Modifications of Circadian Cortisol Rhythm by Cyclic and Continuous Total Enteral Nutrition

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Summary To clarify the relationship between the pattern of diet intake and the circadian adrenocortical rhythm, we measured plasma cortisol levels at 4-h intervals over a 24-h period in 18 patients who were in the vegetative state and had been receiving total enteral nutrition (TEN) for 4 weeks. One group of 6 patients was given a liquid diet intraduodenally and continuously throughout a day (continuous TEN), whereas the two other groups received their daily enteral feeding during a restricted time of day, either in the daytime from 0800 h to 2000 h (diurnal TEN, 6 patients) or in the nighttime from 2000 h to 0800 h (nocturnal TEN, 6 patients). In patients with diurnal TEN, there was a clear cortisol rhythm with a peak at 0800 h, whose pattern was quite similar to the well-established cortisol rhythm in normal subjects. Patients with nocturnal TEN also showed a cortisol rhythm, but the peak appeared at 1600 h. There was no appreciable difference in the amplitude of the rhythm between the two groups. Patients with continuous TEN did not show any consistent circadian cortisol rhythms. Plasma levels of glucose, insulin, and free fatty acids also showed circadian fluctuations corresponding to the pattern of diet infusion in the groups with diurnal and nocturnal TEN, and remained almost constant throughout a day in the group with continuous TEN. We conclude from these results that the timing of diet intake may have a synchronizing effect on the circadian cortisol rhythm in man, as it does in laboratory animals.

Key Words circadian rhythm, plasma cortisol, plasma glucose, plasma free fatty acids, plasma insulin, total enteral nutrition
Since the initial report of Pincus (1) that the excretion of urinary ketosteroids shows a diurnal change, the circadian rhythmicity of adrenocortical activity has been widely accepted in man and also in laboratory animals. Although there is evidence that the circadian adrenocortical rhythm is controlled by some endogenous mechanism, it is not clear, particularly in man, what serves as the environmental synchronizer or entrainer for setting the phase of the endogenous rhythm (2). During the past decade the role of timing of food provision as an entrainer has been proposed for laboratory rodents. This is based on the findings that in rats (3–5) and mice (6) the phase of the plasma corticosterone rhythm shifts when the feeding time is changed and that the peak of the corticosterone level appeared just before the feeding time regardless of lighting conditions. In human subjects, however, such a synchronizing role of meal intake is controversial.

Several authors did not observe such an effect of meals on human plasma cortisol pattern and were doubtful about the relationship between the peak and feeding time (7–9). On the other hand, Quigley and Yen (10) demonstrated a dramatic surge of cortisol immediately after food intake at mid-day but not at evening. These observations were confirmed by Follenius et al. (11), and suggest that food intake at morning and/or noon hour may act as an exogenous synchronizer and amplifier for cortisol secretion. To investigate whether the pattern of dietary intake actually influences the human circadian cortisol rhythm, in the present study we measured plasma cortisol levels at 4-h intervals for 24 h in patients who had been kept under various schedules of total enteral nutrition. Circadian fluctuations of the plasma levels of glucose, free fatty acids, and insulin were also examined.

**METHODS**

Subjects. The subjects of this study were 4 male and 14 female hospitalized patients (57–87 years old) having either cerebral infarction or cerebral bleeding. Although all patients were unconscious and in vegetative states (Glasgow coma scale of 3–5), they were free of any specific diseases, such as infectious diseases, renal dysfunction, hypertension, or endocrine and metabolic diseases. Clinical profiles of the patients are summarized in Table 1. Informed consent for the circadian study was obtained from the family of each patient. The procedure was in accord with the ethical standards of the Committee on Human Experimentation of this university.

Schedules of total enteral nutrition. Nutritional support of the patients was achieved by total enteral nutrition (TEN): that is, they were given a chemically defined diet (Elental, Morishita Pharm., Osaka) as the only source of nutrition through a chronically inserted nasoduodenal tube. The composition of the diet was 79.4% dextrin, 17.6% amino acids, 0.6% soybean oil, 2% minerals, and 0.4% vitamins on a weight basis. The diet was dissolved in water at the concentration of 0.21–0.27 g/ml (0.8–1.0 kcal/ml).

The patients were randomly divided into 3 groups of 6 patients each (Table J. Nutr. Sci. Vitaminol.
Table 1. Clinical profiles of the groups with diurnal, nocturnal, and continuous total enteral nutrition (TEN).

<table>
<thead>
<tr>
<th>Schedule of TEN</th>
<th>Diurnal</th>
<th>Continuous</th>
<th>Nocturnal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Sex (M : F)</td>
<td>1 : 5</td>
<td>2 : 4</td>
<td>1 : 5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>69.5 ± 2.4</td>
<td>65.2 ± 4.1</td>
<td>71.3 ± 3.9</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>133 ± 6</td>
<td>136 ± 6</td>
<td>136 ± 6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>71 ± 4</td>
<td>66 ± 4</td>
<td>71 ± 5</td>
</tr>
<tr>
<td>Red blood cell (× 10^12/mm³)</td>
<td>399 ± 10</td>
<td>405 ± 10</td>
<td>403 ± 8</td>
</tr>
<tr>
<td>Plasma total protein (g/100 ml)</td>
<td>7.4 ± 0.5</td>
<td>7.1 ± 0.1</td>
<td>7.6 ± 0.2</td>
</tr>
<tr>
<td>Plasma urea nitrogen (mg/100 ml)</td>
<td>10.4 ± 3.1</td>
<td>11.8 ± 1.9</td>
<td>12.1 ± 2.2</td>
</tr>
<tr>
<td>Plasma creatinine (mg/100 ml)</td>
<td>0.62 ± 0.11</td>
<td>0.65 ± 0.09</td>
<td>0.71 ± 0.10</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/100 ml)</td>
<td>190 ± 23</td>
<td>192 ± 15</td>
<td>213 ± 11</td>
</tr>
<tr>
<td>Plasma GOT (IU/liter)</td>
<td>18.6 ± 2.7</td>
<td>22.1 ± 1.9</td>
<td>20.6 ± 2.4</td>
</tr>
<tr>
<td>Plasma GPT (IU/liter)</td>
<td>19.4 ± 3.5</td>
<td>20.8 ± 2.6</td>
<td>18.7 ± 1.8</td>
</tr>
</tbody>
</table>

Blood pressure and plasma values were within the normal range in every group.

1) In one group, the diet was infused continuously at the rate of 50–63 ml/h to give 1,200–1,500 kcal a day (continuous TEN). In the two other groups, the diet was infused only during a restricted time of day, either in the daytime from 0800 h to 2000 h (diurnal TEN) or in the nighttime from 2000 h to 0800 h (nocturnal TEN). It was given every day at the rate of 100–125 ml/h to give the same amount of the diet per day as that in continuous TEN.

Illumination was provided by natural light and also by fluorescent strip lights that were usually turned on at 0700 h and off at 2100 h.

Sample collection and assays. After 4 weeks on the intake of TEN, blood samples were taken from an antecubital vein at 4-h intervals over a 24-h period. Plasma levels of cortisol and insulin were determined by respective radioimmunoassay kits (Midori-Jyuji, Tokyo). Plasma glucose was measured by a glucose oxidase kit (Boehringer-Yamanouchi, Tokyo) and plasma free fatty acids (NEFA) by the method of Laurell and Tibbling (12).

Data analysis. Values are expressed as mean ± SEM. Data were analyzed first by analysis of variance to examine whether there was any significant effect of clock time. Then, the cosinor method (13) was used for detection and quantitative characterization of circadian rhythms. In brief, a cosine curve with a period of 24 h was fitted to the data using the method of least squares, and the following parameters were estimated: mesor (24-h rhythm-adjusted mean), acrophase (peak concentration time), and amplitude. The differences of the parameters between the diurnal and nocturnal TEN groups were tested by Student's t-test.
RESULTS

Figure 1 shows 24-h profiles of blood glucose, NEFA, and insulin in patients with continuous, diurnal, and nocturnal TEN. Blood glucose levels were higher during the daytime in the diurnal TEN group, whereas in the nocturnal TEN group they were higher during the nighttime. Similarly, blood insulin levels remarkably increased immediately after the start of diet infusion in both groups of diurnal and nocturnal TEN. In the continuous TEN group, however, blood glucose and insulin levels remained fairly constant. Blood NEFA levels decreased during the time of diet infusion in the diurnal and nocturnal TEN groups, but remained almost constant in the continuous TEN group.

The 24-h fluctuations of blood glucose, insulin, and NEFA were characterized further by the cosinor analysis. As summarized in Table 2, significant 24-h rhythms were detected in the diurnal and nocturnal TEN groups, but not in the continuous TEN group. Although the amplitude of the rhythm was not significantly different between the diurnal and nocturnal TEN groups, the acrophase differed by about 12 h between the two groups (13.5 h for glucose, 11 h for insulin, and 11.4 h for NEFA).

Figure 2 shows the 24-h patterns of plasma cortisol. The mean plasma cortisol
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Fig. 2. Circadian rhythms of plasma cortisol levels in the diurnal (top), continuous (middle), and nocturnal (bottom) TEN groups. Values are expressed as % of the 24-h mean. Hatched bars indicate the times of diet infusion. P values are those from ANOVA, n.s., not significant.

levels of the diurnal TEN group showed a significant circadian rhythm, with a peak of 414±55 nM at 0800 h and a nadir of 171±28 nM at 2000 h. A significant cortisol rhythm was also found in the nocturnal TEN group, but the peak (524±130 nM) and nadir (337±108 nM) appeared at 1600 h and 0000 h, respectively. The cosinor analysis revealed that the difference in the acrophase between the diurnal and nocturnal TEN groups was about 6.2 h (Table 2). There was no significant difference in the amplitude and the mesor of the two groups. Plasma cortisol did not show any significant circadian rhythmic changes in the continuous TEN group.

DISCUSSION

Although the circadian pattern of plasma corticosteroid levels is known to be influenced by a number of factors, such as light, sleep, stress, and meals, it still remains unclear which is the major determinant or entrainer of the cortisol rhythm in man. This is attributed to the difficulty in the studies on man to dissociate the possible factors from each other and to examine effects of one factor independently of those of others. In order to overcome this problem and to assess the role of

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Table 2. Characteristics of circadian rhythms of plasma glucose, insulin, NEFA, and cortisol.

<table>
<thead>
<tr>
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<th>Mesor</th>
<th>Acrophase (clock time)</th>
<th>% amplitude</th>
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<tbody>
<tr>
<td>Diurnal TEN (n = 6)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Glucose (mm)</td>
<td>9.05 ± 0.72</td>
<td>154 ± 0041</td>
<td>35.9 ± 6.3*</td>
</tr>
<tr>
<td>Insulin (pM)</td>
<td>141 ± 22</td>
<td>1612 ± 0042</td>
<td>134.4 ± 24.6*</td>
</tr>
<tr>
<td>NEFA (g/liter)</td>
<td>2.33 ± 0.23</td>
<td>0657 ± 0108</td>
<td>143.4 ± 42.8**</td>
</tr>
<tr>
<td>Cortisol (nM)</td>
<td>279 ± 19</td>
<td>0911 ± 0048</td>
<td>36.1 ± 7.6*</td>
</tr>
<tr>
<td>Nocturnal TEN (n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mm)</td>
<td>6.05 ± 0.22</td>
<td>0511 ± 0036</td>
<td>22.0 ± 3.4*</td>
</tr>
<tr>
<td>Insulin (pM)</td>
<td>80 ± 8</td>
<td>0315 ± 0021</td>
<td>99.2 ± 9.1*</td>
</tr>
<tr>
<td>NEFA (g/liter)</td>
<td>3.35 ± 0.21</td>
<td>1820 ± 0030</td>
<td>54.5 ± 7.2*</td>
</tr>
<tr>
<td>Cortisol (nM)</td>
<td>411 ± 47</td>
<td>1522 ± 0052</td>
<td>21.3 ± 4.9*</td>
</tr>
</tbody>
</table>

The amplitude is expressed as % change from the mesor. Statistical significance of each circadian rhythm was determined by an F-test based on the zero-amplitude hypothesis (13). Values for continuous TEN are not shown, because no significant circadian rhythms were detected in every substance. * p < 0.001, ** p < 0.01

nutrient intake in the regulation of the cortisol rhythm, in the present study we chose as subjects hospitalized patients who were being fed a liquid diet entirely through a nasoduodenal tube. In one group of patients, the diet was infused continuously, whereas in the other two groups it was restricted to a period either during the daytime (0800–2000 h) or during the nighttime (2000–0800 h) every day. The former (continuous TEN) is the most commonly used schedule for total enteral nutrition, but the latter, which are known as cyclic TEN, have also been recommended in some cases (14).

Since glucose, NEFA, and insulin are the representative substances whose plasma level changes rapidly in response to feeding and fasting, we examined first 24-h profiles of the plasma level of these substances to confirm actual patterns of nutrient intake and metabolism. As expected, a considerable rise of plasma levels of glucose and insulin and a concomitant drop of plasma NEFA level were observed during the time of diet infusion (Fig. 1). In contrast, the plasma level of these parameters was kept fairly constant when the diet was infused continuously throughout a day. All these results not only confirm the pattern of diet infusion, but also imply that the activities of digestion, absorption, and metabolism of nutrients are almost normal in every group of patients. This was also supported by the fact that the laboratory data for general profiles of the patients were normal in all groups (Table 1).

Our study demonstrated that in diurnal TEN there is a clear cortisol rhythm with a peak at 0800 h, the time being coincident with the start of diet infusion (Fig. 2). This pattern is quite similar to the well-established cortisol rhythm seen in
normal subjects (2). This is not a surprising result because the schedule of diurnal TEN can be regarded as a mimicry of diurnal feeding habits of normal man. However, the cortisol rhythm was delayed by some 8 h in nocturnal TEN to have a peak at 1600 h, 4 h before the start of diet infusion. The cosinor analysis also revealed an about 6-h shift of the acrophase without appreciable change in the amplitude in nocturnal TEN (Table 2). Thus, the phase of the cortisol rhythm shifts on changing the time of diet infusion. These effects of diet intake are similar to those demonstrated in rodents (3–6), suggesting a role of diet intake as an entrainer of the circadian cortisol rhythm. The importance of the cyclic intake of nutrients for the maintenance of the cortisol rhythm was also supported by our observation that the rhythm disappeared in continuous TEN.

It was thus demonstrated that nutrient intake possibly serves as an entrainer of the circadian adrenocortical rhythm in man, as it does in laboratory animals. The present study, however, failed to confirm a constant and stable phase relationship between the cortisol rhythm and the entrainer: that is, the difference between the acrophase and the time of the start of diet infusion was not identical in diurnal and nocturnal TEN (Fig. 2, Table 2). Thus, a 12-h shift of the diet infusion time changed the acrophase by only 6–8 h, not 12 h. This was a contrast to the 24-h fluctuations of plasma glucose, NEFA, and insulin, whose acrophase shifted by about 12 h on changing the diet infusion time by 12 h. Although reasons for the apparent instability of cortisol rhythm is unclear at present, it should be noted that plasma cortisol level itself and its circadian pattern are influenced, more or less, by various factors other than nutrient intake. Cycles of light and darkness may serve as an additional entrainer, as suggested by Orth and Island (15). It seems also possible that some form of social interactions such as daily care by physicians and nurses modifies the cortisol rhythm. Further studies are needed to clarify roles of these factors.

Utilizing frequent blood sampling at 10-min intervals or less, several authors reported that meal intake causes a rapid but rather transient rise of plasma cortisol (10, 11, 16). In the present study, however, plasma cortisol levels were high before the start of diet infusion, and decreased gradually as the diet was infused. This apparent discrepancy may be due to a longer interval between blood samplings (4 h) in our study, which is not frequent enough to detect a postprandial surge of cortisol secretion. It is thus likely that meal intake has two different influences on the adrenocortical system: one is a direct postprandial effect and the other a signal as an entrainer, and that the postprandial effect on cortisol secretion is superimposed on the entraining effect. This supposition is quite consistent with a recent report of Honma et al. (17), who demonstrated in rats that temporal patterns of plasma corticosterone level are determined not only by a factor associated with endogenous circadian oscillation systems but also by a direct postprandial effect of food itself.

Parenteral feeding, like TEN and total intravenous alimentation, is commonly carried out on a schedule of continuous 24-h infusion of nutrients, whereas the normal pattern of food intake in humans is usually confined to the daytime hours.
and thus is cyclic in nature. A schedule of cyclic parenteral feeding has sometimes
been recommended to reduce complications seen with continuous parenteral
nutrition or to provide time during which the patients are free from the nutrient
infusion so that they may engage in some other activities (14, 18). Considering a
close relationship between the feeding pattern and circadian rhythm of various
endocrine and metabolic activities, it is rational to imagine that the metabolic fate
and the efficiency of nutrients are also influenced by the pattern of parenteral
nutrition. In this connection, of special interest is the observation that an intermittent
feeding regimen is better to maintain nitrogen balance than a continuous one when
feeding patients postoperatively the same amount of diet via a nasogastric tube (19).
There is also a report that squirrel monkeys lose more weight when kept under
continuous intravenous feeding than when given the same amount of nutrients only
during a 12-h period every day (20). These results suggest that cyclic feeding is
nutritionally more beneficial than continuous feeding. It is likely that the effects
of feeding schedule on nitrogen balance and body weight change may be intimately
associated with those of the circadian rhythm of endocrine and metabolic functions.

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