Circadian modulation of glucose and insulin responses to meals: relationship to cortisol rhythm

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Van Cauter, Eve, E. Timothy Shapiro, Hartmut Tillil, and Kenneth S. Polonsky. Circadian modulation of glucose and insulin responses to meals: relationship to cortisol rhythm. Am. J. Physiol. 262 (Endocrinol. Metab. 25): E467-E475, 1992.—To determine whether glucose and insulin responses to a mixed meal are influenced by time of day irrespective of duration of prior fast, eight normal subjects (4 males, 4 females) were studied on two separate occasions, involving ingestion of identical meals at either 6- or 12-h intervals. The 24-h profiles of plasma glucose, serum insulin, and plasma C-peptide were obtained at 20-min intervals. Plasma cortisol levels were measured on each sample to evaluate possible relationship between diurnal variations in metabolic responses and circadian rhythm of cortisol. Rates of secretion of insulin and cortisol were mathematically derived from peripheral concentrations by deconvolution using two-compartment models for clearance kinetics. Postmeal responses of glucose, insulin, and insulin secretion rate were evaluated by calculating maximum postmeal increment, total area under curve, area under curve for 2 h after meal ingestion, and total duration of response. Postmeal cortisol responses were quantified by increment in plasma level and amount secreted in postmeal pulse. For glucose responses, irrespective of duration of prior fast, all four parameters characterizing the response were significantly greater in the evening than in the morning, with total area under curve and 2-h area under curve being approximately twofold larger in the evening than in the morning. Time of day did not significantly influence maximum postmeal increment in insulin secretion rate or duration of insulin secretory response, but total and 2-h areas under curve were 25–50% greater in the evening than in the morning. Meal ingestion was followed by a significant pulse of cortisol secretion in 37 of 40 cases. Magnitude of morning-to-evening increase in insulin response to meals was correlated with magnitude of morning-to-evening decrease in cortisol level. We conclude that glucose and insulin responses to mixed meals in normal adults of both sexes are profoundly modulated by circadian rhythmicity and that this diurnal variation in meal tolerance may be mediated at least partially by circulating cortisol concentrations. In the evening, failure of insulin secretion to increase in proportion to changes in postmeal glucose responses suggests that, in addition to circadian changes in insulin sensitivity, there may be a circadian variation in responsiveness of β-cells to glucose.

circadian rhythmicity; food intake; glucose tolerance; insulin secretion

THE ROLE OF CIRCADIAN RHYTHMICITY in modulating human endocrine function is well established for systems directly dependent on the hypothalamopituitary axis. It is often assumed that the function of more peripheral endocrine systems, such as the pancreas, is not significantly influenced by central mechanisms responsible for circadian timing. However, in normal humans, a series of studies have suggested that the set point of glucose regulation may be under circadian control. Indeed, glucose tolerance to an oral glucose load or to a single intravenous injection of glucose is decreased in the afternoon compared with the morning (8). With the use of experimental designs involving constant glucose infusion for 24–30 h, we have shown that glucose tolerance continues to deteriorate as the evening progresses and reaches a minimum around midsleep (19, 22). In a recent study, we have further demonstrated that the diurnal variation in glucose levels during constant glucose infusion is paralleled by a similar variation of insulin secretion, which is inversely related to the circadian rhythm of cortisol concentrations (21). These observations obtained during constant glucose infusion suggest that there may be a systematic circadian variation in the glucose and insulin responses to a mixed meal, with larger responses in the evening than in the morning, and that this effect of time of day on meal responses could be partially mediated by cortisol. The existence of such a consistent effect of time of day on meal tolerance could be of importance in the design of meal schedules, which would optimize glucose control for subjects with impaired glucose tolerance and in the development of strategies to cope with conditions of abnormal circadian rhythmicity (i.e., “jet lag” or shift work), which are frequently associated with gastrointestinal disorders.

In all previous studies designed to examine the possible effects of time of day on meal tolerance, the response to the morning meal, which was presented after a 10- to 12-h fast, was compared with the response to the evening meal, which was presented after a 4- to 5-h fast. Thus, when morning vs. evening differences in meal responses were observed, it could not be determined whether they reflected differences in the duration of prior fast or true circadian modulation. Reduced carbohydrate tolerance to mixed meals absorbed later in the day has been shown in some, but not all, studies involving identical meals given at various times of day after varying durations of prior fast (1, 10, 12, 13, 17). The presence and magnitude of a diurnal variation seemed to be dependent on meal size and composition (1, 12, 17). Two studies by the same group have indicated that the effects of time of day may be more prominent in women than in men (1, 12). It is noteworthy that, in all these previous studies, the “evening” meal was actually given in the late afternoon, between 1630 and 1800 h, at a time when, according to our 24-h studies during constant glucose infusion (19, 22), glucose tolerance has only begun to deteriorate. Thus larger and more consistent effects of time of day may occur when the evening meal is consumed later, as is often the case under ordinary conditions of daily living.

The present study was therefore undertaken to com-
pare the responses to identical meals given at 0800 and at 2000 h while controlling for the duration of the prior fast. To delineate the size, duration, and dynamics of the meal responses, peripheral levels of glucose, insulin, and C-peptide were determined at 20-min intervals over a 24-h span, and insulin secretion rates were derived from the C-peptide levels by deconvolution. Cortisol levels were measured on all samples to evaluate the relationship between diurnal variations in metabolic responses to meals and the circadian periodicity of cortisol secretion.

SUBJECTS AND METHODS

Subjects

Eight normal subjects, four men and four women aged 22–35 yr, were studied. All were of normal weight (body mass index 21.5 ± 2.2 (SD) kg/m², range 18.8–24.5 kg/m²), and none had a personal or family history of diabetes. Fasting glucose and insulin levels averaged 4.94 ± 0.16 (SD) mmol/l and 45.9 ± 4.3 pmol/l, respectively. Shift workers or subjects who had experienced a transmeridian flight <6 wk before the study were excluded. The protocol was approved by the Institutional Review Board of the University of Chicago, and all subjects gave written informed consent.

Protocol

Each subject participated in three different studies. The order of the studies was randomized.

Twenty-four-hour study with meals at 6-h intervals. After a 6-h fast, the subjects were admitted in the Clinical Research Center at 1800 h and, starting 2 h later, ingested five identical 500-kcal meals presented at 6-h intervals over a 36-h period. Thus meals were served at 2000 h, 0200 h (nocturnal meal), 0800 h (morning meal), 1400 h (lunch meal), and 2000 h (evening meal). Meal composition was 43% carbohydrate, 39% protein, and 18% fat. All meals were entirely consumed within 30 min. The subjects slept in dimly lit rooms 22 h to 0000 h but were awakened at 0200 h to consume the nocturnal meal. The hand with the sampling catheter was kept in a heating blanket to ensure arterialization of the venous blood sample. Arterialized blood samples were taken at 20-min intervals for 24 h starting at 0600 h, i.e., 2 h before the morning meal.

Twenty-four-hour study with meals at 7-h intervals. After a 6-h fast, the subjects were admitted at 1800 h and ingested three identical meals at 12-h intervals over a 36-h period. Thus the meals were served at 2000 h, 0800 h (morning meal), and 2000 h (evening meal). The subjects were awakened for 45 min at 0200 h to control for the sleep loss involved in the study with meals at 6-h intervals. In all other respects the protocol was identical to that in the study with meals at 6-h intervals.

Determination of individual C-peptide kinetics. To obtain accurate estimates of insulin secretion, the kinetics of C-peptide were determined in each subject as previously described in detail (15). Briefly, endogenous C-peptide secretion was suppressed by means of a primed intravenous somatostatin infusion (500 µg/h; Bachem Fine Chemicals, Torrance, CA), and a 150-µg bolus injection of biosynthetic human C-peptide (Eli Lilly, Indianapolis, IN) was administered. Plasma C-peptide levels were measured at 1- to 3-min intervals over the first 20 min, at 5-min intervals over the following 40 min, and at 10- to 20-min intervals over the following 2 h. For each individual, the rate constants describing the distribution and metabolism of C-peptide were derived from the analysis of the decay curve using a two-compartment mathematical model (15). Mean ± SE values for the short and long half-lives of C-peptide were 4.8 ± 0.2 and 33.6 ± 1.9 min, respectively, mean volume of distribution was 4,090 ± 180 ml, and the fraction of decay associated with the short half-life was 0.78 ± 0.01.

Assays

Serum insulin was assayed by a double-antibody technique (11) with an intra-assay coefficient of variation averaging 6% throughout the range of measured concentrations. Plasma C-peptide levels were determined using a previously described radioimmunoassay (6) with an average intra-assay coefficient of variation of 4%. Plasma cortisol levels were measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA) with an average intra-assay coefficient of variation of 5%. All samples from the same subject were analysed in duplicate in the same assay. Plasma glucose concentrations were measured in duplicate with a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH) with a coefficient of variation of <3%.

Data Analysis

Derivation of insulin secretory rates. The rates of production of C-peptide, and, by inference, the insulin secretory rates, were mathematically derived from the plasma C-peptide concentrations using a two-compartment model for C-peptide distribution and metabolism, with individual parameter values derived from the decay curve observed after the bolus injection of C-peptide. This calculation, commonly described as “deconvolution,” involves the integration of equations described by Eaton et al. (5). We have demonstrated that this procedure provides accurate estimates of insulin secretion, even under non-steady-state conditions (15).

Estimation of endogenous insulin clearance. In most situations in which insulin clearance is measured, the endogenous secretion rate of the peptide is not known, and the metabolic clearance rate is derived as the ratio of the exogenous infusion rate of the peptide and its steady-state plasma concentration. In the present study, endogenous insulin secretion was estimated from plasma C-peptide concentrations as described above, and the clearance of endogenously secreted insulin during each meal was calculated as the ratio of the area under the curve (AUC) of insulin secretory rate and the AUC of simultaneously measured peripheral serum insulin concentration as previously described (18).

Postmeal responses in plasma glucose and insulin secretion. The basal level in each individual study was taken to be the morning premeal level. For each meal ingested during the sampling period, the premeal levels of plasma glucose, serum insulin, and insulin secretion were calculated as the mean of the levels during the hour preceding meal presentation. The maximum increments of plasma glucose, serum insulin, and insulin secretion rate were defined as the difference between the maximum level attained after the meal and the premeal level. Because caloric intake was only 1,000 or 1,500 kcal during the sampling period, premeal levels of plasma glucose, serum insulin, and insulin secretion were generally lower in the evening than in the morning. The duration of the response was calculated as the time interval between meal presentation and return to premeal level or basal level, whichever came first. The postmeal responses were estimated as the total AUC above the premeal level. In addition, to evaluate differences in the short-term response to the meal, the AUC for the first 2 h after the meal were similarly calculated.

Analysis of pulses of serum insulin, plasma glucose, plasma cortisol, and insulin secretion. To identify significant pulses of serum insulin, plasma glucose, plasma cortisol, and insulin secretion, each individual 24-h profile was analyzed using Ultra.
a computerized pulse-detection algorithm previously described and validated (20). The general principle of this algorithm is the elimination of all peaks for which either the increment (difference between level at the peak and level at the preceding trough) or the decrement (difference between level at the peak and level at the following trough) do not exceed a certain threshold related to measurement error. For serum insulin, plasma glucose, and plasma cortisol, this threshold was set at twice the intra-assay coefficient of variation, a level which, in view of our previous estimations of the frequency and magnitude of pulses of these blood constituents (14, 19, 22), is expected to optimally balance false-positive and false-negative errors (20). For insulin secretory rates, a more conservative threshold of three times the intra-assay coefficient of variation of C-peptide determinations was used to take into account the amplification of noise involved in the deconvolution procedure.

Postmeal responses in plasma cortisol. For each meal, the premeal plasma cortisol level was calculated as the mean of the levels measured during the hour preceding meal presentation. Cortisol secretory rates were mathematically derived from the plasma cortisol concentrations using a two-compartment model for cortisol distribution and metabolism (3). Thus the same deconvolution procedure as that used to estimate insulin secretion was applied to the estimation of cortisol secretory rates, except that, instead of using individually derived kinetic parameters, the parameter values for the disappearance kinetics of cortisol were obtained from previously published studies (3). The volume of distribution, short half-life, and fraction of decay associated with the short half-life were taken to be 41.1% of body weight, 5 min, and 0.80, respectively, for all subjects. For each subject, the long half-life was adjusted in the previously reported physiological range of 65-90 min (3) by an iterative process designed to minimize the number of negative secretory rates. On average, the long half-life was 72.5 ± 5.3 min, and the number of significantly negative secretory rates was 0.25 ± 0.20 across all 16 studies, i.e., 0.4% of all calculated secretory rates. The error associated with each secretory rate, which results from measurement error on plasma levels, was derived following the theory of error propagation, and the process of pulse identification was repeated for the profile of cortisol secretory rates using a threshold of two times the calculated error. The profiles of cortisol secretory rates generally included more pulses than the profile of plasma concentrations, because the deconvolution procedure revealed that a single large pulse of plasma concentration may reflect two successive pulses of secretion. A postmeal cortisol response was considered to be present if a peak of cortisol secretion rate occurred within 60 min (i.e., 3 sampling intervals) after meal presentation, and the magnitude of the response was estimated by integrating the secretory rates observed in two representative subjects during the studies with meals presented at 6- and 12-h intervals. A clear increase in the levels of glucose, insulin, and insulin secretion was observed after each meal. A significant pulse in plasma cortisol was observed after 35 min, and insulin secretion was observed after each meal. A significant pulse in plasma cortisol was observed after 35 min with meals at 12-h intervals (left) and during study with meals at 6-h intervals (right). Large upward arrows, times of meal presentation; downward arrows, significant pulses of cortisol, glucose, insulin, and ISR. M. meal.

Fig. 1. Twenty-four-hour profiles of plasma cortisol, glucose, serum insulin, and insulin secretion rates (ISR) in subject M2 during study with meals at 12-h intervals (left) and during study with meals at 6-h intervals (right). Large upward arrows, times of meal presentation; downward arrows, significant pulses of cortisol, glucose, insulin, and ISR. M. meal.

RESULTS

Figures 1 and 2 depict the profiles of plasma cortisol, plasma glucose, serum insulin, and calculated insulin secretory rates observed in two representative subjects during the studies with meals presented at 6- and 12-h intervals. A clear increase in the levels of glucose, insulin, and insulin secretion was observed after each meal. A significant pulse in plasma cortisol was observed after 35 min of the total of 40 meals presented during the 2 studies. Pulses in peripheral concentrations of glucose, cortisol, and insulin, as well as insulin secretory rates, were evident in response to meals as well as during the periods separating the meals.

The mean profiles of plasma cortisol, plasma glucose, serum insulin, and insulin secretion rates for the two studies are shown in Fig. 3. The estimated postmeal responses are represented by the shaded areas.

Postmeal Glucose Responses

Four measures of the postmeal glucose responses were used to evaluate the effect of time of day, including the maximum postmeal increment, the AUC during the en-
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The number of significant glucose pulses in the 6 h after meal presentation averaged 1.8 ± 0.6 (SD). The frequency of glucose pulses was similar in both studies and did not appear influenced by time of day.
Postmeal Responses in Insulin Secretion Rate

As for glucose, the duration of the interval between meals was not found significant in determining the characteristics of postmeal responses in insulin secretion. Maximal postmeal increments of insulin secretion rate were similar in the morning and in the evening in both studies (Table 1). In the study with meals at 12-h intervals, the total AUC for insulin secretion were ~25% larger in the evening than in the morning. In the study with meals at 6-h intervals, the AUC were ~50% larger in the evening than in the morning. As was the case for glucose, the enhancement of the morning-to-evening difference was related to smaller morning responses when meals were presented at 6-h, rather than 12-h, intervals. Even though the total duration of the response tended to be longer in the evening, analysis of the 2-h AUC indicated that the effect of time of day was primarily due to increased insulin secretion during the first 2 h after the meal.

Pulses of insulin secretion after meal presentation were significantly more frequent than pulses of glucose, averaging 3.2 ± 1.2 (SD) in the 6-h interval after the meal (i.e., ~1 pulse every 2 h). There was no difference in pulse frequency between studies or according to time of day.

Postmeal Responses in Plasma Insulin

As shown in Table 1, the quantitative characteristics of the postmeal responses in plasma insulin showed trends similar to those observed for the insulin secretory rates (i.e., higher total and 2-h AUC in the evening than in the morning and prolonged duration of response), but these failed to reach statistical significance. Estimations of endogenous insulin clearance during the morning and evening meal suggested that the absence of circadian variation in postmeal insulin responses could be due to increased insulin clearance in the evening. Indeed, a tendency for increased insulin clearance in the evening was present in the study with meals at 12-h intervals (1.42 ± 0.16 l/min in the morning vs. 1.59 ± 0.16 l/min in the evening; P < 0.10) and was highly significant in the study with meals at 6-h intervals (1.22 ± 0.18 l/min in the morning vs. 1.81 ± 0.31 l/min in the evening; P < 0.01).

The frequency of pulses of plasma insulin after meal presentation was similar to that observed for the insulin secretory rates and averaged 3.7 ± 1.1 (SD) pulses in the 6-h interval after the meal. There was no difference in the frequency of plasma insulin pulses between studies or according to time of day.

Postmeal Cortisol Responses

As exemplified by the cortisol depicted in Figs. 1 and 2, postmeal rises in cortisol concentrations were usually clearly identifiable. However, in a few instances, deconvolution analysis was necessary to detect postmeal rises in secretion, which were not apparent in the profile of plasma concentrations. An example is shown in Fig. 4, which illustrates the profile of plasma concentrations and the calculated profile of secretory rates in subject M5 during the study with meals at 12-h intervals. The evening meal was presented at a time when plasma cortisol levels were declining after the occurrence of a large spontaneous pulse. No pulse of plasma concentration occurred after the meal. However, examination of the profile of secretory rates reveals that, at the time of presentation of the evening meal, the secretory process that had caused the large pulse of plasma concentration was terminated and meal ingestion was followed by a smaller secretory pulse. Due to the prolonged disappearance kinetics of the hormone, this meal-related pulse failed to result in an elevation of plasma level. In total, a significant pulse of cortisol secretion was identified after 37 of the 40 meals presented. One of the exceptions is shown in Fig. 4. Indeed, in this individual study, the morning meal was presented when cortisol levels were peaking and only resulted in a small nonsignificant rise in cortisol secretion. The cortisol profiles presented in Figs. 1, 2, and 4 also demonstrate that numerous (i.e., 4–10) cortisol pulses occurred independently of meals and

Table 1. Characteristics of responses to meal

<table>
<thead>
<tr>
<th></th>
<th>Meals at 12-h Intervals</th>
<th>Meals at 6-h Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning</td>
<td>Evening</td>
</tr>
<tr>
<td><strong>Glucose response to meal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum increment, mmol/l</td>
<td>2.4±0.2</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>Total AUC, mmol·l⁻¹·min</td>
<td>190.6±18.1</td>
<td>224.9±33.6</td>
</tr>
<tr>
<td>2-h AUC, mmol·l⁻¹·min ·min</td>
<td>111.3±18.7</td>
<td>203.5±15.9</td>
</tr>
<tr>
<td>Duration of response, min</td>
<td>93±16</td>
<td>155±13</td>
</tr>
<tr>
<td><strong>Serum insulin response to meal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum increment, pmol/l</td>
<td>281±23</td>
<td>238±99</td>
</tr>
<tr>
<td>Total AUC, pmol·l⁻¹·min</td>
<td>21.53±2.57</td>
<td>25.79±2.53</td>
</tr>
<tr>
<td>2-h AUC, pmol·l⁻¹·min ·min</td>
<td>14.95±1.52</td>
<td>17.65±1.90</td>
</tr>
<tr>
<td>Duration of response, min</td>
<td>220±30</td>
<td>263±33</td>
</tr>
<tr>
<td><strong>Insulin secretory response to meal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum increment, pmol/min</td>
<td>353±34</td>
<td>343±34</td>
</tr>
<tr>
<td>Total AUC, pmol·min</td>
<td>28.25±4.25</td>
<td>35.62±3.63</td>
</tr>
<tr>
<td>2-h AUC, pmol·min ·min</td>
<td>21.80±3.05</td>
<td>26.67±3.03</td>
</tr>
<tr>
<td>Duration of response, min</td>
<td>283±17</td>
<td>263±34</td>
</tr>
</tbody>
</table>

Values are means ± SE. AUC, area under curve; NS, not significant.
CIRCADIAN VARIATION IN MEAL RESPONSES

Fig. 4. Profiles of plasma cortisol (top) and calculated profile of cortisol secretory rate (bottom) obtained in subject M5 during study with meals at 12-h intervals. Upward arrows, times of meal presentation; downward arrows, significant pulses of cortisol concentration and of cortisol secretory rates. Note morning meal was given at time when cortisol levels were already peaking and did not elicit significant postmeal rise. Evening meal was given at time of declining cortisol concentrations and resulted in shoulder, rather than peak, of cortisol concentrations. Deconvolution calculations revealed existence of secretory response to meal.

Table 2. Postmeal cortisol responses

<table>
<thead>
<tr>
<th></th>
<th>Meals at 12-h Intervals</th>
<th>P</th>
<th>Meals at 6-h Intervals</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morning</td>
<td>Evening</td>
<td></td>
<td>Morning</td>
</tr>
<tr>
<td>Premeal plasma level, nmol/l</td>
<td>364±66</td>
<td>168±33</td>
<td>&lt;0.001</td>
<td>312±59</td>
</tr>
<tr>
<td>Increment in concentration, nmol/l</td>
<td>177±52</td>
<td>113±36</td>
<td>&lt;0.02</td>
<td>270±69</td>
</tr>
<tr>
<td>Amount secreted, μmol</td>
<td>43.0±12.1</td>
<td>18.4±5.8</td>
<td>&lt;0.001</td>
<td>55.1±10.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Magnitude of postmeal cortisol response was estimated by integrating cortisol secretory rates over duration of postmeal pulse.

Sex Differences in Postmeal Responses

Table 2 gives the quantitative characteristics of the postmeal cortisol responses. Irrespective of the interval between meals, the premeal level, the postmeal increase in plasma level, and the amount of cortisol secreted in response to the meal were all significantly lower in the evening than in the morning. In the morning, the amount of cortisol secreted in response to the meal was positively correlated with the magnitude of the postmeal responses in plasma glucose, serum insulin, and insulin secretion (Table 3). In the evening, there were no significant correlations between the magnitude of the cortisol response and the magnitude of the postmeal metabolic responses (Table 3).

To determine whether the effect of time of day on the magnitude of the postmeal glucose and insulin responses was related to the diurnal variation in cortisol concentrations, correlations between the morning-to-evening increases in postmeal responses in plasma glucose, serum insulin, and insulin secretion and the morning-to-evening decline of cortisol levels were calculated. On average, the postmeal responses in plasma glucose, serum insulin, and insulin secretion, as estimated by the 2-h AUC, increased by 166 ± 57, 20 ± 9, and 49 ± 11%, respectively, from morning to evening. Premeal cortisol levels decreased from 0800 to 2000 h in 14 of 16 individual studies, and the relative decline averaged 33 ± 16% across all studies. As shown in Fig. 5, the magnitude of the morning-to-evening increase in meal response was positively correlated with the magnitude of the morning-to-evening decline of premeal cortisol levels for serum insulin ($P < 0.01$) and insulin secretion ($P < 0.05$). The correlation was not significant for plasma glucose ($P < 0.11$). These correlations were not dependent on the two studies where cortisol levels were higher, rather than lower, at 2000 than at 0800 h and which were thus associated with "negative" decreases from morning to evening (as shown on the abscissa of Fig. 5). Indeed, when the cortisol decrease in these two studies was considered to be zero, the coefficients of correlation were 0.421 ($P < 0.11$), 0.614 ($P < 0.01$), and 0.511 ($P < 0.05$) for plasma glucose, serum insulin, and insulin secretion, respectively.

Sex Differences in Postmeal Responses

Previous reports (1, 12) have indicated that the effects of time of day on glucose and insulin responses to meal may be sex dependent. In the present study, the 2-h AUC for glucose and insulin secretion were larger in the evening than in the morning in all 16 individual studies, irrespective of the sex of the subject. However, the magnitude of the effect of time of day had a tendency to be larger in women than in men. In men ($n = 4$), the evening glucose response, as estimated by the 2-h AUC, was on
average 57 ± 19% higher than the morning response in the study with meals at 12-h intervals and 96 ± 43% higher than the morning response in the study with meals at 6-h intervals. The corresponding values for women \((n = 4)\) were 167 ± 57% \((P < 0.06\) compared with men\) and 172 ± 214% \((P = 0.17\) compared with men\). Similarly, for insulin secretion, the postmeal responses in men in the evening were 15 ± 6 and 40 ± 22% higher than in the morning in the studies with meals at 12- and 6-h intervals, respectively. In contrast, in women, the corresponding values were 40 ± 16% \((P < 0.06\) compared with men\) and 101 ± 12% \((P < 0.02\) compared with men\).

These sex differences in magnitude of the diurnal variation in meal response of glucose and insulin secretion were paralleled by a sex difference in the magnitude of the morning-to-evening cortisol decrease, which was significantly larger in women than in men. Indeed, in women, the relative morning-to-evening decrease averaged 69 ± 6% whereas in men, it was only 27 ± 9% \((P < 0.01\)\), when the decrease was considered to be zero in the two studies where cortisol levels were higher at 2000 than at 0800 h.

**DISCUSSION**

The present study was undertaken to determine whether glucose and insulin responses to meals are influenced by circadian timing. The protocol was specifically designed to control for a number of potentially confounding variables that could influence the outcome and interpretation of the data. To avoid differences in plasma glucose and insulin, which could result from differences in the type of food, subjects were given meals that were identical in composition and caloric content but were presented at different times of the day. The period of fasting before each meal was either 12 or 6 h, respectively, and was kept constant irrespective of the time of day at which the meal was consumed. An equal number of male and female subjects were studied. To address the possibility that the deterioration in glucose tolerance after ingestion of a mixed meal may only reach a sufficient magnitude to be clearly detectable later in the evening, our subjects were given the evening meal at 2000 rather than in the late afternoon as was done in previous studies.

The results clearly demonstrate that the glucose response to a meal is substantially greater when the meal is eaten in the evening rather than the morning. This marked effect of time of day is robust and, in the present study, all measures of the postprandial glucose response were greater in the evening than in the morning, including the maximum increment, total area under the curve, the area under the curve during the 2 h after meal presentation, and the duration of the glucose response. The effect of time of day was statistically significant irrespective of whether there was a 6- or 12-h period of fasting before administration of the meal, although there was a tendency for the differences to be greater with the shorter duration of fasting, which is more comparable with normal meal schedules. Surprisingly, the large increase in glucose response was not associated with a commensurate increase in insulin concentrations or insulin secretion rates. Thus, although a trend toward greater postprandial responses in plasma insulin in the
evening was apparent, the difference failed to reach statistical significance. Increased insulin clearance in the evening appeared to be partially responsible for the absence of circadian variation in magnitude of the plasma insulin response. Indeed, the areas under the curve for the insulin secretion rates derived by deconvolution of peripheral C-peptide were increased in the evening, but to a lesser extent than the postprandial glucose responses. The evening vs. morning differences in maximum increment and duration of the response were not significant. The failure of insulin secretion rates to increase in proportion to the observed increase in glucose raises the possibility that, in addition to diurnal changes in insulin sensitivity, there may be diurnal changes in the set point at which $\beta$-cells respond to glucose, with reduced responsiveness in the evening resulting in increased plasma glucose concentrations.

Earlier studies had shown that meal ingestion may evoke short-term cortisol rises that are more pronounced when the meal is eaten around noon than in the evening (7, 16). The present results show that meal-related increases in cortisol levels also occur consistently in the morning and that the amount of cortisol secreted in response to a morning meal is positively correlated with the magnitude of the postprandial glucose and insulin responses. Furthermore, the magnitude of the morning-to-evening increase in postmeal insulin response was positively correlated with the magnitude of the morning-to-evening decline of cortisol levels. These findings are in agreement with our recent observation during constant glucose infusion of an inverse temporal relationship between the circadian variation of glucose levels and insulin secretion, on the one hand, and that of plasma cortisol, on the other hand (21). Under these experimental conditions, the amplitude of the diurnal variation in insulin secretion was significantly correlated with amplitude of the cortisol rhythm (21). Thus both studies suggest that the cortisol rhythm may mediate, at least partially, the diurnal variation in carbohydrate tolerance. However, this concept is difficult to reconcile with current notions on cortisol counterregulatory mechanisms, which would predict that low cortisol levels in the evening would be associated with increased, rather than decreased, insulin sensitivity.

Other pathways by which the centrally generated circadian signal could modulate postprandial glucose and insulin responses include neural transmission and endocrine factors outside of the corticotropic axis. Gastrointestinal hormones could be implicated, but, because the morning-to-evening differences in meal responses parallel the diurnal variations in glucose tolerance seen after intravenous glucose administration (8, 19, 22), it appears unlikely that they are the primary cause of circadian changes in meal tolerance. Diurnal variations in concentrations of counterregulatory hormones other than cortisol could conceivably be responsible for circadian modulation of the set point of glucose regulation. However, peripheral levels of glucagon (19), growth hormone (23), and catecholamines (9) do not undergo consistent diurnal variations during the waking portion of the 24-h cycle.

The meals given in the present study were of relatively small size (500 kcal) and were not of particularly high carbohydrate content (43%). With this type of meal, the glucose responses in the evening were approximately twofold larger than those observed in the morning. The careful studies of Service et al. (17), who studied the effect of meal size, and of Nuttall et al. (12), who studied the effect of meal composition, indicate that the effect of time of day would have been even more pronounced if larger meals of higher carbohydrate content had been given. Taken together with the present findings, these studies suggest that, depending on meal size and composition, the postprandial glucose response in the evening could be at least fivefold larger than in the morning.

Studies in human subjects living in isolation units have shown that the duration of intermeal intervals are correlated with the length of the "free-running" circadian period (2), indicating a role for the circadian system in the timing of food ingestion in the absence of social constraints. Records of the spontaneous food intake of adults living in normal environments have revealed that the size and composition of meals also undergo diurnal changes, with increasing meal sizes and a shift from high carbohydrate to high fat and protein as the day progresses (4). The current analyses show that this intricate control of feeding behavior by circadian rhythmicity is matched by circadian variations in metabolic responses to meals. Further understanding of the mechanism linking circadian rhythmicity, feeding behavior, and metabolic responses to food could lead to the development of optimal dietary regimens for patients with impaired glucose tolerance and diabetes as well as for individuals submitted to conditions of abnormal circadian rhythmicity, such as jet lag and shift work.

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REFERENCES


