris, France). The intra-assay coefficient of variation was less than 1%. Plasma C-peptide was determined in duplicate by RIA using commercially available kits (CIS – France). The intra-assay coefficient of variation was 4.8%. The sensitivity of the assay was 0.05 nmol/l.

Statistical analysis

Results are presented as individual profiles for glucose, insulin, and C-peptide. For each experiment, the peak levels and the time-lags were calculated. The time for reversion to fasting basal levels was also noted.

Spectral analysis was performed (BMDP1T and BMDP2T programs, BMDP Statistical Software, University of California Press, Berkeley, Calif, USA) to document the existence of rhythmic patterns in the postprandial plasma glucose, insulin, and C-peptide levels. The Fast Fourier Transformation method of the autocorrelation function and a Blackman and Tukey smoothing window were used on the filtered data in assessing the spectral density function [15, 16]. A difference filter, defined as $X_d(t) = X(t) - X(t-1)$ was used to remove low frequency components. The band width was 0.025 cycle/min and the frequency spacing was 0.002 cycle/min. The spectral density indicates the variability function which is broken down into harmonic components with given periods and, thus, reveals the strength of the oscillatory pattern. The filtered data were used to determine the relationship between insulin, C-peptide and glucose by means of cross-spectral estimation. The Fast Fourier Transformation of the cross-correlation function was used to compute coherency and phase spectrum. The coherency spectrum measures the correlation between the two data series at each frequency.

The Mann Whitney U test [17] was used to assess the statistical significance of differences between the results obtained after the midday and evening meals.

Results

Postprandial glucose and insulin profiles

Figure 1 shows the postprandial glucose and insulin profiles in 4-min samples from the four subjects after the midday meal, and Figure 2a and 2b give the profiles obtained after the evening meal. The results from the eight subjects are summarised in Table 1. The pre-prandial glucose and insulin levels were similar at midday and in the evening.

Postprandial glucose profiles were characterised by the existence of a first blood glucose response followed by a varying number of secondary increases and decreases, usually of lower magnitude than the first peak. While two of the subjects only exhibited one major peak, followed by a little fluctuation, three to six secondary peaks were observed in the other six subjects. The first blood glucose peak, which occurred with a mean time-lag of 48 min (range=26–70 min) after the meal reached a mean level of 7.8 mmol/l (6.3–9.7 mmol/l). The second glucose increment, which was observed in the eight subjects was generally lower than the first peak (mean levels: 6.2 mmol/l; range: 5.3–7.3 mmol/l) and occurred with a mean time-lag of 152 min (70–286 min) after the meal. The time for reversion to the fasting basal levels after the meal was as high as 346 min (260–500 min).

A high concordance between glucose and insulin profiles was observed in the eight subjects. However, fluctuations with shorter periodicities were superimposed on some segments of the longer fluctuations. The first glucose increment was accompanied by an insulin increase consisting in a first pulse occurring very

Fig. 1. Postprandial plasma glucose and insulin profiles in 4-min blood samples from 4 subjects after the midday meal

rapidly after the meal, followed by a smoother increment. The amplitude of the first insulin increase was 55 mU/l (27–102 mU/l). The second increase reached 43 mU/l (32–60 mU/l). The time for reversion to the fasting basal levels was similar to that for glucose (353 min). Glucose and insulin fluctuations were generally higher and more prolonged after the evening meal than in the afternoon, but this difference was only significant for the first insulin peak.

Cross-correlation analyses of the filtered data revealed that the changes in plasma glucose were nearly synchronous with the plasma insulin fluctuations.

Spectral analysis of postprandial glucose and insulin profiles

Spectral analysis revealed the existence of an oscillatory pattern in the postprandial profiles of glucose and insulin and also the predominant periodicity. Results are given in Table 1.

Significant periodic oscillations in glucose were found in all but one subject. The predominant period for each experiment varied from 51 to 112 min. In

Fig. 2. **a** Postprandial plasma glucose and insulin profiles in 4-min blood samples from 2 subjects after the evening meal. **b** Postprandial plasma glucose and insulin profiles in 4-min blood samples from 2 subjects after the evening meal

Table 1. Results of analysis of plasma glucose and insulin levels in 4-min blood samples after the midday and the evening meals

Subjects	Glucose (mmol/l)								Insulin (mU/l)							
	Base-line level	Number of peaks	Peak 1 level	Peak 1 Time lag (min)	Peak 2 level	Peak 2 Time lag (min)	Time for reversion to baseline (min)	Period of oscillations (min)	Base-line level	Number of peaks	Peak 1 level	Peak 1 Time lag (min)	Peak 2 level	Peak 2 Time lag (min)	Time for reversion to baseline (min)	Period of oscillations (min)
12.15 hours meal																
S1	4.6	4-5	6.3	46	5.6	154	330	72 (34)	5.7	4-5	36	58	41	154	365	34 (72)
S2	4.9	3-4	6.4	26	6.0	102	260	64 (19)	10.0	3	27	14	33	102	220	19 (57)
S3	4.8	2	7.9	34	6.8	70	260	51 (23)	6.6	2	35	30	33	74	260	- (51)
S4	4.3	3-4	8.2	62	6.4	110	460	85	6.3	4	34	42	47	106	340	73
19.15 hours meal																
S1	4.4	4	6.8	30	7.3	162	300	96 (32)	13.9	4	74	37	60	150	360	96 (32)
S2	4.6	4	8.5	50	6.8	170	360	102	8.0	5	78	58	55	162	480	102
S3	4.3	2	8.6	62	5.9	162	300	112 (22)	5.1	2	55	86	45	162	300	- (21)
S4	4.5	3	9.7	70	5.3	286	500	60	1.9	3	102	145	32	258	500	60

Brackets indicate secondary peaks of the spectral density function

some experiments (5 of them) more than one period was identified. In these cases, the second strongest period ranged from 19 to 34 min.

The spectral density functions corresponding to the

insulin profiles only inconsistently revealed a significant oscillatory pattern with a periodicity longer than 50 min. When both the strongest and second strongest periods were considered, spectral analyses revealed

Fig. 3. Concomitant plasma glucose, insulin and C-peptide profiles in 4-min blood samples from subject S1, with the corresponding spectral density functions

significant periods in the 51 to 102 min range, comparable to that of glucose, for 7 subjects. In addition, significant shorter frequencies were sometimes present for insulin (with periods of 19–32 min). The previously described 8–14 min oscillations were superimposed on some segments of the longer fluctuations, especially some time after the meal. The profiles in Figure 2 illustrate such additional rapid oscillations.

C-peptide profiles

C-peptide was measured in two subjects after the mid-day meal and in two other subjects after the evening meal. Figure 3 illustrates, for one subject, the concomitant glucose, insulin, C-peptide profiles in blood taken at 4-min intervals from 11.30 to 20.00 hours, with the corresponding spectral density functions.

The C-peptide profiles were very similar to the insulin ones in each of the four subjects. Spectral density of the C-peptide profiles revealed significant oscillations with periods similar to those observed for insulin. In addition to the 51–112 min oscillations, oscillations with significant periods of 20–30 min and 9–14 min were observed. The cross-correlation study of C-peptide and insulin revealed a high coherency for the different periods. The phase angle corresponding to these periods was about 0, indicating that insulin and C-peptide are highly correlated in these frequency bands and that their oscillations are in phase.

Discussion

This study establishes that, in normal subjects, postprandial glucose and insulin profiles cannot be considered as a smoothed curve of simple harmonic motion,

but they exhibit one or more secondary peaks, which, in some cases, give an oscillatory pattern. The glucose oscillations, varying in number in each subject, display a periodicity of 51 to 112 min, which is similar to that previously described in man during continuous enteral nutrition [8]. The oscillations amplitude is highest immediately after meals, decreasing and becoming less regular with time, especially during the night. They are accompanied by similar, although less smooth insulin fluctuations. Preprandial glucose and insulin levels are only reached 5–6 h after feeding.

Two or more glucose peaks after an oral glucose load have occasionally been reported in normal man [4–7]. In our study this phenomenon was very frequent in subjects after the meal. High-frequency blood sampling, (at 4-min intervals) over more than 8 h was of critical importance in identifying this 59–110 min oscillatory pattern. In most of the studies which sought to define the glucose and insulin postprandial profiles blood-sampling intervals were longer, generally 30 min, and the experiments lasted no more than 240 min. Moreover, results were mostly presented as averages of the individual values. Since the fluctuations occur with different time periods in normal subjects, they were usually smoothed out, leading to an artefactual pattern [18].

At present, the mechanisms underlying the oscillations can but be subject to conjecture. Self-limiting cycles with over-correction of plasma glucose levels by insulin might be responsible for transient postprandial oscillations. Since C-peptide is a good indicator of pancreatic secretion [19, 20] the existence of synchronous oscillations of C-peptide and insulin suggests variations in pancreatic secretion rather than cyclical changes in the insulin degradation rate. Oscillations with periods of more than 1 h have been described in

man for other pancreatic hormones, such as pancreatic polypeptide [21]. In fasting dogs, insulin, glucagon and somatostatin oscillated in portal and peripheral blood with a 50–120 period, which suggests a periodic secretion of these hormones [22].

The rate of appearance of oral glucose in the portal bloodstream might also play a major role in the postprandial plasma glucose and insulin fluctuations. It was recently demonstrated [13] that ^{14}C glucose continued to appear 210 min after an oral glucose charge in normal man and that total rate of glucose appearance had a clear biphasic time pattern, with an initial peak 30 min and a second peak at 180 min, which approximately corresponds to the time of occurrence of the two first postprandial peaks described in this study.

Gut factors might be expected to increase the tendency towards an oscillatory response. The gastric inhibitory peptide, which is probably the major component of the entero-insular axis [23, 24], is responsible for significantly higher insulin levels after intrajejunal [24] or orally [25] than after intravenously administered glucose. The role of multiphasic gastric emptying cannot be ruled out. Plasma glucose and insulin oscillations may be associated with the gastric motility cycles which have been described in man [26], with the gastric acid oscillations [21], or with the interdigestive myoelectric complexes observed in the dog [27]. The central nervous system might also help to generate the oscillatory pattern, since oscillations have been described for plasma noradrenaline levels in man [28]. These physiological rhythmic processes have all been reported to have a 90–100 min period, but further investigations are necessary to determine their possible relationships.

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