Circadian Variation of the Blood Glucose, Plasma Insulin and Human Growth Hormone Levels in Response to an Oral Glucose Load in Normal Subjects

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SUMMARY

Circadian variations in blood glucose, plasma insulin and human growth hormone response were studied in six healthy males who received 100 gm. oral glucose loads at 6 a.m., noon, 6 p.m., and midnight. The tests were conducted at seven day intervals, and each was preceded by a ten hour fast. During the three days before each test the subjects received meals containing no less than 300 gm. carbohydrate per day. Blood samples were drawn at 0, 15, 30, 60, 90, 120, and 180 minutes. A clear circadian variation occurred in the blood glucose levels, with lower values in the morning and higher values at 6 p.m. and midnight. The insulin profiles showed a trend toward lower afternoon and night values, with a noon peak. The afternoon insulin-glucose ratios were significantly lower. HGH values were inconsistent and tended toward higher afternoon and night basal levels. The results confirm the existence of a circadian variation in the blood glucose response to oral glucose loads in healthy men. This might in turn result from a circadian variation in the insulin response, probably secondary to changes in the pancreatic β cell sensitivity to glucose. This basic mechanism is believed to sustain the conditioning influence of other hormones, HGH being one of them. DIABETES *23*:132-37, February, 1974.

As deeper insight is gained into the concept of circadian rhythms, their role in the elucidation of physiologic and pathologic phenomena becomes increasingly important. Circadian rhythms have been described for various hormones, such as HGH,[1] cortisol,[2] ACTH,[3] epinephrine[4] and others. The regulation of blood glucose is further complicated by other factors involved in its rhythm: glucose absorption rates, pancreatic β cell sensitivity to different blood glucose levels,[5] peripheral resistance. There is experimental evidence that circadian variations of the blood glucose and plasma insulin levels occur in animals in connection with light-darkness cycles.[6] Other recent findings include a circadian rhythm in the insulin response of the β cells to glucose[7] and variations in the effects of insulin on protein synthesis[8] and on the glycogen content of muscle.[9] The findings in healthy humans are, however, contradictory. In studies of fasting and postprandial blood glucose levels, some authors have found diurnal variations to occur, with greater rises in blood glucose after lunch than after breakfast.[10,11] These results could not be reproduced in other series.[12] With respect to circadian variations in the blood glucose response following an oral glucose load, the highest values were found in tests conducted in the afternoon, with minimal values occurring in the morning.[13] The behavior of the insulin response is apparently more consistent than that of blood sugar, since various authors have reported a peak response during the morning hours.[12,13]

The purpose of this study was to gain a thorough view of the circadian variations in the blood glucose, plasma insulin and HGH response of healthy subjects undergoing standard oral glucose tolerance tests. The experimental design used in this study involved an attempt to achieve uniformity in the tests and thus escape the influence of individual factors likely to alter the results, which are probably responsible, at least in part, for the contradictions noted above.

MATERIAL AND METHOD

The subjects were six males twenty to thirty-nine years old (mean ± S.E., 31.0 ± 7.0 yrs.) who

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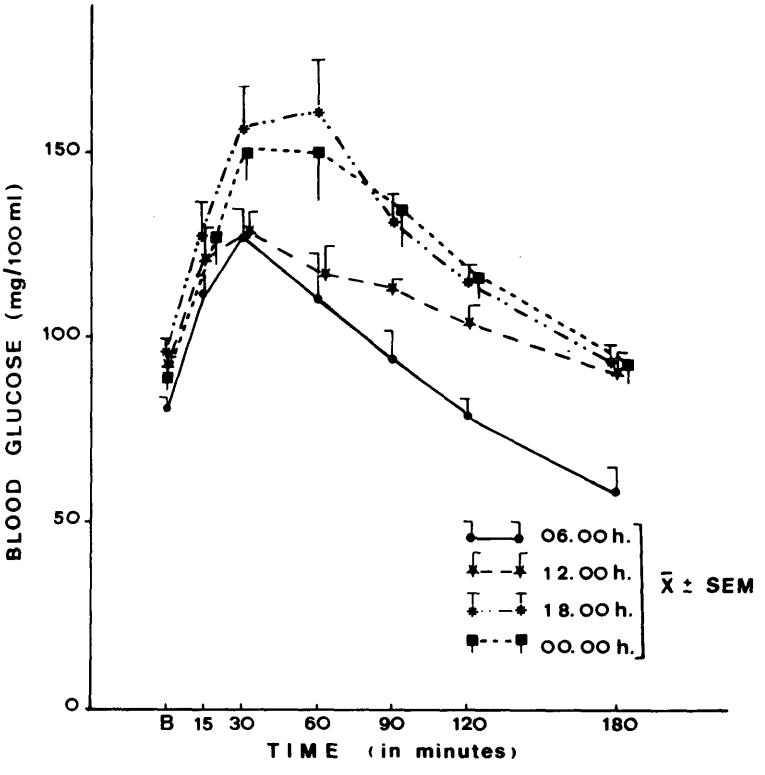
weighed the ideal value (according to age and height) ± 10 per cent. All were healthy, by both clinical and humoral standards, and from families without a history of diabetes. None was receiving any kind of drug therapy. The height of the subjects ranged from 165 to 180 cm. (mean ± S.E., 172.2 ± 7.2 cm.) and their weight from 65 to 79 kg. (mean ± S.E., 71.8 ± 5.6 kg.). Each individual had four oral glucose tolerance tests with 100 gm. glucose diluted in 500 ml. of water at room temperature, ingested in five minutes. The tests were performed at 6 a.m., noon, 6 p.m., and midnight and were separated from each other by seven day intervals. Each scheduled test was conducted on all six subjects simultaneously and was preceded by a ten hour fast, during which only water ingestion was allowed. This fast, in turn, was preceded by a standard meal. During the three days before each test, the subjects ingested meals containing no less than 300 gm. carbohydrate. Physical activity remained at normal levels throughout the entire study period.

Blood samples were drawn by venipuncture immediately before each test and 15, 30, 60, 90, 120, and 180 minutes after the beginning of glucose ingestion. Determinations included: blood glucose by the orthotoluidine method,[14] plasma insulin by the method of Herbert et al.[15] and human growth hormone according to Catt et al.[16] The insulin-glucose ratios[17] were calculated for each subject at each moment of every test.

The results were analyzed using the *t* test for paired samples, and a bidirectional criterion (2 p) was adopted for levels of significance.

RESULTS

Figure 1 illustrates the blood glucose profiles ob-



2p between \ TIME (in minutes)	0	15	30	60	90	120	180
06.00 - 12.00	<.02	ns	ns	ns	<.10	<.01	<.01
06.00 - 18.00	<.02	ns	<.10	<.05	<.01	<.01	<.01
06.00 - 00.00	=.05	ns	<.10	<.10	<.02	<.01	<.01
12.00 - 18.00	ns	ns	<.05	<.02	<.05	ns	ns
12.00 - 00.00	ns	ns	<.10	<.10	<.10	ns	ns
18.00 - 00.00	ns	ns	ns	ns	ns	ns	ns

FIGURE 1

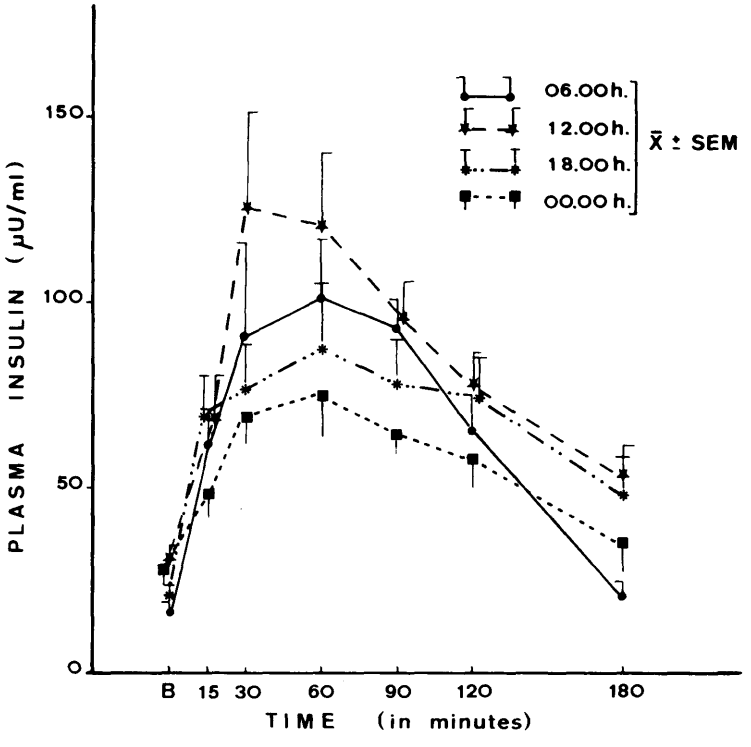
Blood glucose levels ($\bar{x}$ ± S.E.M.), basal and after 100 gm. glucose orally in six healthy young males at four different times of the day. Levels of significance of the differences between the schedule times for each period of the tests.

tained at the four scheduled times, as well as the levels of significance of the differences among them. When the 6 a.m. values are chosen as a reference, the fasting values (always in the normal range) are significantly higher at noon, 6 p.m. and midnight. The rising slopes of the 6 a.m. and noon curves are similar, whereas that of the 6 p.m. curve reaches higher values, extending for up to sixty minutes after glucose ingestion. The rise in blood sugar at midnight is similar to that observed at 6 p.m., but does not reach statistical significance upon comparison with the 6 a.m. and noon curves. The descending slopes are significantly higher at noon, 6 p.m. and midnight than at 6 a.m. Only in this last curve did the 180 minute blood glucose level fall below the baseline (2 p β vs. 180 min. < 0.02).

Figure 2 illustrates the plasma insulin profiles and the levels of significance of the differences among the various tests. The basal 6 a.m. values are significantly lower than the basal noon and midnight values. The poststimulation insulin response shows, when compared to morning values, a trend toward higher levels at noon and lower at 6 p.m. and midnight at 30, 60, and 90 minutes. These differences, however, are without statistical significance. After 120 minutes, the morning insulin levels fall more rapidly, and the 180 minute values of the noon and 6 p.m. tests are significantly higher than those obtained in the morning.

Figure 3 shows the insulin-glucose ratios found in all four tests and the levels of significance of their differences. The various noon values are similar to the 6 a.m. profile, except for the 180 minute level, which is significantly higher at noon. The 6 p.m. and midnight values, in turn, are lower during the first 120 minutes.

The HGH levels are shown in figure 4. The results were highly variable, with a trend toward lower basal



2p between \ TIME(in minutes)	0	15	30	60	90	120	180
6.00 - 12.00	<.02	ns	ns	ns	ns	ns	<.01
6.00 - 18.00	ns	ns	ns	ns	ns	ns	<.02
6.00 - 00.00	<.05	ns	ns	ns	<.02	ns	ns
12.00 - 18.00	<.10	ns	ns	ns	ns	ns	ns
12.00 - 00.00	ns	ns	<.10	ns	<.05	ns	ns
18.00 - 00.00	ns	ns	ns	ns	ns	ns	ns

FIGURE 2

Serum immunoreactive insulin ($\bar{x} \pm$ S.E.M.), basal and after 100 gm. glucose orally, in six healthy young males at four different times of the day. Levels of significance of the differences between the schedule times for each period of the tests.

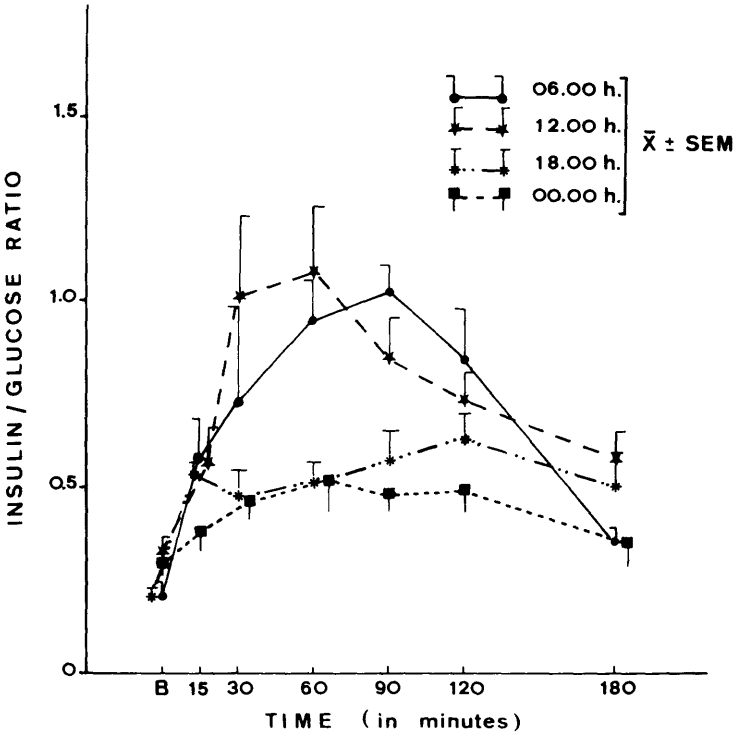


FIGURE 3

Insulin-glucose ratios ($\bar{x} \pm$ S.E.M.), basal and after 100 gm. glucose orally, in six healthy young males at four different times of the day. Levels of significance of the differences between the schedule times for each period of the tests.

2p between \ TIME (in minutes)	0	15	30	60	90	120	180
06.00 - 12.00	<.10	ns	ns	ns	ns	ns	<.05
06.00 - 18.00	ns	ns	ns	<.05	<.01	ns	ns
06.00 - 00.00	ns	ns	ns	<.02	<.01	<.10	ns
12.00 - 18.00	=.05	ns	ns	<.10	ns	ns	ns
12.00 - 00.00	ns	ns	<.10	<.10	<.02	<.10	<.10
18.00 - 00.00	<.10	<.10	ns	ns	ns	ns	ns

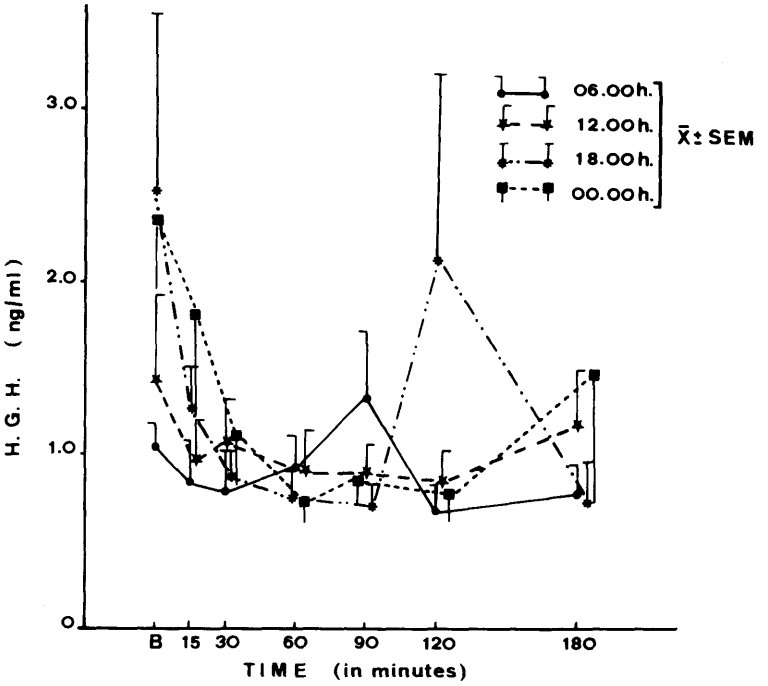
values at 6 a.m. and higher at 6 p.m. and midnight. These trends, however, were not statistically significant.

DISCUSSION

The blood glucose profiles obtained following ingestion of 100 gm. glucose at different times of the day exhibit a clear variation, characterized by lower levels at 6 a.m. and higher levels at 6 p.m. and midnight. A typical feature of the noon curve is a rising slope similar to that found in the 6 a.m. profile, but with a much slower fall.

The modulation of the various blood glucose responses must be predominantly ruled by insulin secretion and peripheral resistance. In comparison with the 6 a.m. curve, the insulin response in this study was similar or slightly higher at noon and lower at 6 p.m, and midnight. The insulin-glucose relationship, more graphically expressed as the insulin-glucose ratio, shows lowest values in the afternoon and night tests. The variation in the insulin response to glucose might explain the higher blood glucose levels found at 6 p.m. and midnight. This variation might result from circadian changes in the pancreatic β cell sensitivity to glucose, occurring as greater responsiveness during the morning, and subsiding toward the night. In this respect, the present findings confirm those of other authors both in the experimental field[6,7] and in clinical studies, although a different method was applied in the latter.[13]

Although the variations in insulin secretion explain most of the 6 p.m. and midnight findings, they do not suffice to explain the behavior of the blood glucose profile elicited at noon. The pancreatic response at noon was evidently similar to (or even higher than) the 6 a.m. response. The difference in the blood glucose curves found at these two times might be ascribed,



2p between \ TIME (in minutes)	0	15	30	60	90	120	180
06.00 - 12.00	ns	ns	ns	ns	ns	ns	ns
06.00 - 18.00	ns	ns	ns	ns	ns	ns	ns
06.00 - 00.00	ns	ns	ns	ns	ns	ns	ns
12.00 - 18.00	ns	ns	ns	ns	ns	ns	ns
12.00 - 00.00	ns	ns	ns	ns	ns	ns	ns
18.00 - 00.00	ns	ns	ns	ns	ns	ns	ns

FIGURE 4

Human growth hormone levels ($\bar{x} \pm$ S.E.M.), basal and after 100 gm. glucose orally, in six healthy young males at four different times of the day. Levels of significance of the differences between the schedule times.

therefore, to peripheral resistance factors. In this respect, the human growth hormone levels found in this study exhibited an inconsistent behavior, in spite of a trend toward higher afternoon and night basal values. Findings of others[1] could confirm this tendency of HGH.

The following conclusions may be drawn from the discussion above:

1. Under the present study conditions, the blood glucose and plasma insulin levels elicited by an oral glucose load exhibit a circadian variation.

2. The blood glucose response could be chiefly dependent on the pancreatic β cell responsiveness, which declines in the afternoon and night.

3. Although the human growth hormone levels found are not sufficiently consistent to allow definitive conclusions, HGH, by acting on peripheral glucose uptake and on insulin synthesis, might condition the blood glucose and plasma insulin responses. In this regard, other hormones with known circadian secretion rhythms, such as glucocorticoids[2] and catecholamines,[4] might also play a role in conditioning the insulin response to glucose.

4. Last, the rate of glucose absorption did not appear to play an important role under the conditions of this study. Indeed, the blood glucose rising slopes were similar at all four test times during the initial fifteen minutes. However, the possible role of intestinal factors in conditioning the degree of insulin response awaits elucidation.

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REFERENCES

[1]Quabbe, H. J., Schilling, E., and Helge, E.: Pattern of growth hormone secretion during a 24-hour fast in normal adults. J. Clin. Endocrinol. Metab. *26*:1173, 1966.

[2]Nichols, C. T., and Tyler, F. H.: Diurnal variation in adrenal

cortical function. Annu. Rev. Med. *18*:313, 1967.

[3]Sorge, F., Mentsel, G., Schuman, G., Brendell, A., and Pfeiffer, E. F.: Pardiabetikern und Fettsuchtigen, abstract 119. First Meeting European Ass. Study of Diabetes, Montecatini, 1965.

[4]Patkai, P.: The diurnal rhythm of adrenaline secretion in subjects with different working habits. Acta Physiol. Scand. *81*:30, 1971.

[5]Lerner, R. L., and Porte, D.: Relationship between intravenous glucose loads, insulin responses and glucose disappearance rate. J. Clin. Endocrinol. Metab. *33*:409, 1971.

[6]Gagliardino, J. J., and Hernández, R. E.: Circadian variation of the serum glucose and immunoreactive insulin levels. Endocrinology *88*:1529, 1971.

[7]Gagliardino, J. J., and Hernández, R. E.: Relationship between differential responsiveness of pancreatic beta cell and the circadian variation of serum IRI levels. Endocrinology *91*:822, 1972.

[8]Gagliardino, J. J., and Rebolledo, O. R.: Hormonal control of protein synthesis in muscle: An approach through the study of its circadian rhythm. Horm. Metab. Res. *4*:278, 1972.

[9]Gagliardino, J. J., and Pessaq, M. T.: 24 hr. variation of insulin effectiveness on muscle glycogen synthesis. J. Endocrinol. In press.

[10]Gordon, T.: Glucose Tolerance of Adults. National Center of Health Statistics, Series 11, No. 2, U.S.P.H.S. Publication, 1964.

[11]Wilkerson, H. L. C., and Krall, L. P.: Diabetes in a New England town. J.A.M.A. *135*:209, 1957.

[12]Malherbe, C., de Gasparo, M., de Hertogh, R., and Hoet, J. J.: Circadian variations of blood sugar and plasma insulin levels in man. Diabetologia 5:397, 1969.

[13]Jarrett, R. J., Baker, I. A., Keen, H., and Oakley, N. W.: Diurnal variation in oral glucose tolerance; blood sugar and plasma insulin levels, morning, afternoon and evening. Br. Med. J. *1*:199, 1972.

[14]Dubowski, K. M.: An O-toluidine method for body fluid glucose determination. Clin. Chem. *8*:215, 1962.

[15]Herbert, V., Lau, K., Gottlieb, S., and Bleicher, S.: Coated charcoal immunoassay of insulin. J. Clin. Endocrinol. Metab. *25*:1375, 1965.

[16]Catt, K. J., Tregear, G. W., Burger, H. G., and Skermer, C.: Antibody-coated tube method for radioimmunoassay of human growth hormone. Clin. Chem. Acta *27*:267, 1970.

[17]Perley, M., and Kipnis, D. M.: Plasma insulin responses to glucose and tolbutamide of normal weight and obese diabetic and nondiabetic subjects. Diabetes *15*:867, 1966.