Biphasic Patterns of Peripheral Insulin and Glucose Levels After Lunch in Normal Subjects

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The dynamic relationship of glucose concentrations and insulin secretion during the postabsorptive state is complex and has been associated with a variety of cyclic rhythms. To study the pattern of insulin and glucose response immediately after a mixed meal, we collected blood every 15 min from 0730 to 1645 h from eight normal resting men (age 24.9 ± 2.1 yr). They took identically constituted mixed meals at 0800 and 1145 h. Concentrations of glucose and insulin were measured in samples taken throughout the study, whereas levels of C-peptide, glucagon, and α-NH_2 were determined in samples taken after 1130 h only. Computer-assisted analysis was used to identify significant increments and declines in concentrations and to quantify the coincidence of peaks of glucose, C-peptide, glucagon, and α-NH_2 with peaks of insulin. Coefficients of correlation between data points were calculated for each individual.

The patterns of blood insulin and glucose after breakfast and lunch were different. After breakfast, a single simultaneous peak in insulin and glucose occurred ~60 min after starting the meal. In contrast, the pattern after lunch in seven of the eight subjects was clearly biphasic. There were secondary, significant coincident peaks in serum insulin, glucose, and C-peptide occurring 1.75–2.25 h after the meal was served. The secondary peak appeared unrelated to the late absorption of protein because it was not associated with consistent changes in serum α-NH_2 concentration. Erratic variations characterized the postlunch pattern of glucagon levels, excluding a role for this counterregulatory hormone in the control of the biphasic insulin and glucose response. Of the measured substances, only glucose, insulin, and C-peptide demonstrated significant cross-correlations. These data indicate that in this well-defined group of healthy volunteers, the response of plasma glucose, serum insulin, and C-peptide to lunch is biphasic. *Diabetes Care* 10:293–99, 1987

The dynamics of insulin secretion and glucose homeostasis during basal and poststimulatory conditions are complex and incompletely understood. Variations in peripheral insulin concentrations are not solely dependent on the action of various pancreatic β-cell secretagogues (1–7). During the basal state, it has been shown in humans (3–5,7), monkeys (2,6), and dogs (5) that insulin levels fluctuate rhythmically with a period of ~9–13 min.

Complex patterns of oscillations in serum insulin and plasma glucose concentrations have also been found after the ingestion of oral glucose (8). Kraegen et al. (9), for example, measured insulin and glucose levels in blood samples taken every 10 min during a 50-g oral glucose tolerance test in 12 normal subjects. Relatively late and large secondary coincident peaks in insulin and glucose occurred among all subjects.

In a preliminary study designed to establish reference values for serum insulin and plasma glucose levels among ambulatory normal subjects consuming standard mixed meals, we observed secondary peaks in these compounds, especially after lunch (10). In this study we have statistically evaluated these secondary peaks to determine whether they correlate with changes in levels of related substrates or of glucagon. Our data demonstrate that a secondary, synchronous increment in serum insulin and plasma glucose levels, unrelated to direct nutrient absorption, is a consistent and normal response after the mixed noon meal.

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MATERIALS AND METHODS

Subjects. Eight normal-weight men, aged 24.9 ± 2.1 yr (mean ± SD) were studied (11). They were free of acute or chronic disease and had taken no medication for 3 wk before the study. Inclusion criteria included willingness to eat a standard American Diabetes Association diet; absence of abnormalities on routine hemogram, urinalysis, or liver and kidney function tests; and no first-degree relative with a diagnosis of diabetes mellitus. Subjects were instructed to ingest a balanced diet that included 300 g/day carbohydrate for at least 3 days before entry. The mean ± SD weight of the group as a percentage of the ideal body weight was 102.9 ± 2.0% (11).

Subjects were admitted to the Clinical Research Center (CRC) after obtaining informed consent. They were asked to refrain from vigorous exercise for 72 h before entry. Food was limited to what was served to them at the CRC. The composition of the meals was according to the recommendations of the ADA (60% CHO, 20% fat, 20% protein, 20–25 g/day crude dietary fiber), and calories for each subject were based on 1.2 times the basal energy requirement (12). Meals were served at 0800, 1145, and 1645 h, and there was a snack at 2000 h. The distribution of calories among the meals and snack was 25, 20, 40, and 15%, respectively. The subjects finished the entire meal within 30 min after it was served.

No testing was performed on study day 1 (admission). On the 2nd day, a 75-g oral glucose tolerance test was performed after an overnight fast. After sampling and 3 h after the oral glucose, the subject was given the routine breakfast, and the meal plan was resumed as described above. Plasma glucose concentrations before and during the test were normal for each subject according to the criteria of the National Diabetes Study Group (13). No further testing took place on study day 3, which was intended to ensure uniform dietary preparation and regulation of activity for the test period (day 4).

On day 4 at 0630 h, an intravenous line was placed in a forearm vein to allow frequent blood withdrawal. The line was kept patent by the slow infusion of normal saline. The diet plan on this day only was changed to ensure that the subjects received identically constituted meals for breakfast and lunch. Thus, the only difference between the meals was the time of ingestion. The meals, served at 0800 and 1145 h, consisted of 240 g of low-fat milk, 82 g raw orange sections (without skin), 60 g ground beef (after cooking), 50 g whole wheat bread, 10 g corn oil margarine, 100 g tomato, 10 g lettuce, 67 g apple slices (with skin), and 69 g banana slices. The subjects were observed during meal times to ensure that the entire meal was eaten within 20–30 min after serving. The test period lasted until 1645 h. Subjects were in the recumbent or sitting position in bed, and activities were limited to reading, watching television, or quiet conversation throughout the test period. Blood was withdrawn every 15 min throughout the study.

Analytical methods. Plasma glucose was analyzed by the automated glucose oxidase method. Serum insulin (14) and plasma C-peptide (15) were measured by radioimmunoassay. Plasma glucagon was measured by radioimmunoassay with the 30K antiserum of Unger et al. (16). Plasma α-NH_2 concentrations were measured photometrically (17). The intra-assay coefficients of variation (C.V.s) for glucose, insulin, C-peptide, glucagon, and α-NH_2 were 2, 4.5, 4, 4.9, and 5%, respectively. The corresponding interassay C.V.s were 3, 7, 9.2, 9, and 8%, respectively.

Statistical analyses. For each of the measured plasma constituents and for each individual, the mean, SD, and C.V. of all data points were calculated. Computer-assisted analysis of the data was used to identify significant peaks by previously described methods (18–24). The significance of the peaks was determined in terms of relative increments and declines. An increase or decrease in concentration was considered significant if it exceeded, in relative terms, twice the intra-assay C.V. A peak was considered significant if both its increment and subsequent decline were significant. Every peak was characterized by the time of occurrence and the height of its maximum. Coincidence of an insulin peak with a peak of another variable was defined as occurrence of the two peaks at the same sampling time ±15 min.

TABLE 1
Means, SDs, and coefficients of variation (C.V.s)

	Insulin (n = 38)		Glucose (n = 38)		C-peptide (n = 22)		α-NH_2 (n = 22)		Glucagon (n = 22)	
Subject	Mean ± SD (μU/ml)	C.V. (%)	Mean ± SD (mg/dl)	C.V. (%)	Mean ± SD (pm/dl)	C.V. (%)	Mean ± SD (mg/dl)	C.V. (%)	Mean ± SD (pg/ml)	C.V. (%)
1	32.2 ± 25.6	80	100 ± 11	11	0.88 ± 0.39	45	14.2 ± 3.7	7	45 ± 10	22
2	32.3 ± 23.0	71	102 ± 16	16	1.20 ± 0.47	39	7.03 ± 0.64	9	63 ± 9	14
3	27.7 ± 19.9	72	99 ± 15	15	1.12 ± 0.44	39			128 ± 14	11
4	36.4 ± 27.0	75	93 ± 17	18	1.19 ± 0.41	34	6.11 ± 0.67	11	54 ± 7	13
5	11.3 ± 17.0	67	98 ± 20	20	0.91 ± 0.3	43	5.23 ± 0.43	8	42 ± 9	21
6	21.6 ± 11.3	51	95 ± 12	12	1.21 ± 0.29	24	7.09 ± 0.41	6	72 ± 12	16
7	23.5 ± 17.7	75	88 ± 9	11	1.03 ± 0.36	35	6.06 ± 1.14	19	44 ± 10	22
8	29.0 ± 22.0	76	101 ± 16	16	0.97 ± 0.48	49	6.29 ± 0.62	10	76 ± 7	9
Group mean		70 ± 9		14 ± 13		39 ± 7		11 ± 5		15 ± 6

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Coefficients of correlation between data points were calculated for all possible pairs of measured blood constituents and for every individual. For each profile, the area under the postmeal glucose and insulin curve was estimated as the sum of glucose and insulin levels during the 3-h period beginning at either 0800 or 1145 h. All statistical tests were two tailed. The maximum probability level for significance was .05. Group results were expressed as means ± SD. The methods of pulse identification and statistical evaluation used in this study have been compared with other published methods of pulse identification (25). A detailed description of the pulse analysis method is provided by Van Cauter (26). Among the salient features of the pulse analysis methodology used are the following. *1*) It is very sensitive because it uses a small threshold (2 C.V.s rather than 3 C.V.s used in several other methods). 2) It does not produce an unacceptably high rate of false positives because the criteria of significance of a peak are applied to both the ascending and declining limb. 3) There is no limit to the number of data points that can be included in an ascending or declining limb so that slowly rising or falling levels are adequately evaluated.

RESULTS

Table 1 lists the mean, SD, and C.V. for each subject and profile of measured hormone or substrate. The C.V.s were highest for insulin, reflecting sharp peaks over relatively low basal levels. The variability of C-peptide was considerably less than that of insulin (39 ± 7 vs. 70 ± 9%, respectively, $P < .01$). The concentrations of glucose, glucagon, and α-NH_2 were more stable, with average C.V.s of ≤15%.

The profiles of plasma glucose and serum insulin in the entire group of subjects are shown in Fig. 1. The principal peaks immediately after the two meals were clearly defined and occurred within 1.45 h after starting the meal. During the morning hours, one or more additional glucose and insulin peaks, which were asynchronous and of considerably lesser magnitude, were seen. After lunch, secondary peaks of larger magnitude than those found during the morning hours were observed between 1300 and 1430 h. In the eight subjects, there were 41 significant glucose peaks and 40 significant insulin peaks. Thirty-two of these were coincident (i.e., a concomitance ratio of 78% for glucose vs. insulin

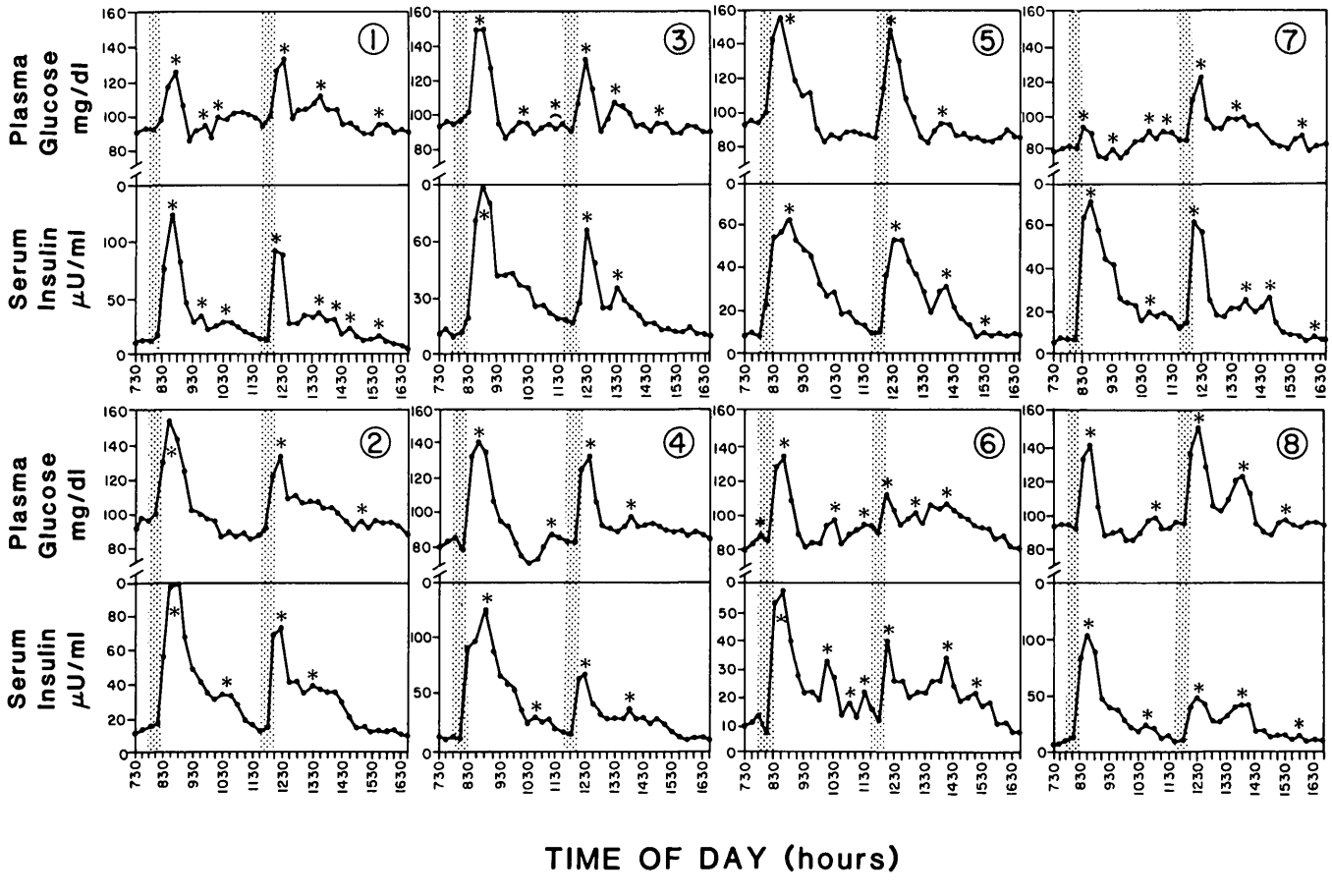

FIG. 1. Profiles of plasma glucose and serum insulin in each of 8 subjects. Subject numbers correspond to those in Table 1. Meal times are represented by *shaded areas*. *Asterisks* indicate significant peaks as defined in text. Note differing scales. In subject 3, *asterisk* before lunch denotes significance of each of 3 *points* before lunch as designated by *curved line*. Biphasic response to lunch is evident in all but 1 subject (no. 2).

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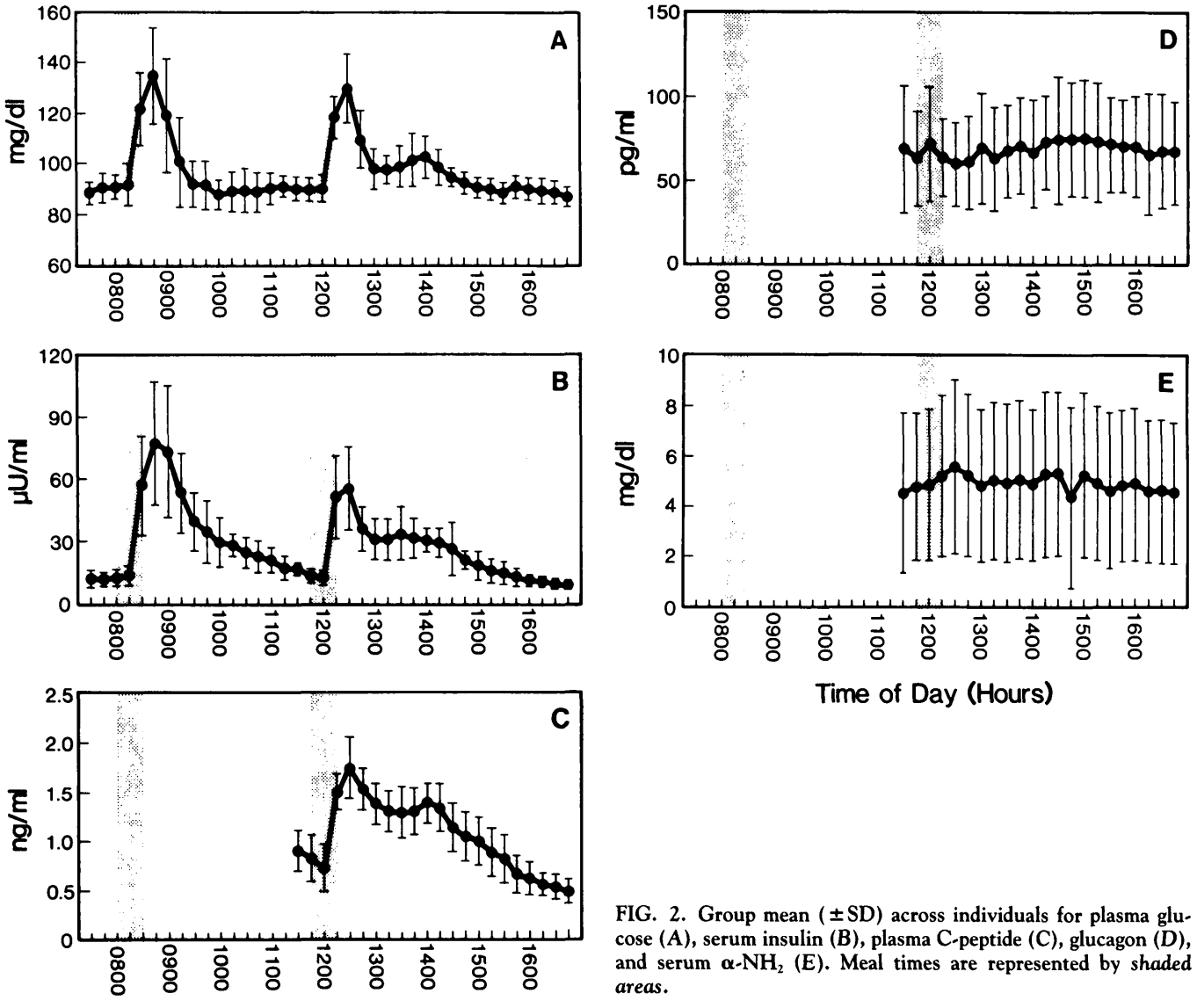

FIG. 2. Group mean (±SD) across individuals for plasma glucose (A), serum insulin (B), plasma C-peptide (C), glucagon (D), and serum α-NH_2 (E). Meal times are represented by *shaded areas*.

and of 80% for insulin vs. glucose). Of the 9 glucose peaks that did not coincide with an insulin peak, 4 were coincident with an insulin "shoulder," and 1 was coincident with a nonsignificant insulin peak. Thus, a correspondence between a glucose peak and a coincident peak or shoulder of insulin was observed in 90% of the cases. Similarly, of the 8 insulin peaks that did not coincide with a glucose peak, 5 were coincident with a glucose shoulder, suggesting a correspondence between insulin and glucose changes in 37 of 40 (92.5%) cases. Among the 32 coincident and significant glucose and insulin peaks, the maxima coincided exactly in 21 of 32 (66%) cases, the glucose maximum preceded the insulin maximum by 15 min in 7 of 32 (22%) cases, and the insulin maximum preceded the glucose maximum in 4 of 32 (12%) cases. Thus, we were unable to show a lag or lead relationship between insulin and glucose changes, which appear to be coincident. The mean profiles across individuals of plasma glucose, serum insulin, plasma C-peptide, plasma glucagon, and plasma α-NH_2 concentrations are shown in Fig. 2.

In all subjects the plasma glucose concentrations showed a detectable increment in the first sample drawn after starting each meal (Fig. 2A), with the highest values occurring 60 min after the beginning of breakfast. The glucose response to lunch (mean peak value 133 ± 13 mg/dl) was not significantly different from that after breakfast (mean peak value 137 ± 20 mg/dl, $P > .10$). The areas under the postmeal curves were also similar (mean area 1347.6 ± 99.5 after lunch vs. 1290.6 ± 111.3 after breakfast, $P > .10$), whereas the pattern of changes differed between the two meals. After lunch, in addition to the peak occurring 60 min after the meal, another peak occurred between 1330 and 1400 h, i.e.,

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1.75–2.25 h after the meal was served. All but one subject exhibited this biphasic response to lunch. In contrast, after breakfast, glucose levels after the major postmeal elevation were scattered throughout the period of 0945 to 1130 h and did not result in a consistent biphasic response.

Figure 2*B* shows the corresponding average pattern in serum insulin concentrations. The similarity with the pattern of glucose levels was evident. All subjects had a major postmeal peak after breakfast and after lunch. After lunch, a secondary significant insulin rise of smaller magnitude was observed 1–2 h after the major postmeal peak in all subjects. In contrast, after breakfast, secondary peaks were found in only six subjects and occurred sporadically in the 0945-to-1130 h period without apparent interindividual synchronization and without consistent association with a coincident glucose peak. In contrast to glucose, the initial insulin response after lunch was significantly less than after the identical meal taken at breakfast (mean peak value 60.5 ± 19.5 after lunch vs. 82 ± 26 μU/ml after breakfast, $P < .01$). Similarly, the integrated response over the 3-h period after meals, as estimated by the area under the curve, was also larger after breakfast (514.8 ± 108.3) than after lunch (397.0 ± 63.5, $P < .01$). The calculation of the ratios of insulin peak to glucose peak after breakfast (0.68 ± 0.20) and after lunch (0.45 ± 0.15) further indicated a difference in the response of insulin to a given glucose load at different times of the day ($P < .01$). The pattern of C-peptide levels paralleled those of insulin and glucose (Fig. 2*C*), suggesting that the biphasic response of insulin to lunch was due to increased β-cell secretion rather than to altered clearance.

The possibility that the secondary rise in plasma glucose might have occurred in response to the effects of an insulin counterregulatory hormone was also investigated. Measurements of plasma glucagon, performed on samples drawn between 1130 and 1645 h, showed random, erratic variations of plasma glucagon in all subjects throughout the afternoon. No consistent postmeal pattern emerged (Fig. 2*D*), excluding a role for glucagon in the control of the biphasic postlunch glucose and insulin responses.

The secondary rise in postlunch glucose pattern could also have been related to delayed absorption of a portion of the meal such as protein. We therefore measured the concentration of α-NH_2 during the period of 1130 to 1645 h. All subjects had a significant rise in the concentration of α-NH_2 with a peak between 1215 and 1230 h, clearly attributable to nutrient absorption. The rest of the profile reflected random fluctuations in concentration, thereby excluding the possibility that a delayed absorption of a component of the meal accounted for the secondary glucose peak.

On average, the fluctuations of glucose, insulin, and C-peptide were highly correlated. Thus, the coefficients of correlation between insulin and glucose, C-peptide and glucose, and insulin and C-peptide were .78 ± .18, .79 ± .08, and .88 ± .05, respectively (all significant at $P < .001$). In contrast, glucagon and α-NH_2 did not correlate with glucose, insulin, or C-peptide.

DISCUSSION

Our observations show that changes in insulin and glucose after a mixed meal are complex. After lunch, in addition to the expected immediate postmeal peak, secondary statistically significant coincident peaks in insulin and glucose concentrations occurred in all but one of the subjects. In contrast with the inconsistent insulin and glucose elevations observed after the early postbreakfast peak, the secondary postlunch peaks were of greater magnitude and occurred predictably 1.75–2.25 h after the meal was served. These biphasic postlunch patterns may reflect a phenomenon similar to that detected by Kraegen et al. (9) during a 50-g oral glucose tolerance test administered to previously fasting normal subjects. In this earlier study, secondary and even tertiary coincident peaks in blood insulin and glucose levels appeared during a similar observation period.

The aim of this study was to document and analyze movements in metabolic hormone and substrate concentrations under carefully controlled conditions in properly prepared normal subjects, a prerequisite to further experimentation designed to further evaluate and analyze these phenomena. Some of our data readily rule out certain possible explanations for the secondary postprandial peaks in insulin and glucose. For example, the fact that a secondary peak in C-peptide concentration was coincident with that of insulin implicated increased β-cell secretion rather than decreased peripheral insulin clearance (27). Analysis of glucagon profiles did not suggest that glucose and insulin fluctuations were caused by or related to glucagon changes. Furthermore, the secondary insulin peaks did not seem to be related to asynchronous absorption and appearance of glucose and α-NH_2, both of which are β-cell secretagogues.

Although our data indicate that biphasic or multiphasic patterns in insulin and glucose concentrations should be considered a normal response to lunch and are not restricted to the response to oral glucose, further investigation is required to determine whether such patterns also occur after meals other than lunch. Between 1.5 and 2.5 h after meals were served, there were significant and coincident peaks in insulin and glucose in one of eight subjects after breakfast but in seven subjects after lunch. Thus, it should be noted that patterns of insulin and glucose qualitatively compatible with the hypothesis of a biphasic response to breakfast were occasionally observed in our study. Such patterns resulted in mean insulin and glucose curves showing asymmetric early postbreakfast peaks. These early peaks, compared with those after lunch, had a broader declining rather than ascending portion. However, when secondary postbreakfast peaks were observed, their timing was inconsistent, and their magnitudes were often near or below our criteria for significance. Nevertheless, the possibility that a biphasic response after breakfast similar to that observed after lunch but of lesser magnitude may have occurred cannot be excluded.

It must, however, be emphasized that we studied a rela-

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tively homogeneous group of young volunteers. Further studies are necessary to evaluate the extent to which this biphasic response is a normal phenomenon in other groups, e.g., women and obese but normoglycemic subjects, and whether disturbances of this pattern are present in abnormal states, particularly diabetes.

The fact that a relatively straightforward observation such as the biphasic response to lunch described here has not been previously reported is probably due to inadequate sampling frequencies and lack of detailed data analysis. For example, in some reports describing normal insulin and glucose levels throughout the day, blood sampling was performed at hourly or half-hourly intervals. Because the secondary glucose and insulin postlunch peaks occurred within ~15 min, such data were probably too widely spaced to observe them. Furthermore, the practice of averaging data from a group of subjects without prior analysis of each individual profile may obscure secondary peaks because their times of occurrence vary slightly among subjects. This may have been the case in the study by Rizza et al. (28), who showed a relatively large shoulder rather than a distinct secondary peak after lunch. Thus, appropriate methods must be applied to differentiate significant peaks from methodologic variation and random, noncoincident changes in hormone and substrate concentrations (22).

On the other hand, although the morning and noon meals given in this study were identical, there were other differences between the meals that may have effected the subsequent response. First, the time elapsed since the previous meal was 12 h for breakfast but only 4 h for lunch. Thus, the shorter period of fasting before lunch may be accompanied by, for example, relatively increased hepatic and muscle glycogen stores. It is possible that the glycogen-replete liver either takes up less or produces more glucose after lunch than after breakfast and thereby contributes to or causes the fluxes in blood glucose. Second, there are important differences in the hormonal milieu between early and late morning hours (29). For example, the concentrations of several insulin counterregulatory hormones, e.g., cortisol, are greater in the early morning, which may cause or contribute to relative insulin resistance (30–32). Despite our inability to demonstrate asynchronous absorption of carbohydrate and protein, it is entirely possible that at least glucose is absorbed from the gut in a biphasic or multiphasic manner. This important possibility should be differentiated from changes in peripheral glucose disappearance or hepatic glucose production. Complex kinetic studies employing the ingestion and intravenous infusion of labeled glucose tracer will be necessary to distinguish among these intriguing possibilities. Finally, because this investigation was not continued beyond 1645 h, we cannot dismiss the possibilities that there may be additional peaks after meals, that this phenomenon might be present after the evening meal, and that meals might provoke a dampened oscillatory insulin and glucose response pattern.

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