Fasting Early Morning Rise in Peripheral Insulin: Evidence of the Dawn Phenomenon in Nondiabetes

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The dawn phenomenon, a tendency for glucose to rise between 0500 and 0800 h in subjects with diabetes, is also reflected as an increase in insulin required to maintain normoglycemia during closed-loop insulin infusion. Individuals without diabetes have minimal or absent rises in early morning glucose. To test the hypothesis that the absence of early morning glucose increases in subjects without diabetes is due to an increase in insulin levels, we measured insulin levels from 2400 to 0800 h in four male and two female volunteers. Subjects were on an unrestricted diet with three main meals and one bedtime snack at 2100 h. Blood samples were collected continuously in hourly pools by a constant-rate withdrawal pump. We observed the following: (1) hourly integrated concentration of glucose was stable from 2400 to 0800 h (range of mean plasma values, 94.5–97.3 mg/dl), and (2) hourly integrated concentration of insulin increased from the 0300–0400 (4.6 μU/ml) to the 0700–0800-h pool (6.2 μU/ml) ($P < 0.05$). The observed increase in insulin in the early morning hours despite stable levels of glucose indicates a temporally increased insulin need in nondiabetic individuals similar to that found in individuals with diabetes. The mechanism underlying this increased insulin need may be similar in diabetes and nondiabetes, with the ensuing rise in glucose being dependent on the availability of compensatory insulin.

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Some individuals with insulin-dependent diabetes have a tendency for blood glucose to rise in the early morning—the dawn phenomenon.[1,2] This rise persists despite continuous infusion of insulin at constant rates[3] but can be blunted if the rate of infusion is increased starting at about 0500 h.[4] Individuals with diabetes also have a circadian rhythm in blood glucose levels[5–8] with the ascending phase beginning at night and continuing to the morning. The change in insulin need associated with the dawn phenomenon may reflect part of this glucose cycle, that is, its ascending phase and peak.

In contrast, individuals without diabetes have minimal[9] or no rhythm[8,10] in blood glucose levels. We hypothesized that their early morning glucose stability is due to increased insulin levels during this period. Previous works examining this basic point of insulin physiology are inconclusive.[8,10,11] Such studies have been limited by two facts. First, peripheral insulin levels are low in the fasting state, and measurements of low insulin values with standard radioimmunoassays are associated with large random error, limiting the detection of small changes in the insulin level. Second, a rise in insulin level, which is of short duration and low magnitude, may be obscured by high-frequency cyclical variations in insulin levels, which normally occur,[12] unless continuous sampling techniques are used.

The purpose of this study was to test the hypothesis that peripheral insulin levels increase in the preprandial early morning period in nondiabetic subjects. Several techniques were used to decrease the random error related to sampling and analysis of insulin, therefore improving precision of results and the power to detect small but significant peripheral insulin changes.

METHODS

Six white volunteers free of diabetes as assessed by the National Diabetes Data Group criteria[13] were admitted to the Clinical Research Unit at the North Carolina Memorial Hospital after informed consent was obtained (Table 1). All studies were performed in December 1981, except that of subject 5, which occurred in July 1982. Both female participants were studied after the 23rd day of their menstrual cycle.

TABLE 1
Clinical information on participants

Subject	Sex	Age	Weight (kg)	Height (cm)
1	F	33	50	165
2	M	39	74	192
3	F	29	52	163
4	M	23	67	178
5	M	22	71	173
6	M	32	62	180

Subjects were on a diet unrestricted in content but with fixed times for all meals. Evening snack was at 2100 h (with the exception of subject 5, who had his evening snack at 2200 h), and breakfast at 0800 h. An evening snack was included to make study conditions comparable to those used in subsequent investigation of individuals with type I (insulin-dependent) diabetes. Lights were turned off at 2330 h and turned on at 0645 h. During the hours of light, subjects went about their usual activities; during the dark, they were encouraged to sleep. Between 2200 and 2330 h, a nonthrombogenic catheter was introduced in a forearm vein and kept through the next morning. Blood was withdrawn continuously at a rate of 8–10 ml/h using a portable peristaltic pump (Model ML6, Cormed, Inc., Middlepoint, New York), and samples were collected hourly. Insulin and glucose values therefore represent the average level occurring during each hour period. This approach minimizes the random fluctuations due to sampling and the short duration (13 min) cycles in insulin recently described in nondiabetes.[12] Samples were collected on ice and removed from the pump without touching the participants, thus avoiding sleep disturbances.

Plasma glucose was determined by an automated ferrocyanide method[14] (AutoAnalyzer, Technicon, Tarrytown, New York). Insulin was measured by radioimmunoassay[15] with human insulin standards. Intra-assay variations assessed with low (5.9 ± 1.0 μU/ml; mean ± SD) and median (23.8 ± 1.2 μU/ml; mean ± SD) level insulin pools were 18% and 8.8%, respectively. Between-assay variations at low and high insulin levels were 16.7% and 5.1%, respectively.[16]

The statistical significance of the increase in insulin levels from the nadir at night to the peak before breakfast was tested with a two-sided paired t test (alpha = 0.05).

RESULTS

Mean hourly integrated insulin and glucose concentrations for all six patients from 2400 to 0800 h are shown in Figure 1. All insulin levels shown in this figure were determined in the same assay, using 100 μl of plasma. While glucose remained stable, insulin initially fell, reaching a plateau at 0200–0300 h. Insulin then rose, starting at about 0500–0600 h and continuing through 0700–0800 h.

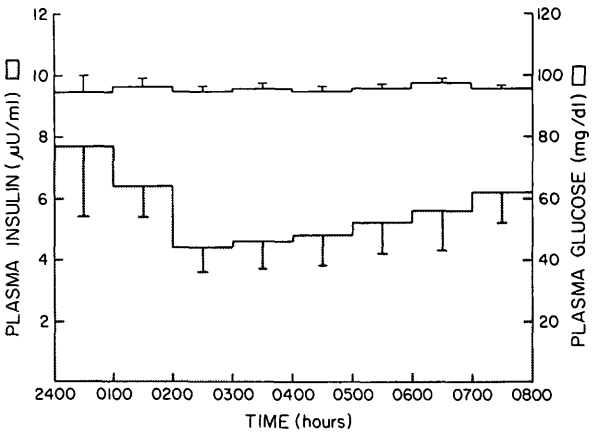

FIG. 1. Mean ± SEM of hourly integrated concentration of plasma insulin (bottom) and glucose in six volunteers free of diabetes. Insulin determinations were done with standard assay (100 μl of plasma).

Because the insulin levels were low and in a range with a high coefficient of variation, we reassayed the samples obtained at 0300–0400, 0600–0700, and 0700–0800 h using twice (200 μl) the initial volume. All samples were measured in the same assay. Values fell on the standard curve within the range of 8.2–20.6 μU/ml and were then halved to adjust for the double volume of plasma entered in the assay. The 0600–0700 and 0700–0800-h mean insulin values are higher than the 0300–0400-h ones in both assays (Figure 2). The smaller magnitude of the SEM for measurements made on the 0300–0400-h samples using 200 μl as compared with 100 μl of plasma indicates that the double-volume assay is more precise. Insulin increased between 0300–0400 and 0600–0700 h by 21.7% and 25.9% ($P > 0.10$ and $P < 0.05$) and between 0300–0400 and 0700–0800 h by 34.8% and 27.6% ($P < 0.025$ and $P < 0.005$) for the single- and double-volume assays, respectively. Their differences reached statistical

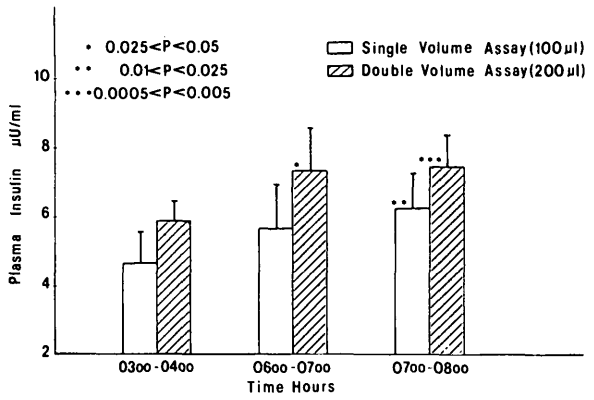

FIG. 2. Mean ± SEM of 1-h integrated concentration of insulin at the beginning and end of the overnight fasting period using standard (100 μl) and double-volume plasma assays. Results from the latter assay have been halved.

TABLE 2
Individual insulin levels during an overnight fast in six subjects free of diabetes

	Insulin level (μU/ml)							
	0300–0400 h		0400–0500 h	0500–0600 h	0600–0700 h		0700–0800 h	
Subject	100 μl*	200 μl*	100 μl	100 μl	100 μl	200 μl	100 μl	200 μl
1	6.1	7.1	6.7	5.6	6.9	10.4	8.1	8.8
2	2.6	4.1	5.0	4.5	5.8	4.8	5.7	4.9
3	6.2	4.8	3.4	5.4	3.9	4.9	5.0	5.7
4	7.3	8.0	8.4	9.7	11:0	11.4	9.9	10.3
5	2.4	5.6	2.7	2.5	2.8	6.9	4.5	8.7
6	2.9	4.9	2.3	4.1	3.3	5.3	3.7	6.2
Mean	4.6	5.8	4.8	5.3	5.6	7.3	6.2	7.4
SEM	0.9	0.6	1.0	1.0	1.3	1.2	1.0	0.9

*Volume of plasma used in assay; results from the assay using 200 μl of plasma have been halved to adjust for the double volume.

significance by the 0700–0800-h period with the regular assay and at both intervals with the double-volume assay.

While insulin levels increased in the early morning in all subjects, the individual timings for the insulin changes differed. Table 2 displays the individual insulin values obtained in both assays from the 0300–0400 to the 0700–0800-h periods. With the regular insulin assay, a tangible increase is seen more consistently at the 0700–0800-h period (subjects 1, 3, 5, and 6). Subjects 2 and 4 had an earlier rise. With the double-volume assay, an increase by 0600–0700 h was seen in all subjects except subject 3, whose increase was seen only by the 0700–0800-h period.

DISCUSSION

The results of the study demonstrate that, in individuals without diabetes, serum insulin levels change markedly between 2400 and 0800 h despite constant glucose levels. In accordance with these changes, three periods can be identified: first, a decline; second, a plateau; and third, a rise. The fall in insulin (2400–0200 h) may reflect the ending phase of glucose disposal following bedtime snack ingestion. The ensuing plateau (0200–0500 h) probably indicates a true fasting steady state. The rise in insulin that followed occurred despite the continuing fasting state. This rise in insulin level associated with constant glucose levels can only be explained by an increase in insulin need early in the morning. Whether this increase in insulin-to-glucose ratio early in the morning reflects an increase in hepatic glucose production, a decrease in glucose utilization, or both, needs to be investigated further.

The exact time at which the fasting insulin rise commences and peaks cannot be concluded from this study, since hourly averages were used. Furthermore, measurements of the fasting rise in insulin beyond 0800 h were precluded by the interruption of the fast. Nevertheless, the approximate timing of the fasting insulin rise observed here is similar to the timing of increased fasting insulin need seen in type I diabetes.[4] Also, the time characteristics found here strongly suggest that this insulin rise is part of the ascending phase of a circadian rhythm in insulin level in individuals without diabetes.[17]

The results of this study provide further insight into the "dawn phenomenon" and suggest that both individuals with and without diabetes experience a similar chronobiologic phenomenon—that is, an increased need for insulin in the early morning. In healthy individuals, the increased insulin need is met by an endogenous increase in insulin levels. In type I diabetes, the inability to compensate for the increased insulin need is manifested by a rise in glucose, unless compensation is provided by exogenous insulin. Thus, although additional mechanisms related to exogenous insulin may be at play in individuals with insulin-treated diabetes, any mechanism postulated to explain this chronobiologic phenomenon should be applicable to individuals both with and without diabetes.

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