A Comparison of Circadian Rhythms in Feeding, Plasma Insulin, Glucose and Glucagon and Pancreatic Insulin and Glucagon between Normal and Diabetic Chinese Hamsters

William VanSickle
Western Michigan University

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A COMPARISON OF CIRCADIAN RHYTHMS IN FEEDING, PLASMA INSULIN, GLUCOSE AND GLUCAGON AND PANCREATIC INSULIN AND GLUCAGON BETWEEN NORMAL AND DIABETIC CHINESE HAMSTERS

by

William VanSickle

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of the
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There are three people who made decisive contributions to my graduate education and this thesis: Dr. Leonard Beuving, my graduate advisor and Dr. Jean Lawrence, both of Western Michigan University and Dr. George Gerritsen of The Upjohn Company, with special reference to the Chinese hamsters he most generously supplied. In completing this thesis I have found all their criticisms and suggestions extremely helpful.

William VanSickle
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Western Michigan University, M.A., 1977
Physiology

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INTRODUCTION

Aggravation or amelioration of diabetic symptoms is often associated with changes in food intake. Recent experiments indicate that different biochemical and physiological variables related to the diabetic symptoms of spontaneously diabetic animals are involved in these dietary effects. It has been shown that modifying total caloric intake and dietary composition affects the mitotic activity of beta cells and the levels of serum insulin in the diabetic mutant mouse (1,2). Food restriction stabilizes blood glucose and plasma insulin and increases the concentration of pancreatic insulin in the young db mouse (3) and reverses the resistance of adipose tissue to insulin in older KK mice (4). It has been reported that binding of insulin to receptors can be returned toward normal in diabetics by dietary regulation (5). In addition, a diabetic syndrome can be induced in the sand rat by modifying dietary composition (6,7) and can be prevented in the C3F1 (Wellesley hybrid) mouse by restricting caloric intake (8). These studies using animal models of diabetes may eventually help explain the therapeutic effects of dietary regulation that is used to treat diabetes in humans.

The Chinese hamster (Cricetus griseus) is among the preferred animal models used to study diabetes (9,10). Extensive inbreeding has fixed the genetic composition in a homozygous state (11). The diabetic hamster has a relatively short life span that is reduced by diabetes (12) and exhibits a variety of metabolic, hormonal (13,14,15) and
histologic pathologies (16,17,18 and 19) that appear to be similar to those found in human diabetics.

It has been demonstrated that the diabetic Chinese hamster consumes more food over a period of 24 hours than does the non-diabetic hamster, but has a net retention of calories similar to the normal hamster (20). The severity of the disease in this animal, as measured by blood sugar, has been reported to be responsive to manipulation of dietary composition and caloric intake (21). Food restriction retards the onset and reduces the severity of diabetic symptoms in hyperphagic Chinese hamsters born of two ketogenic parents (22). There is a suggestion that substitution of a low-fat diet for a high-fat diet will reduce glycosuria and eliminate ketonuria in ketogenic Chinese hamsters (23). Overnight fasted, refed, but not ad lib fed, diabetic Chinese hamsters have reduced levels of plasma insulin (20) and elevated levels of plasma glucagon (24). Pancreatic glucagon concentrations have been reported to be higher in the diabetic than in the non-diabetic hamster fed ad lib (23,24).

These studies demonstrate that, like other animal models of diabetes, the diabetic syndrome in the Chinese hamster is influenced by changes in the diet. The observations that relate excessive food consumption to severity or onset of symptoms suggests that faulty regulation of food intake may be contributing to the manifestation of clinical diabetes. It would therefore be of interest to obtain more information about the diabetic hamster's feeding behavior and to relate to this behavior those metabolic and endocrine variables that are involved in diabetes.
The diabetic Chinese hamster's feeding habits and the aberrations in blood and tissue chemistry have not been adequately characterized for the normal, free-feeding situation. Therefore, the purpose of this investigation was to describe 24-hour variations in feeding and to relate plasma insulin and glucose, and pancreatic insulin to these feeding habits of diabetic and non-diabetic Chinese hamsters. As glucagon, a glycogenolytic and gluconeogenic hormone, has been suggested to be involved in diabetic hyperglycemic in the Chinese hamster (24), the circulating and pancreatic concentration of this hormone was also measured.

MATERIALS AND METHODS

Adult, inbred, diabetic and non-diabetic hamsters were obtained from The Upjohn Company. Non-diabetic hamsters came from sublines that had been bred for lack of glycosuria. The diabetic and the non-diabetic control animals were balanced within group and between groups for age and sex. The diabetic animals all had similar duration of glycosuria (7.1 ± 2.0 months). All animals were housed individually in 11 ½ x 7 ½ x 5" deep plastic mouse cages (Maryland Plastics) containing wood shaving for bedding material. Purina Mouse Breeder Chow (20) and tap water were provided ad lib throughout this investigation. All animals were housed in a temperature (32°C) and humidity controlled room under a 12:12 hour light-dark cycle, with lights on at 7:00 a.m. A small map light with a pink painted bulb was used as the only source of illumination whenever it was necessary to enter the animal room during the dark phase of the lighting cycle. The animals were handled as little as possible but
the investigator entered the animal room frequently. At least fifteen days adaptation was allowed prior to investigation.

A twenty-four hour pattern of food intake was determined for seventeen diabetic and ten control hamsters by measuring the amount of food eaten during eight, non-overlapping three-hour intervals. These measurements were taken at random over a period of nine days in order to determine if the feeding patterns were constant (29). It was observed that food spillage was negligible so that only the food remaining in the hopper at the end of a three-hour feeding period was weighed. A single measurement of total 24-hour food consumption and body weight was taken for each of these 27 animals at the completion of this study.

Blood samples and the entire pancreas were obtained from mixed groups of diabetic and control animals that were sacrificed within 0.5 hours of the following time periods: 7:00 a.m., 10:00 a.m., 4:00 p.m., 10:00 p.m. and 1:00 a.m. These time periods corresponded to the high and low points and three periods of intermediate food intake. Animals were individually removed from the animal room to an adjacent room where they were quickly weighed, bled via the orbital sinus and decapitated. Blood was collected in chilled test tubes that contained 375 KIU trasylol, a proteolysis inhibitor (FBA Pharmaceuticals, Inc.) in 45 mg Na$_2$EDTA (Eastman Kodak Company) in a volume of 0.075 ml. Each blood sample was centrifuged and the plasma removed and frozen at -20°C until assayed for glucose, insulin and glucagon. The pancreas' were removed and immediately frozen on dry ice. Pancreatic insulin and glucagon were extracted into acid.
alcohol by the method of Gerritsen (13) as modified by Wyse (personal communication). The tissue homogenates were stored at 4°C for 72 hours during which they were stirred once at 48 hours. They were centrifuged at 4°C and 1.0 ml aliquots neutralized to a pH of 7-8 with 100 μl of 20% (v/v) NH₄OH. The neutralized extracts were centrifuged to remove precipitates and diluted by a factor of 100 with the appropriate assay buffer. Fifty and 100 μl aliquots of the diluted extracts were assayed for insulin and/or glucagon. All insulin and glucagon determinations were made in a single respective assay.

Plasma glucose was determined using 0.050 ml aliquots by the glucose oxidase method. Immunoreactive insulin was determined using a modification of the cellulose immunoassay procedure of Falloona and Unger (26). ¹²⁵I-insulin was obtained from Nuclear International Corp.; ¹²⁵I-glucagon from New England Nuclear Corp.; beef insulin standard Lot #P4609 and porcine glucagon standard Lot #258-B30-138-4 from Eli Lilly Research Laboratories; and glucagon antiserum 30K from R. Unger. Total plasma volume was estimated as a percentage of the body weight (27).

Two-tailed student's t-test with consideration given to unequal n's and variances and unpaired observation where applicable was used to compare means. Analysis of variance for repeating measures (28) was used to compare feeding patterns and simple correlations were also determined for some of the endpoints measured.
RESULTS

As can be seen in Figure 1 diabetic and non-diabetic Chinese hamster exhibit a circadian (a single peak and nadir occurring once about every 24 hours) pattern of food consumption. There was an extremely significant ($p < .0001$) variation in the quantities of food consumed when nocturnal and daylight feeding was compared. There was no difference ($p > .60$) between group in this pattern.

Both diabetic and control groups ate significantly more food during the first 3 hours of darkness than they did during the last 3 hours of light (diabetics: 0.57 gms vs. 0.27 gms $p < .001$; controls: 0.41 gms vs. 0.17 gms $p < .05$). The diabetics tended to eat more than the controls for 7 of the 8, 3-hour intervals so that by 24 hours they had consumed significantly more food (Table 1).

<table>
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Twenty-Four Hour Food Consumption and Body Weights (grams; mean ± S.E.) of Diabetic and Control Chinese Hampsters

<table>
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<th>n</th>
<th>Food Intake</th>
<th>Body Weights</th>
</tr>
</thead>
<tbody>
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<td>Diabetics</td>
<td>16</td>
<td>3.86 ± 0.15</td>
<td>29.6 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p &lt; .01$</td>
<td>N.S.</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>3.24 ± 0.13</td>
<td>30.2 ± 1.5</td>
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N.S. = $p > .05$
In Figure 2, the levels of plasma glucose are compared between groups at 5 different time periods during a 24-hour period. High intra-group variation, consistently high levels, and no apparent changes during the time periods examined was observed for the diabetic group. However, the non-diabetic group showed marked elevation of plasma glucose during the nocturnal phase as compared with the daylight phase of the feeding cycle.
Diabetics -○; controls -○. (mean ± S.E.) Numbers = n's. N.S. = non-significant. 10:p.m. and 1:a.m. values differ from 10:a.m. and 4:p.m. values (p < .01) for controls.

Plasma insulin (Figure 3) was elevated at 10:00 p.m. as compared to both 10:00 a.m. and 4:00 p.m. in the control group. A significant difference in plasma insulin was observed between the two groups at 10:00 p.m. At 4:00 p.m. plasma insulin in the diabetics was reduced compared with the 7:00 a.m. and 10:00 a.m. values.
Plasma Insulin Concentrations For 5 Time Intervals During A 24-Hour Period.

Diabetics - ●; controls - ○. (mean ± S.E.). 10:p.m. value higher than 4:p.m. (p < .05) and 10:a.m. (p < .01) values for controls. 4:p.m. value lower than 7:a.m. and 10:a.m. (p < .05) value for diabetics.

Plasma glucagon levels are presented in Figure 4. At 1:00 a.m. plasma glucagon concentrations are higher than the 4:00 p.m. value for both groups. Also the control group exhibited higher levels at 10:00 a.m. than at 4:00 p.m. There was no statistically meaningful difference at any of the 5 time periods between either the diabetic of non-diabetic groups.
FIGURE 4

Plasma Glucagon Concentrations For 5 Time Intervals During A 24-Hour Period.

Diabetics - ○; controls - ○. (mean ± S.E.) 1:a.m. values for both groups higher than their 4:p.m. values (p < .05). 10:a.m. value differs from 4:p.m. value (p < .01) for controls.

Significant differences in pancreatic glucagon content between diabetic and control groups were found only at 10:00 p.m. and 7:00 a.m. (Figure 5). At 10:00 p.m. a reduction in pancreatic glucagon content compared with the 10:00 a.m. value was observed only in the control group.
FIGURE 5

Pancreatic Glucagon Concentrations During A 24-Hour Period.

Diabetics - ○; controls - □. (mean ± S.E.) 10:00 p.m. value lower than 10:00 a.m. value (p < .05) for controls.

The insulin content of the pancreas was significantly lower in the diabetics compared with the non-diabetics at all 5 time periods and there were no statistically significant changes in levels over time for either group (Figure 6).

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Diabetics - •; controls - 0. (mean ± S.E.)

The body weights of both groups did not significantly vary over the observation periods and remained similar to those values given in Table 1.

Plasma insulin and glucose were weakly but significantly correlated in the control group ($r = 0.56$, $p < 0.01$). This correlation was significantly higher than that for the diabetic group (0.56 vs. 0.03, $p < 0.05$). No significant correlations between food intake and/or the other pancreatic or plasma variables were found.
DISCUSSION

The normal Chinese hamster exhibits a circadian rhythm in food consumption that is typical of nocturnally feeding rodents (34). It is evident that although the diabetic Chinese hamsters consumed more food than the normal hamsters over a period of 24 hours, their feeding pattern was the same. Since the description of 24-hour feeding patterns was obtained as a composite of 3-hour food consumption measurements, it is possible that a finer analysis (such as could be obtained by electronically monitoring food consumption) could have revealed subtle differences between diabetics and non-diabetics in feeding behavior. Nevertheless, the average quantity of food consumed varied with time to form a cyclic pattern that was similar for both diabetic and non-diabetic hamsters. The eight periods of food consumption were taken at random over nine days so that if differences existed between groups in frequency or phase of the feeding cycle there should have been several aberrant values for 3-hour food consumption (29). Because a circadian pattern of feeding did emerge for both groups, it is unlikely that such phase or frequency shifts occurred during these nine days.

The relatively short interval (3 hours) over which measurements of food intake were taken and the high intragroup variations prevented significant differences between groups to be observed at any of the individual time intervals. However, the diabetics tended to consume more food than the controls for most of the feeding cycle.
This suggests that a change in reference level or baseline or feeding has occurred. That such a change has developed is supported by the fact that the diabetics consumed a greater quantity of food over 24 hours than did the non-diabetics. The consequences of excessive food consumption is the aggravation of the diabetic syndrome (22,23) but the causes are not apparent. Excessive food consumption (hyperphagia) often results from procedures that produce gross functional abnormalities in those brain areas that integrate feeding behavior (30,31) but these procedures also result in a loss of rhythmic eating habits (31,32). Since the diabetics in this study exhibited a rhythmic pattern of eating similar to the non-diabetics, it is unlikely that their hyperphagia was due to abnormal CNS integration of feeding. The hyperphagia may be related to the morphological aberrations in the small intestines of the diabetic hamster observed by Diani (19). Abnormal gut motility, the absorption of nutrients, or possibly altered nervous feedback from the digestive tract may contribute to excessive food intake, but there is little information upon which to determine a cause and effect relationship.

It should be noted that the amount of food consumed per unit of time is a single characteristic among many (30,31), that comprise an animal's feeding behavior and should not be used as a test for abnormal control of feeding. The relatively normal feeding pattern of the diabetics observed in this study and the fact that the diabetic hamster has a net retention of calories from the ingested food similar to the non-diabetic (20) suggests that food intake regulation per se in the diabetic Chinese hamster is normal.
Feeding provides an animal with required nutrients, and circulating insulin and glucagon help to maintain a relative homeostatic consistency in the extracellular availability of these nutrients. Since the quantity of food ingested changes with time, fluctuations in the levels of circulating glucose, insulin and glucagon coordinated with feeding, may be expected to occur. These fluctuations were observed in the normal Chinese hamster and they suggest that carbohydrate metabolism in this animal is subject to similar 24-hour variations as measured in other laboratory animals and humans (34, 35, and 36). In the diabetic hamster, however, the variations in plasma insulin and glucose concentrations did not appear to be coordinated with the feeding pattern. In these animals the levels of plasma immunoreactive insulin did not increase with the increased nocturnal eating. It remained at a statistically similar level until the daylight nadir in eating occurred, at which time the levels dropped. Plasma glucose concentrations remained high, and there was no observed variation with feeding. Plasma insulin concentrations did not seem to affect the plasma glucose levels and neither appeared to affect the feeding pattern. Because feeding patterns were similar in both diabetic and non-diabetic groups even though plasma insulin and glucose and pancreatic insulin concentrations were dissimilar suggests that none of these factors, at the concentrations measured in this study, exert a significant physiological effect on the feeding pattern of the Chinese hamster.

Under experimental conditions insulin secretion in response to an insulinogenic stimulus has often been observed to be abnormal in
the fasted diabetic hamster in vivo and in perfused islets or whole pancreas in vitro. In this study plasma insulin concentrations were significantly below normal during a period of time when food consumption was increasing. Since the animals used in this study were not subjected to any experimental manipulations and were living under normal, free-feeding laboratory conditions, the suggestion that insulin secretion to insulogenic stimuli is abnormal in the diabetic hamster is strongly supported.

There was no observable difference in plasma glucagon concentrations between groups at any of the 5 time periods. It is possible then, that the reported abnormal in vitro secretion of glucagon (23) in the diabetics may not be reflected in the plasma concentration of this hormone. Since the circulating concentration determines the extent to which glucagon elevates blood sugar, it is doubtful that the plasma levels per se are significantly contributing to the hyperglycemia during those time periods in which blood samples were obtained. The increased plasma concentrations of glucagon for both groups at 1:00 a.m. compared with the 4:00 p.m. levels, and the reduced pancreatic levels at 10:00 p.m. relative to the 10:00 a.m. values in the controls suggests that the secretion and storage of this hormone may vary with the feeding cycle. Differences between diabetics and controls in pancreatic glucagon levels at only two of the five time intervals suggests that the reproducibility of this observation, like plasma insulin, is at least partially dependent upon the time of day and/or the period of the feeding cycle during which the ad lib fed hamsters are killed.
The levels of circulating insulin, glucose and glucagon and the biochemical behavior of the pancreatic islets in the whole animal under physiological conditions must be viewed as being dependent upon multiple and changing physiological and environmental stimuli rather than simple, univariant stimulus-response interactions. Other factors, such as adrenal steroids and catecholamines certainly influence the parameters measured in this investigation. The purpose of this study was to provide at least a partial description of the diabetic Chinese hamster's feeding behavior and of the variations in the levels of plasma insulin, glucose and glucagon that occur with ad lib feeding. These data are not meant to be taken as evidence of simplistic relationships existing between feeding, insulin, glucose and glucagon. They describe, at least in the controls, the normal physiological status of these substances in the ad lib feeding hamster.

Since these data indicate that food consumption and the levels of circulating insulin, glucose, and glucagon are not constant over time, they add confirmation to the validity of the assumptions of a need to control for circadian variations in behavioral and physiological investigations.
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