Timed Daily Thermopulses Alter the Circadian Hormonal Regulation of Glucose and Lipid Metabolism.

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Timed daily thermopulses alter the circadian hormonal regulation of glucose and lipid metabolism

de Souza, Christopher Joseph, Ph.D.
The Louisiana State University and Agricultural and Mechanical Col., 1991
A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University
Agricultural and Mechanical College
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requirements for the degree of
Doctor of Philosophy

in
The Department of Zoology and Physiology

by
Christopher J. de Souza
B.Sc., University of Bombay, 1981
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M.S., Louisiana State University, 1985
May 1991
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Most of all my sincerest thanks to my parents, wife and daughter. Without their constant help and unwavering support and love this and all my previous endeavors would not have been possible.
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Abstract

Studies were made in Syrian hamsters and Holtzman rats to examine roles for circadian rhythms in the control of body fats stores. Baseline and circadian rhythms of plasma insulin varied seasonally in the Syrian hamster. Baseline concentrations of the hormone were almost 4 times greater in scotosensitive female hamsters held on short daily photoperiods when they were fat than in lean animals at other seasons and other photoperiodic schedules. Additionally, plasma insulin levels in fat hamsters, but not in lean animals, peaked at light onset when lipogenic responsiveness to insulin is greatest.

The circadian rhythms of plasma insulin are not a result of an endogenous rhythmicity of B-cell secretion. Perifused Syrian hamster islets in the presence of glucose did not secrete the hormone in a circadian manner. Instead, islets lost their ability to respond to glucose when exposed to a constant level of the secretagogue over a 48-hour period. This islet desensitization was preventable if the glucose stimulus was provided in a pulsatile manner. The response to glucose pulses did not reveal a circadian periodicity.

In Holtzman rats, daily 2-hour increases in the ambient temperature (thermopulses) provided 16 hours after light onset (TP-16) for 14 days consistently decreased retroperitoneal fat stores. Thermopulses provided at other times of day were largely ineffective. TP-16 treatment also decreased food consumption and basal levels of plasma insulin and increased glucose tolerance and tissue sensitivity to insulin. However, plasma glucose levels were unaltered. The robust circadian rhythms of plasma insulin and corticosterone present in controls (nonthermopulsed) were obliterated by the TP-16 treatment whereas those of prolactin, body temperature and locomotor activity were unaltered. Conversely, thermopulse treatment at light onset (TP-0) was ineffective in altering any of the parameters studied. These studies support a role for circadian neuroendocrine interactions in the control of lipid and glucose metabolism.
Introduction

Body fat content cannot be solely attributed to excess energy intake over energy output. Oftentimes dramatic increases in body fat stores are associated with little if any increase in calorie intake and in some cases may even be accompanied by increased locomotor activity. For example, body fat content is greatest in birds during the migratory period, a time when locomotor activity is intense and food is harder to obtain (Meier and Fivizzani, 1981). Seasonal increases in body fat stores occur in scotosensitive hamsters held on short daily photoperiods although food consumption is unaltered (Bartness and Wade, 1984). Studies of nonseasonal animals also indicate that the regulation of body fat stores is not solely dependent on food consumption. Genetically obese Zucker rats restricted to a caloric intake equal to that of their lean littermates still become obese (Zucker, 1967). Studies of humans indicate that food consumption is no different between lean and obese individuals (Stefanik et al., 1959; Maxfield and Konishi, 1966; McCarthy, M.C. 1966; Kromhout, 1983). Furthermore, bromocriptine administration in several rodent species (Cincotta and Meier, 1989) and humans (Meier, Cincotta and Lovell, submitted) resulted in a decrease in body fat stores without reducing food consumption. Similar decreases in body fat stores without a decrease in food consumption were observed in pigs treated with bromocriptine (Southern et al., 1990), ractopamine (Anderson et al., 1987; Watkins et al., 1988) or cimaterol (Moser et al., 1986; Cromwell et al., 1988). Hence, although food consumption is a contributory factor it is not the only determinant of body fat stores.

Noninsulin dependent diabetes (NIDDM) or type-II diabetes is the result of increased tissue resistance to insulin (Mondon, et al., 1980; Olefsky and Kolterman, 1981; DeFronzo, 1982; Stern and Haffner, 1986). Because of it's decreased effectiveness to lower blood glucose concentrations, greater circulating levels of the hormone are required to maintain normoglycemia. In the initial stages of the disease the elevation in plasma glucose levels resulting from increased insulin resistance can be counteracted by increased
insulin secretion resulting from B-cell hypersecretion. As the disease progresses, increases in plasma insulin may become necessary. This can be provided by further increasing insulin secretion through the use of oral hypoglycemic agents such as glyburide and tolbutamide (Grodsky et al., 1977; Gylfe et al., 1984), or by insulin injections. However, the ensuing hyperinsulinemia, although necessary for cellular glucose uptake and metabolism, also enhances lipogenesis and fat content. Insulin is a potent lipogenic hormone. Indeed, along with insulin resistance and hyperinsulinemia, obesity is usually associated with type-II diabetes (Mrosovsky, 1976; Jeanreanud, 1978; Berstein et al., 1981). Whereas exercise (Goodman and Ruderman, 1979; Mondon et al., 1980; Kraegen et al., 1989; Wasserman et al., 1989) and calorie restricted diets (Reaven et al., 1983; Berger et al., 1975; Goodman and Ruderman, 1979) have both had some success in reducing insulin resistance and lowering circulating levels of insulin, their effects are transient and last only as long as the treatment does. Despite decades of research into these serious health problems, an effective cure for insulin resistance and obesity are still wanting.

Results from studies pertaining to the neuroendocrine regulation of body fat stores and insulin sensitivity in seasonal animals may help provide solutions for the control of obesity and insulin resistance. Many vertebrate species undergo dramatic annual changes in body fat stores (Berthould, 1975; Meier, 1976; Mrosovsky, 1976; Young, 1976; Pond, 1978; Dark and Zucker, 1985). Results from studies of the Syrian hamster indicate that seasonal changes in body fat are accompanied by seasonal changes in insulin resistance (de Souza and Meier, 1987; Cincotta et al., 1989). These seasonal changes are thought to result from altered phase relations between circadian rhythms, especially those of plasma corticosterone and prolactin. The phase relationship between these 2 hormones differs in seasonally fat and lean animals (Fivizzani and Meier, 1977; Meier, 1972; Meier et al., 1978; Meier et al., 1981; Meier, 1984). Timed daily injections of these two hormones in a phase relation that simulates their phase relation in the plasma of lean or fat animals produce the appropriate decrease or increase in fat stores within 10-14 days (Meier and Martin,
1971; Meier et al., 1971; Meier and Burns, 1976; Cincotta et al., 1989). Hence, a 12-hour relation (prolactin injections given 12 hours after corticosterone injections) that simulate spring conditions induces spring conditions out of season (Meier and Martin, 1971; Martin and Meier, 1973).

The daily rhythms of plasma corticosterone and prolactin are thought to be expressions of circadian neural oscillators. Injections of these 2 hormones are thought to reset two controlling circadian oscillators and their multiple neural and hormonal circadian expressions (Meier et al., 1971; Miller and Meier, 1983a). Similar alterations in body fat stores can be produced by properly timed injections of neurotransmitter precursors, namely 5-HTP (serotonin precursor) and DOPA (dopamine precursor) (Miller and Meier, 1983b; Meier and Wilson, 1985; Emata et al., 1985; Wilson and Meier, 1989).

Several environmental stimuli provided daily have also been demonstrated to produce variable physiological and metabolic changes depending on the time of day the stimuli are provided. These stimuli include brief periods of handling and mechanical agitation (Meier et al., 1973; Horseman et al., 1976; Weld and Meier, 1984), periodic availability of food (Spieler et al., 1977) and daily intervals of increased ambient temperature (Ferrell and Meier, 1981; Noeske and Meier, 1983; Weld and Meier, 1984). Although it may be argued that the daily increases in ambient temperature mediate their effects by altering the metabolism of these ectothermic species (fish and lizards), similar effects have been produced in endotherms (rodents) (Waldrop and Meier, 1985). Timed daily environmental stimuli induce physiological and metabolic changes apparently by altering the temporal interaction between circadian rhythms (Meier et al., 1973; Ferrell and Meier, 1981).

Recent studies in hamsters and rats provide further evidence that alteration of the phase relationships between circadian rhythms result in long term reversals of obesity and insulin resistance. Administration of prolactin and corticosteroids in a 0-hour relationship in hamsters (Cincotta et al., 1989) and a 4-hour relationship in rats (Cincotta and Meier,
submitted) resulted in decreased body fat stores in obese animals. Furthermore, plasma insulin levels were decreased without a concomitant increase in glucose levels, indicating an increase in tissue sensitivity to insulin. This conclusion was supported by findings of greater hypoglycemic responses to exogenous insulin administration in the hormone-treated animals. Hence, the treatment resulted in animals with physiological and metabolic conditions equivalent to those of nonobese animals of the same species. The effects of this treatment were long lasting in that they persisted for several months after termination of the treatment (Cincotta et al., 1989; Cincotta and Meier, submitted).

Despite decades of research on the role of insulin in carbohydrate and lipid metabolism little effort has been directed towards a regulatory role for circadian insulin rhythms even though circadian rhythms of the hormone exist in many if not all mammalian species (Gagliardino and Hernandez, 1971; Bellinger et al., 1975; Van Sickle et al., 1981). The basis for plasma insulin rhythms are speculative but may be the result of endogenous rhythms of B-cell secretion or of humoral and/or neural mediated changes in B-cell responsiveness to stimuli such as glucose. In vitro studies examining the possibility of B-cell rhythmicity have not been made.

Hypoglycemic and lipogenic responses to insulin also vary during a day (Cincotta and Meier, 1984, 1985). Based on a theory of "Temporal Synergism", maximal physiologic and metabolic effects are achieved if stimulus and response rhythms peak at the same time of day; other circadian temporal relations produce lesser effects (Meier and Russo, 1984). Hence, variations in the phase relation between the rhythm of insulin (stimulus) and the rhythms of tissue responsiveness (hypoglycemic and lipogenic) to the hormone would be expected to result in different physiological and metabolic conditions.

The Syrian hamster is a seasonal animal and it's reproductive system and body fat stores exhibit very prominent annual changes. In female hamsters, body fat store changes are inversely related to changes in the reproductive system (Bartness and Wade, 1984). Alterations of body fat stores also occurred in Syrian hamsters exposed to timed daily
increases in ambient temperature (thermopulse) (Waldrop and Meier, 1986). Examination of the role of the most important lipogenic hormone (i.e., insulin) in these seasonal animals and thermopulse induced changes in body fat stores are inadequate. Hence, plasma insulin levels and the phase relationships of plasma hormonal circadian rhythms were investigated with respect to the annual cycle and thermopulse treatment.
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February 25, 1991

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Chapter 1

Circadian and seasonal variations of plasma insulin and cortisol concentrations in the Syrian hamster, *Mesocricetus auratus*. 
CIRCADIAN AND SEASONAL VARIATIONS OF PLASMA INSULIN AND CORTISOL CONCENTRATIONS IN THE SYRIAN HAMSTER, *MESOCRICETUS AURATUS*

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(First received March 1986; accepted in revised form September 1986)

Abstract—Circadian rhythms of plasma insulin, cortisol, and glucose concentrations were examined in scotosensitive (reproductively sensitive to inhibitory effects of short daylengths) and scotorefractory male and female Syrian hamsters (*Mesocricetus auratus*) maintained on short (LD 10:14) and long (LD 14:10) daylengths. The baseline concentration (mean of all values obtained every 4 hr six times of day) of insulin was much greater in female than in male scotosensitive hamsters kept on short daylengths. These differences in insulin concentration may account for the observed heavy fat stores in female and low fat stores in male scotosensitive hamsters kept on short daylengths. The baseline concentrations of cortisol were approximately equal in both scotosensitive and scotorefractory males held on short and long daylengths, but were relatively low in females held on short daylengths and especially high in scotorefractory females held on long daylengths.

The plasma concentrations of both cortisol and insulin varied throughout the day in many of the groups tested. However, the variations were not equivalent. The circadian variations of cortisol were similar irrespective of sex, seasonal condition and daylength. Peak concentrations generally occurred about 12 hr after light onset. In contrast, the circadian variations of insulin differed markedly. For example in male hamsters, robust daily variations were found in scotosensitive hamsters held on short daylengths but not on long daylengths and in scotorefractory hamsters held on long daylengths but not on short daylengths. Furthermore, the daily peak occurred during the light in the scotosensitive hamsters and during the dark in the scotorefractory animals. Neither the daily feeding pattern (about 40% consumed during dark) nor the daily variations of glucose concentration varied appreciably with seasonal condition or daylength. They do not appear to determine nor directly reflect the variations in cortisol and glucose concentrations. It is postulated that the daily rhythms of cortisol and insulin are regulated by different neural pacemaker systems and that changes in the phase relations of circadian systems account in part for seasonal changes in body fat stores.

Key words—Circadian, seasonal variation, insulin rhythm, cortisol rhythm, Syrian hamster.

Introduction

The Syrian hamster, *Mesocricetus auratus*, exhibits a distinct seasonal cycle associated with reproductive and body weight changes. Although seasonal changes in daylength are an important environmental synchronizer, the seasonal cycle is controlled as well by an endogenous mechanism such that daylength is interpreted differently during the year. In scotosensitive hamsters (during winter when maintained on natural daylengths), short daylengths, (<12 hr light) have inhibitory effects on the reproductive system. This sensitivity is lost within 20 weeks of short daylengths, and the reproductive system recrudesces (1). Scotorefractory hamsters regain sensitivity to short daylengths after 11 or more weeks of long daily photoperiods (2). Short daily photoperiods also stimulate body weights and lipid mass in scotosensitive, but not in scotorefractory, female hamsters (3, 4).

Circadian rhythms have been implicated in photoperiodic control of reproductive readiness in many species (5), including the Syrian hamster (6). A circadian basis for the endogenous seasonal mechanism (seasonality) has also been proposed (7, 8). The essence of this hypothesis is that seasonal changes in reproductive and metabolic conditions are the net consequence of changes in the phase relations of neuroendocrine rhythms. It is based in part on observations that the phases of some rhythms, such as plasma corticosteroid concentrations, vary little with...
season whereas those of other rhythms, such as plasma prolactin concentrations, may vary markedly.

Insulin is thought to be the most important anabolic and lipogenic hormone in mammals and might be expected to play a major role in seasonal changes in body weight and fat stores. Circadian rhythms of plasma insulin concentration have been reported in rats (9, 10). In addition circadian rhythms of hypoglycemic and lipogenic responsiveness to insulin have been demonstrated in hamsters (11). Accordingly any change in the phase relationship between the circadian rhythms of plasma insulin concentration and of lipogenic responsiveness to insulin might be expected to influence the net accumulation of body lipid. Such an interaction of stimulus and response rhythms has been reported with respect to corticosteroid and prolactin activities (8, 12).

Therefore the present study was designed to examine circadian variations of plasma insulin and cortisol concentrations in scotosensitive and scotorefractory Syrian hamsters maintained on short and long daylengths and to determine possible seasonal changes in their daily rhythms.

Materials and Methods

Adult male and female Syrian hamsters, Mesocricetus auratus, were obtained from a closed colony introduced by Dr George C. Kent at Louisiana State University in 1942. At the time of birth animals were exposed to long daily photoperiods (LD 14 : 10), lights on at 0800. Thereafter the photoperiod was adjusted periodically to simulate seasonal differences in daylength. Temperature was maintained constant at 23 ± 2°C. Food (Rodent Laboratory Chow No. 5001, Ralston Purina Co.) and water were available ad libitum. Siblings were segregated from the mother approximately one month after birth. Males and females were separated and placed in hanging cages (40.5 cm × 24.5 cm × 18.0 cm), three animals per cage.

Animals were bled for hormone and glucose assays using the eye orbit puncture technique (13). Approximately 1 ml of blood was obtained in about 1.5 min from each animal. This technique did not cause any significant stress-related increase in plasma cortisol as indicated by similar concentrations in blood collected from guillotined and eye orbit punctured animals (unpublished data). Blood was collected in heparinised glass tubes and centrifuged in a Beckman J-6B centrifuge for 15 min at 3000 rpm and 4°C. Plasma was decanted and stored at −15 ± 1°C until assayed for insulin, cortisol and glucose concentrations.

Corticosterone and cortisol are the two major adrenal corticosteroids found in the Syrian hamster. The baseline plasma concentrations of cortisol are greater (14), but the phase of their rhythms approximate one another (15).

Insulin and cortisol were assayed in duplicate using radioimmunoassay (RIA) kits purchased from Cambridge Medical Diagnostic Co. (Billerica, MA). Insulin standards (porcine insulin in borate buffer) were acquired with the kit. Cortisol standards were prepared using a stock solution of cortisol (No. H-4001, Sigma Chemical Co.) dissolved in ethanol (10 μg/ml). Aliquots were air dried and diluted with hamster plasma stripped of cortisol with Norit-A (16). Radioactivity was counted using a Beckman Gamma 5500 counter. Standard curves were plotted and plasma hormone values calculated using a computerised RIA program. Glucose was assayed enzymatically using a Sigma No. 15-UV glucose kit.

Twenty-four-hr feeding rhythms were determined in all animals the week before blood collection, by measuring food removed from each cage of three animals every 6 hr (0600, 1200, 1800 and 2400). After correction for spillage, food consumption was expressed in terms of gm/animal by dividing food consumed/cage by the number of animals in each cage.

Variations among treatment groups were examined by analysis of variance. Comparisons between groups were made with the Student–Neuman-Keul's test and the Student's t-test. Any value more than two standard deviations from the mean was excluded from the data and statistical analyses.

Experiment 1

Thirty-six male and 36 female adult hamsters
Insulin and Cortisol Rhythms

born in early July and maintained on LD 14:10 from birth were bled at six times of day (six males and six females at each time) beginning at light onset during the first week of October. The plasma was assayed for insulin, cortisol and glucose concentrations. Although reproductively stimulated on long daylengths, these hamsters were scotosensitive (SS) in that their gonads regressed when they were exposed to a short photoperiod immediately following blood sampling. They were designated SS-14:10.

Experiment 2

The animals used in this experiment were the same as those used in Experiment 1 except that 32 of the 36 females were used. During the second week of November, after 6 weeks on short daily photoperiods (LD 10:14), the hamsters were bled six times of day (six males and five or six females per time period) for analysis of plasma concentrations of insulin, cortisol and glucose concentrations. Their gonads were regressed indicating they were in a scotosensitive condition. They were designated SS-10:14.

Experiment 3

Thirty-two male and 30 female hamsters, born between July and August on long daylengths, were transferred to short daily photoperiods (LD 10:14) in early October. After 18 weeks the hamsters were apparently scotorefractory as indicated by gonadal recrudescence. They were hence designated SR-10:14. Different groups of animals were bled at 4-hr intervals and the plasma was analysed for insulin, cortisol and glucose concentrations.

Experiment 4

Thirty male and 36 female hamsters born during mid-summer were maintained on long daily photoperiods (LD 14:10) for 12 weeks and then kept on short daily photoperiods (LD 10:14) for a duration of 20 weeks when they were returned to long daily photoperiods (LD 14:10) for 8 weeks. At the time of experimentation (early May 1984), the animals were probably scotorefractory since at least 11 weeks of long daily photoperiods are required to terminate refractoriness (2). These animals were designated SR-14:10. Animals were bled at six times a day (every 4 hr beginning at light onset) and the plasma analysed for insulin concentration.

Thirty male and 30 female adult hamsters, treated similarly as the above mentioned animals, were randomly selected from the colony and bled during the first week of May during the following year, 1985. The plasma was analysed for cortisol and glucose concentrations.

Results

Experiment 1

Food consumption in male and female SS-14:10 hamsters varied during a 24-hr period (ANOVA, $P>0.005$). Food intake was greatest during the latter half of the dark and early part of the light period (Student-Newman-Keul's test, $P<0.05$) (Figure 1b).

Plasma insulin concentration varied in females during a 24-hr period (ANOVA, $P<0.005$). The peak value occurred 16 hr (2000) after the onset of light (Figure 2b). Although plasma insulin concentration did not vary significantly (ANOVA) in males during a 24-hr period, the insulin concentration at the onset of light (0800) was higher than at 16 hr (2400) after light onset (Student's $t$-test, $P<0.01$) (Figure 3b). Mean baseline concentrations (mean of all values during 24-hr) of plasma insulin were $57.8 \pm 3.4 \, \mu U/ml$ in females and $63.6 \pm 2.0 \, \mu U/ml$ in males (Table 1).

Plasma cortisol concentration varied during a 24-hr period in both males and females (ANOVA, $P<0.005$). Peak values occurred at 12 and 20 hr (2000 and 0400) after light onset in females (Figure 4b). Mean baseline concentrations of plasma cortisol were $1.5 \pm 0.3 \, \mu g/dl$ in females and $1.1 \pm 0.2 \, \mu g/dl$ in males (Table 1).

Plasma glucose concentrations were similar throughout a 24-hr period. The mean baseline concentrations were $112.0 \pm 2.4 \, mg/dl$ in females and $102.1 \pm 1.6 \, mg/dl$ in males (Table 1).

Experiment 2

Food consumption in male and female SS-10:14 hamsters varied during a 24-hr period
(ANOVA, \( P<0.005 \)). Food intake was greatest during the dark (Student–Newman–Keul’s test, \( P<0.05 \)) (Figure 1a).

Plasma insulin concentration did not vary significantly during a 24-hr period in either male or female hamsters, probably because of large intra-group variations. There was nevertheless some indication of circadian variations in both sexes (see Figures 2a and 3a). The mean baseline insulin concentration in females (129.3 ± 23.5 \( \mu U/ml \)) was substantially greater (Student’s \( t \)-test, \( P<0.05 \)) than that in males (34.8 ± 7.2 \( \mu U/ml \)) (Table 1).

Another group of male scotosensitive hamsters held on LD 10:14 and similarly treated in our laboratory were tested for circadian variations in plasma insulin concentration (Cincotta, unpublished). A rhythm was observed (ANOVA, \( P<0.05 \)) that was virtually identical, except for less variability, with the values we observed in male SS-10:14 hamsters (Figure 3a). Lower concentrations (20–30 \( \mu U/ml \)) were found during the light and then gradually increased to the greatest concentration (45 \( \mu U/ml \)) late during the dark four hours before light onset.

Plasma cortisol concentration in females varied during a 24-hr period (ANOVA, \( P<0.005 \)); a peak value occurred 16 hr after the onset of light (2400) (Student–Newman–Keul’s test, \( P<0.05 \)) (Figure 4a). There was no significant variation in plasma cortisol concentration in males, perhaps because of large intragroup variations. The value at 12 hr after light onset (2000) may have been somewhat higher (not verified statistically) than those at other times during the 24-hr period (Figure 5a). The plasma cortisol baseline concentration was substantially greater in males (1.2 ± 0.1 \( \mu g/dl \)) than in females (0.3 ± 0.1 \( \mu g/dl \)) Student’s \( t \)-test, \( P<0.01 \)) (Table 1).

Figure 1. Twenty-four hr feeding pattern in male and female scotosensitive and scotorefractory hamsters maintained on short (a and c) and long (b and d) daylengths.
Plasma glucose concentrations in female hamsters varied little, albeit significantly, during a 24-hr period (ANOVA, $P < 0.05$). A peak value occurred 12 hr after light onset ($P < 0.05$). The plasma glucose concentration was constant during a 24-hr period in males. The mean baseline concentration of plasma glucose was $110.0 \pm 1.8$ mg/dl in females and $110.0 \pm 1.9$ ml/dl in males (Table 1).

**Experiment 3**

Food consumption in male and female SR-10:14 hamsters varied during a 24-hr period (ANOVA, $P < 0.005$ and $P < 0.025$, respectively). Maximum feeding occurred during the dark (Student–Newman–Keul's test, $P < 0.05$) (Figure 1c).

Plasma insulin concentration in females varied during a 24-hr period (ANOVA, $P < 0.005$). A peak value occurred at 20 hr after the onset of light (0400) (Student–Newman–Keul's test, $P < 0.05$) (Figure 2c). Plasma insulin concentration was relatively constant during a 24-hr period in males (Figure 3c). The mean baseline concentrations were $30.8 \pm 2.9 \mu$U/ml in females and $39.0 \pm 3.2 \mu$U/ml in males (Table 1).

Another group of scotorefractory male hamsters maintained on LD 10:14 (SR-10:14) were recently examined in our laboratory for a rhythm of plasma insulin concentration (unpublished data). These animals were treated in essentially the same manner as those above except that blood was sampled later (about 10 weeks) following the initiation of scotorefractoriness in hamsters maintained continuously on short daylengths. The results were similar in that there were no significant daily variations in plasma insulin concentration. However, the baseline concentration was lower ($24.3 \pm 1.4 \mu$U/ml) than that above.

Plasma cortisol concentration did not vary significantly (ANOVA) during a 24-hr period in...
Figure 3. Circadian rhythms of plasma insulin concentration in male scotosensitive and scotorefractory hamsters maintained on short (a and c) and long (b and d) day lengths. *Denotes the mean baseline concentrations for a 24-hr period.

Table 1. Baseline concentrations of plasma insulin, cortisol and glucose in male and female Syrian hamsters

<table>
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<td>Insulin</td>
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<td>34.9 ± 2.6a</td>
<td>63.6 ± 2.0b</td>
<td>34.8 ± 7.2ab</td>
<td>30.8 ± 3.2a</td>
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<td>Female</td>
<td>32.1 ± 2.3a</td>
<td>57.8 ± 3.4b</td>
<td>129.3 ± 23.5a</td>
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<td>Cortisol (µg/dl)</td>
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*Treatment consisted of long (LD 14:10) and short (LD 10:14) daily photoperiods in scotorefractory (SR) and scotosensitive (SS) male and female hamsters.  
†Mean ± S.E.  
‡Means with dissimilar lettered superscripts differ by Student's t-test (P < 0.05).
Insulin and Cortisol Rhythms

Scotosensitive Females

Scotorefractory Females

Figure 4. Circadian rhythms of plasma cortisol concentration in female scotosensitive and scotorefractory hamsters maintained on short (a and c) and long (b and d) day lengths. *Denotes the mean baseline concentrations for a 24-hr period.

either males or females (Figure 4c and 5c). However, the plasma cortisol concentration in males at 20 hours after the onset of light (0400) was higher than at 4 hours after light onset (12) (Student's t-test, \( P < 0.05 \)) (Figure 5c). The mean baseline concentrations of plasma cortisol were 0.4 ± 0.1 μg/dl in females and 0.8 ± 0.2 μg/dl in males (Table 1).

Plasma glucose concentration in females varied during a 24-hr period (ANOVA, \( P < 0.01 \)). Higher values occurred during the scotophase; a peak value occurred at light onset (0800) (Student-Newman-Keul’s test, \( P < 0.05 \)). In males the plasma glucose concentrations were relatively constant during a 24-hr period. Mean baseline concentrations were 105.9 ± 1.8 mg/dl in females and 100.8 ± 2.1 mg/dl in males (Table 1).

Experiment 4

Food consumption in male and female SR-14:10 hamsters varied during a 24-hr period (ANOVA, \( P < 0.005 \)). Maximum food intake occurred late during the dark (Student-Newman-Keul’s test, \( P < 0.05 \)) (Figure 1d).

Plasma insulin concentration varied during a 24-hr period in both male and female hamsters (ANOVA, \( P < 0.005 \) and \( P < 0.05 \), respectively). The peak values, however, occurred at different times of day. In females the peak occurred 20 hr after the onset of light (0400) (Figure 2d) (Student-Newman-Keul’s test, \( P < 0.05 \)) whereas in males it occurred 8 hr after light onset (1600) (Figure 3d). The mean baseline concentrations were 32.1 ± 2.3 μU/ml in females and 34.9 ± 2.6 μU/ml in males (Table 1).

Plasma cortisol concentration in female hamsters varied during a 24-hr period (ANOVA, \( P < 0.005 \)) with peak values 8 and 16 hr after light onset (1600 and 2400) (Student-Newman-Keul’s test, \( P < 0.05 \)) (Figure 4d). Plasma cortisol concentrations in males did not vary significant-
Figure S. Circadian rhythms of plasma cortisol concentration in male scotosensitive and scotorefractory hamsters maintained on short (a and c) and long (b and d) daylengths.

• Denotes the mean baseline concentrations for a 24-hr period.

Glucose concentration varied little throughout the 24-hr period in both sexes. The mean baseline concentrations were 113.0 ± 4.2 mg/dl in females and 107.5 ± 4.8 mg/dl in males (Table 1).

Body weights varied with daylength and season (Table 1). There was a steady increase of body weights in males as daylength and seasonal conditions varied according to a seasonal progression (SS-14 : 10 to SS-10 : 14 to SR-10 : 14). This increase might be attributed to maturational changes. Although there was a rise in body weights in scotosensitive females when transferred from LD 14 : 10 to LD 10 : 14, the body weights did not continue to rise as they did in males, and may have decreased in scotorefractory females on LD 10 : 14. Scotosensitive female hamsters were consistently heavier than males (Student's t-test, P < 0.05).

Discussion

The following generalities may be offered concerning differences in baseline plasma concentrations of insulin, cortisol and glucose without respect to circadian variations: insulin concentrations varied with daylength, seasonal condition and sex (Table 1). In females, higher insulin concentrations were found in scotosensitive animals maintained on both short and long daylengths. The concentration was especially high in scotosensitive females kept on short daylengths. In males, the insulin concentration was also higher in scotosensitive animals kept on long daylengths but not in those kept on short daylengths. Baseline concentrations of cortisol
Insulin and cortisol rhythms varied according to sex, and daylength, and little with seasonal condition. In both scotosensitive and scotorefractory females, cortisol was strongly elevated on long daylengths and severely depressed on short daylengths. However, plasma cortisol concentrations were similar in scotosensitive and scotorefractory males held on short and long daylengths (Table 1). Although both insulin and corticosteroids are known to have potent influences on plasma glucose concentration, the dramatic changes in concentrations of these hormones were not reflected by such changes in glucose concentrations.

Although circadian rhythms were not verified statistically in all instances, there were several interesting differences and generalities that can be discerned regarding daily variations of plasma insulin concentrations. With respect to sex, the times of greatest concentrations of insulin differed by as much as 8 to 12 hr between males and females in scotorefractory animals held on long daylengths and in scotosensitive hamsters held on short daylengths (Figures 2 and 3). Although obvious differences exist between scotosensitive and scotorefractory hamsters, the photoperiodic and sex differences obscure any underlying principle concerning these seasonal differences. It should also be noted that seasonality (scotosensitivity and scotorefractoriness) does not necessarily imply static conditions which might be characterized by hormone rhythms having single specific sets of phases and amplitudes. Although circadian variations of plasma insulin concentration similar to the ones we observed have been reconfirmed in male scotosensitive and scotorefractory hamsters on short daylengths (see Results), it would be premature to ascribe a specific rhythm for each seasonal condition. On the other hand, our results with insulin do support the conclusion that some hormone rhythms change seasonally (review, 8).

The daily variations of plasma cortisol concentration were much less variable than those of insulin. Changes in seasonal conditions were not accompanied by obvious changes in the cortisol rhythms (Figures 4 and 5). Daylength also appeared to have little influence, although baseline concentrations were greater in females held on long daylengths than on short daylengths (Table 1). Similar findings have been reported previously in rats (17). Peak values usually occurred near the onset of darkness in hamsters or, perhaps more accurately, about 12 hr after the onset of light. These results are generally consistent with previous findings that indicate peak cortisol concentrations occur near the onset of darkness in nocturnal rodents (18,19) but they do not necessarily support the premise that onset of darkness determines the phase of the plasma cortisol rhythm (20).

The hamsters displayed a feeding pattern with maximum food intake occurring late during the dark. This finding is in agreement with previously published literature on feeding patterns in Syrian hamsters wherein 60% of food was ingested at night (21). Because the feeding pattern was similar in both sexes and varied little with seasonal condition and daylength, it is apparent that changes in the insulin rhythms observed in this study were not caused by differences in the time of feeding. Variations in feeding do not account for the circadian variations in plasma insulin concentration.

Because plasma glucose concentrations varied little during a 24-hr period irrespective of season and daylength, it is also apparent that the changes noted in the circadian variations of plasma insulin and cortisol were not directly related to plasma glucose concentrations. Absence of a correlation between plasma insulin and glucose concentrations seems to run contrary to the literature which maintains an inverse relationship between the two. However, this relation is based on experiments carried out over a short period of time, a few hours usually, where one might expect a direct glucose reaction to a hormone or an endocrine response to glucose changes. Under steady state conditions, glucose homeostasis is not solely under the control of insulin but involves a host of other hormones. Furthermore, a robust circadian rhythm of hypoglycemic responsiveness to insulin has been reported in Syrian hamsters (11). Thus, plasma glucose concentrations reflect tissue responsiveness to insulin as well as insulin concentrations.

Circadian rhythms of insulin and cortisol differed greatly. While circadian variations of
plasma cortisol remained relatively unaltered by changes in daylength and season, those of insulin were profoundly affected. A similar phenomenon was noted with regards to the prolactin and cortisol rhythms in the Syrian hamster (22). The circadian rhythm of cortisol concentration remained relatively constant while that of prolactin underwent phase shifts with changes in daylength and season. There is considerable evidence that circadian time keeping is accomplished by a multioscillator system (23-27) that may be presided over by a secondary as well as a primary circadian pacemaker (8, 28). In this context, the circadian rhythm of cortisol would be controlled by a different pacemaker than the one(s) that control(s) the insulin and prolactin rhythms. There is evidence to indicate that the corticosteroid rhythm is controlled by the suprachiasmatic nucleus (29) whereas the insulin rhythm may be controlled by the ventromedial and lateral hypothalamic nuclei (30, 31).

The time of day when insulin concentration is high may be important in controlling fat synthesis since the hepatic lipogenic and body fat responses to insulin are largely restricted to an 8-10 hr interval near light onset (11). Coincidence of high plasma concentrations of insulin at this time of maximal lipogenic responsiveness might be expected to promote maximal lipogenesis and fat storage. Contrariwise, low insulin concentration during the lipogenic response period (near light onset) would be associated with low levels of lipogenesis and fat stores. Such relations can be examined in our studies because it has been established that introduction of scotosensitive female hamsters to short daylengths promotes fattening (3, 4, 32). The concentrations of insulin are much greater in scotosensitive female hamsters held on short daylengths (LD 10:14) than on long daylengths (LD 14:10) (Figure 2). In scotosensitive male hamsters, on the other hand, short daylengths are ineffective and may even promote a loss of body fat (33, 22). In our study (Figure 3), insulin concentrations of male scotosensitive hamsters subjected to short and long daylengths also varied inversely in these regards with respect to females. Concentrations were greater at light onset in hamsters held on long daylengths (LD 14:10) than on short daylengths (LD 10:14).

References

Insulin and cortisol rhythms


Chapter 2

Pulsatile Glucose-Stimuli Alleviate Long-Term Desensitization to Glucose and the Resultant Inhibition of Insulin Release.
Abstract

The long term effects of continuous and pulsatile glucose stimulation of islets of Langerhans microdissected from Syrian hamsters were examined. In the presence of a continuous glucose stimulus insulin secretion peaked during the first 4-6 hours of stimulation followed by a decrease. In the presence of 11.2 mM glucose a second smaller peak of insulin secretion was observed 14-16 hours after the perifusion started. Irrespective of the glucose concentration insulin secretion then steadily decreased and reached very low levels at the end of the 48 hour perifusion. This decrease in insulin secretion has been attributed to desensitization of the islets to a constant glucose stimulus. However, glucose stimulus provided in a pulsatile manner appeared to alleviate islet desensitization and the marked decrease in insulin secretion was not observed.
Introduction

*In vivo* and *in vitro* studies of insulin secretion in rats are typically characterized by an increase in insulin secretion when the islets are exposed to a constant glucose stimulus for as long as 4 hours. However, if the stimulus is maintained longer, insulin secretion is inhibited. This decrease is thought to be the result of a decrease in sensitivity of the target cells to the stimulus (Bolaffi *et al.*, 1986; Hoenig *et al.*, 1986). This phenomenon is not unique for islets. For example, anterior pituitary gonadotropin secreting cells also become desensitized when exposed to a constant stimulus of GnRH. However, administration of GnRH in a pulsatile manner prevents desensitization (Belchetz *et al.*, 1978; Smith and Vale, 1981).

In the present study the effects of both continuous and pulsatile stimuli on islets microdissected from Syrian hamsters were examined.

Materials & Methods

Two month old male Syrian hamsters (*Mesocricetus auratus*) (Charles Rivers, Wilmington, MA) were maintained on a 14:10 light:dark cycle for at least 2 weeks prior to experimentation. The animals were kept at 22 ± 1 °C and given food and water *ad libitum*. After an overnight fast animals were anesthetized with ether, the pancreas removed onto a wax tray and 12 islets microdissected per animal. Islets from each animal were then loaded into media filled 13 mm filter units (Swinnex-HA) fitted with a 100 micron nylon mesh and sealed at the threads. The units were then attached to a peristaltic multichannel pump (Manostat Corp) with a bubble trap in between. Media was equilibrated with 95:5 O₂:CO₂ and the islet chambers were maintained at 37 ± 1 °C during the perifusion. Perifusion flow rates were set at approximately 4.5 mls/hour. After an initial half hour wash period samples were collected every 30 minutes by a fraction collector modified to collect samples from 10 channels simultaneously. The collector was enclosed in a styrofoam jacket and maintained at 5 ± 1 °C with cooled nitrogen. Samples
were removed every 4 hours and frozen at -20 ± 1 °C till assayed for insulin.

Under sterile conditions RPMI 1640 containing no glucose or glutamine (NIH
Media Unit) was supplemented with 10% heat inactivated fetal bovine serum (Gibco
#2306140), 100 U/ml-100 mcg/ml penicillin-streptomycin (Gibco #6005140) and 25 mcg/ml
fungisone (Gibco #6005295). Appropriate additions of glucose, KCl, caffeine or glyburide
were made to this basal media. The pH was maintained at 7.2.

**Experiment 1:** Islets were perifused with media containing either 5.6 mM (low) or 11.2
mM (high) glucose. Seven and 11 animals were used in the two treatment groups,
respectively. After a 48-hour perifusion period the islets were exposed to a half-hour pulse
of either 50 mM KCl or 30 mM caffeine, and then perifused for an additional 2 hours.

**Experiment 2:** Islets were perifused with media containing 5.6 mM (low) glucose. After 6
hours the chambers were pulsed with media containing 30 mM glucose for half-hour
periods once every 4 hours. A total of 7 animals were used for this glucose pulsing
experiment. Islets exposed to 5.6 mM (low) glucose in experiment 1 served as controls.
Insulin was assayed by radioimmunoassay using ammonium sulfate in a final
concentration of 21% to separate out the bound fraction (Joost and Atwater, 1986).

In order to facilitate data analysis the first 4 samples (2 hours) from each
perifusion during a time of increasing insulin levels were excluded. Data was then grouped
into 3 hour periods and the means determined. Four of these periods were used to test for
differences within and between treatments as a function of time by way of a multivariate
analysis of variance (MANOVA). The four periods were 2-5 (T1), 8-11 (T2), 14-17 (T3) and
44-47 hours (T4) during perifusion. In order to test for differences in basal insulin
secretion between islets pulsed with 30 mM glucose and their controls the same procedure
outlined above was used. However, peak insulin concentrations resulting from the glucose
pulses were eliminated prior to analysis. All other tests for statistical significance were
preformed using Student’s t-test.

Results

**Experiment 1:** Insulin secretion by islets exposed to either low or high glucose stimuli varied with time (p < 0.0001) (Figure 1 and 2). When exposed to a high glucose concentration insulin levels peaked 3-4 hours (T1) after perifusion started. A decrease in insulin secretion followed resulting in a trough 4-6 hours later (T2), *i.e.*, 8-10 hours after the start of the perifusion (p < 0.0001). Insulin levels then gradually increased and significantly higher levels were reached 4-6 hours later (T3), *i.e.*, 14-16 hours after the perifusion started (p < 0.04). The magnitude of this second peak was less than the initial peak at T1 (p < 0.001). Following this second peak insulin levels rapidly decreased to levels lower than the insulin levels during the first 20 hours of perifusion *viz* at T1 (p < 0.0001) and T3 (p < 0.0005).

Under a low glucose stimulus there was a gradual decrease from the beginning (T1) to the end (T4) of perifusion (p < 0.0005). However, neither a trough at T2 nor a second peak at T3 were observed under low glucose stimulation during the first 20 hours of perifusion. Insulin secretory profiles differed between islets exposed to high and low glucose (p < 0.001). The magnitude of the decrease in insulin levels from T1 to T4 was greater for islets exposed to the higher glucose concentrations. However, this difference in the magnitude of the insulin decrease between the 2 treatment groups was predominantly due to differences during the first 20 hours of perifusion. There was no difference in the magnitude of the insulin decrease from T3 to T4 between the 2 treatment groups (p < 0.4). The magnitude of the first peak was greater in the high glucose treatment group (p < 0.002). Additionally, islets treated with a low glucose stimulus did not exhibit a significant trough at T2 nor a second peak at T3.

Exposure of all islets to 0.5 hour of either 50 mM KCl or 30 mM caffeine near the end of the perifusion caused an increase in insulin secretion compared with average
concentrations during the 2-hour period preceding the stimulatory pulse (Student's t-test, p < 0.01).

Experiment 2: The islets exposed to low glucose stimulation in experiment 1 served as controls for experiment 2. As noted above, there was a significant decrease in insulin levels as a function of time during exposure to constant glucose levels and this decrease was greatest beginning approximately 20 hours after the start of perifusion. However, insulin secretion by islets that were pulsed repetitively with 30 mM glucose did not vary significantly as a function of time (Figure 5). Insulin peaked 2-3 hours after perifusion started and stayed at a high level throughout the duration of the perifusion. No significant differences were observed between T1, T2, T3 and T4. Most importantly there was no significant difference between insulin levels at T1 and T4 as evident in figures 3 and 4. That is, insulin secretion from islets exposed to periodic high glucose pulses did not decrease during the 48-hour perifusion period.
Figure 1: Insulin secretory profiles from islets of Langerhans isolated from 2 hamsters.

The islets from the individual hamsters were perifused with either 11.2 mM (X—X) or 5.6 mM (X···X) glucose.
Figure 2: Mean insulin secretory profiles from isolated hamster islets of Langerhans perifused with either 11.2 mM (X—X) glucose (11 animals) or 5.6 mM (X···X) glucose (7 animals).
INSULIN (ng/islet-0.5 hour)

AVG HIGH GLU
AVG LOW GLU

TIME (hours)

T1 T2 T3 T4
Figure 3: Mean insulin secretory profiles from isolated hamster islets of Langerhans perifused with a low glucose medium and either stimulated X——X or not stimulated X···X with half hour pulses of 30 mM glucose every 4 hours starting 6 hours after commencement of the perifusion.
IN S U LIN (ng/islet-0.5 hour)

GLUCOSE PULSES

1St PULSE

AVG LOW GLU

TIME (hours)

INSULIN (ng/islet-0.5 hour)
Figure 4: A linear cubic split-spline regression model ($r^2=0.92$) for the insulin secretory profile from isolated hamster islets perifused with a low glucose medium and stimulated with half-hour 30 mM glucose every 4 hours starting 6 hours after the perifusion started.
INSULIN (ng/islet-0.5 hour)

GLUCOSE PULSES

MODEL CURVE

TIME (hours)
Figure 5: 30 mM glucose stimulated increases in insulin secretion at varying times during a 48 hour perifusion of isolated hamster islets of Langerhans perifused with 5.6 mM glucose in between pulses. The % increase was based on the mean insulin secretion during the 1.5 hours preceding the pulse.
Discussion

Results from this in vitro study of insulin secretion from microdissected hamster islets of Langerhans demonstrate that insulin secretion decreases during 48 hours of perifusion when perfused with a constant glucose stimulus. A similar effect was also found in rats and termed the 3rd phase of insulin secretion (Bolaffi et al., 1986). The decreased secretion was attributed to a desensitization of the islets to a constant glucose stimulus (Bolaffi et al., 1986; Hoenig et al., 1986; Leahy and Weir, 1988; Zawalich et al., 1990). The decrease in rats occurs 3-6 hours after the perifusion has begun irrespective of the magnitude of the glucose stimulus (Bolaffi et al., 1986; Hoenig et al., 1986; Zawalich et al., 1990). However, in hamsters a marked decrease in insulin secretion is observed only 18-20 hours after the onset of the perifusion irrespective of the concentration of the glucose stimulus. Additionally, when hamster islets are exposed to a high concentration of glucose a second peak of insulin secretion occurs 16-18 hours after the onset of the perifusion. The occurrence of this second peak may be unique for hamsters in that it is not known to occur in perfused islets from either rats (Bolaffi et al., 1986) or mice (unpublished data). The biochemical processes involved in the generation of the second peak are yet to be delineated. Thus the secretory profiles of hamster islets prior to desensitization differ from those in rats.

Desensitization of the islets to a constant glucose stimulus cannot be attributed to degeneration of the islets since the secretory response of hamster islets to KCl and caffeine in this experiment and to several other secretagogues in the case of rat islets (Somers et al., 1976; Hoenig et al., 1986;) are preserved. Desensitized rat islets are also capable of responding to a single high glucose stimulus although the magnitude of the response is severely dampened (Lacy et al., 1976; Verspohl et al., 1988). The possibility that a lack of some yet to be determined factor in the perifusion/culture media is the cause of this desensitization seems highly unlikely since rat islets exposed to a hyperglycemic environment in vivo also become desensitized to the hexose (Leahy and Weir, 1988). Nor
can decreased insulin secretion be attributed to alterations in glucose transport (Weir et al.,
1988), insulin biosynthesis (Giroix et al., 1989) or depletion of insulin stores (Bolaffi et al.,
1986 Verspohl et al., 1988).

In experiment 2 islets were perifused with near physiological levels of glucose (5.6 mM) and repeatedly stimulated with pulses of 30 mM glucose. Under these conditions basal insulin secretion, unlike controls, did not decrease significantly during the 48 hours the islets were perifused. Nor were the transient increases in stimulated insulin secretion reduced during the 48 hours. This difference between islets exposed to a constant level of glucose and those exposed to pulsatile glucose stimulation was especially prominent during the last 24 hours of perifusion (Figure 3). However, this is not unique for pancreatic islets. It has been demonstrated that several target cells become desensitized under conditions of continuous stimulation, a phenomenon that can be prevented if the stimulus is administered in a pulsatile manner. For example gonadotrophs from the anterior pituitary become desensitized to stimulatory effects of gonadotropic releasing hormone (GnRH) when exposed to a constant level of the secretagogue resulting in decreased FSH and LH secretion. This decrease in FSH and LH secretion can be prevented by stimulating the gonadotrophs with pulses of GnRH (Belchetz, et al., 1978; Smith and Vale, 1981).

Grill and coworkers (Grill and Cerasi, 1978; Grill, 1981) demonstrated that prior exposure of islets to a high concentration of glucose facilitates glucose induced insulin release during a subsequent glucose stimulation, probably by activating an islet 'memory' for glucose. This 'memory' appears to be short lived in that insulin secretion during a glucose pulse is facilitated if the previous glucose pulse was administered 1 hour earlier but not if the pulse was administered 24 hours earlier (Lacy et al., 1976). It would appear from the results of the pulsatile stimulus experiments reported herein that the 'memory' lasts for at least 4 hours since a glucose pulse administered 48 hours after perifusion started elicited a insulin response no different in magnitude from a response elicited by a pulse administered 6 hours after the perifusion started (Figure 5). Also, by maintaining
the islet 'memory' for the hexose, desensitization is prevented.

The mechanisms by which high glucose pulses avert islet desensitization to glucose stimulation when the hexose is present at a constant level is yet to be determined. However, it appears from this experiment and others (Giroix et al., 1989) that the cyclic AMP system is not involved. The desensitization does not seem to involve Ca\(^{2+}\) or K\(^{+}\) channels directly in as much as desensitization occurs even in the presence of glyburide (de Souza, in preparation) a second generation sulfonylurea. Glyburide induces insulin secretion by activating Ca\(^{2+}\) channels (Couturier and Malaisse, 1980; Hellman, 1981) either directly or indirectly by first inhibiting K\(^{+}\) channels that results in depolarization which activates Ca\(^{2+}\) channels (Atwater, 1980).

It was suggested that protein kinase C (PKC) may play an integral role in inducing islet 'memory' to glucose (Niki et al., 1988). Protein kinase C is an important enzyme involved in the secretion of insulin. PKC is activated when it associates with the plasma membrane and is converted to its Ca\(^{2+}\) sensitive form. PKC translocation to, and association with, the plasma membrane is activated by several agents including glucose, acetylcholine (ACh) and cholecystokinin (CCK). Priming or sensitization of B-cells has been proposed to involve the association of PKC with the plasma membrane (Niki et al., 1988; Zawalich and Rasmussen, 1990). In the presence of an active PKC any subsequent hexose stimulation of the B-cell will result in a greater insulin secretory response (Niki et al., 1988). However, this association of PKC with the plasma membrane is not everlasting (Zawalich and Rasmussen, 1990) and in order to maintain the B-cells in a primed state repeated activation of the PKC system is necessary. One explanation for the results reported here is that exposing the islets to periodic pulses of high glucose keeps the islets primed and prevents desensitization by preventing PKC from disassociating from the plasma membrane and hence maintaining the enzyme in an active state.

In all in vivo and in vitro experiments desensitization of the islets occurs under unchanging glucose conditions. Such an environment in vivo under normal conditions is
probably rare. Glucose levels vary over a 24 hour period (Bailey et al., 1975; de Souza and Meier, 1987). The degree of islet stimulation by glucose and other insulin secretagogues also varies over a 24 hour period. Stimulation tends to be greatest just after feeding and least between meals. Agents such as ACh and CCK can alter the response of the islets to a glucose stimulus enhancing the sensitivity of the islets to the hexose (Zawalich and Dias, 1987; Zawalich et al., 1987). Hence desensitization is probably a product of the experimental design, namely a constant glucose level, as well as of the absence of parasymathetic innervation. However, it remains to be seen if islet desensitization can be alleviated by exposing the islets to varying concentrations of glucose over a 24-hour period and/or of other islet priming agents such as ACh and CCK. The results from the experiments performed herein suggest that if the magnitude of the stimulus is periodically altered, as is the case when the islets were periodically exposed to different glucose levels, desensitization can be reduced or alleviated. Further experimentation is needed to clarify the mechanisms controlling desensitization.
Bibliography


Chapter 3

Alterations in Body Fat Stores and Plasma Insulin Levels in Response to Daily Intervals of Heat Exposure in the Holtzman Rat.
Abstract

The ability of timed daily increases in ambient temperature (from 22±1°C to 40±1°C for 2 hours) to alter body fat stores and other indices often associated with type-II diabetes and atherosclerosis were tested in male Holtzman rats (3-4 months old). The temperature pulses were administered at one of 6 times of day (0, 4, 8, 12, 16 or 20 hours after light onset) for 14 days. Only temperature pulses administered 16 hours after light onset consistently decreased body weights, retroperitoneal fat stores and plasma insulin levels. Subsequently, temperature pulses were administered either 0 (TP-0) or 16 (TP-16) hours after light onset (L:D 12:12). Body weight gains and food consumption were monitored during the course of the experiment. Oxygen consumption, oral glucose tolerance (OGTT), insulin intolerance (IIT), retroperitoneal fat weights and plasma concentrations of insulin, glucose, cholesterol and triglycerides were determined 12 hours after the last temperature pulse. Whereas no differences were observed between the TP-0 group and the constant temperature (22°C) controls, decreases in body weight gains, food consumption, retroperitoneal fat stores, and plasma concentrations of insulin, cholesterol and triglycerides were observed in the TP-16 group. Although changes in plasma glucose during the OGTT were similar when the two treatment groups were compared with their respective controls, glucose tolerance was achieved with less insulin in the TP-16 animals than in their respective controls. Insulin intolerance was lower in the TP-16 group as indicated by a decrease in plasma glucose that was of greater magnitude and longer duration than in controls. These results indicate that timed daily increases in ambient temperature may decrease obesity in part by decreasing plasma insulin levels. Insulin levels were apparently reduced as a consequence of increased tissue sensitivity to insulin (greater glucose tolerance and less insulin intolerance). Because the treatment is effective only at a particular time of day the findings support a role for circadian neuroendocrine interactions in the regulation of these metabolic states.
Introduction

Daily environmental stimuli have been shown to produce variable physiological and metabolic conditions in representative species of all the major vertebrate classes as a function of the time of day the stimuli are provided. These include brief periods of handling and mechanical agitation (Meier et al., 1973; Horseman et al., 1976; Weld and Meier, 1983), periodic availability of food (Spieler et al., 1977) and intervals of increased ambient temperature (Noeske and Meier, 1977, 1983; Ferrell and Meier, 1981; Weld and Meier, 1983). Alterations of body fat stores in Syrian hamsters by timed daily increases in ambient temperature (thermopulses) are of special interest in that they occur in an endothermic species and apparently involve the neuroendocrine system (Waldrop and Meier, 1986).

Insulin resistance and hyperinsulinemia are characteristic features often associated with obesity in type II diabetes (Kahn, 1978; Olefsky and Kolterman, 1981; Stern and Haffner, 1986). These three factors are interrelated, and a reduction in one of these factors can result in decreases in the other two. Similarly obesity is often accompanied by high levels of plasma lipids (Olefsky et al., 1974; Stern and Haffner, 1986). Inasmuch as daily thermopulses alter body fat stores, it is hypothesized that a reduction in body fat stores by administering the thermopulse treatment at the right time of day might also reduce these related features.

Materials and Methods

Male Holtzman rats (3-4 months old) were maintained on a 12:12 light:dark cycle for at least 2 weeks prior to experimentation. The animals were kept at 22 ± 1°C and had free access to food (Purina Rodent Chow) and water. Daily temperature increases (thermopulses) were administered for 14 days by raising the ambient temperature in the temperature chambers from 22° to 40 ± 1°C for 2 hours. The 18° elevation in temperature was achieved within 20 minutes of thermopulse onset. Control animals were maintained at
a constant 22 ± 1°C. At the end of the treatment period animals were anesthetized and placed under heat lamps to prevent anesthesia induced hypothermia. Blood was collected from the orbital sinus into chilled heparinized glass tubes and maintained on ice until it was centrifuged and the plasma harvested. Plasma was stored at -20°C. The animals were then sacrificed with an overdose of the anesthetic (see individual experiments below) and the weight of the retroperitoneal fat stores determined and expressed as a percent of the body weight.

Plasma insulin was assayed by radioimmunoassay utilizing a second antibody in the precipitation step (Welbone and Frazer, 1965). Plasma glucose, cholesterol and triglyceride concentrations were determined enzymatically using commercially available kits (Sigma, St Louis MO).

Results are presented as the mean ± standard error of the mean (SEM). Data were analyzed by Student’s t-test with an a priori alpha value set at p<0.05.

Experiment 1: Six groups of 6 animals each were temperature pulsed beginning at one of 6 times of day (0, 4, 8, 12, 16 and 20 hours after light onset). Pre and post treatment body weights were measured to calculate % changes in body weights. On the 15th day 24 hours after the last thermopulse, the treated animals and an equal number of control (nonthermopulsed) animals were bled and sacrificed. Plasma was assayed for insulin and glucose. Sodium pentobarbital (65 mg/kg body weight) was used to anesthetize and sacrifice the animals.

Experiment 2: This experiment was similar to the first experiment except that only 2 experimental groups of 12 animals each were temperature pulsed at either 0 or 16 hours after light onset. Each treatment group had its own set of 12 control (nonthermopulsed) animals because they were treated at different times. The treated animals along with their respective controls were bled and sacrificed on the 15th day 24 hours after the last
Experiment 3: Two groups of 12 animals each were thermopulsed either 0 (TP-0) or 16 (TP-16) hours after light onset for a period of 14 days. Each treatment group was provided with an equal number of control animals. Pre and post treatment body weights were measured to calculate % changes in body weights. Food consumption was measured over the last 2 days of experimental treatment and expressed as a percent of the body weight. Oxygen consumption was measured 12 hours after the last thermopulse in the treated animals and their respective controls. The animals were then lightly anesthetized with Ketamine (120 mg/kg body weight) and subjected to either an oral glucose tolerance test (OGTT) or an insulin intolerance test (IIT). The initial (0 minute) samples from both these tests provided basal plasma hormonal and metabolite concentrations.

The OGTT was performed after an initial blood sample (0 minute) was obtained. A glucose load (200 mg/100 gms body wt) was administered by syringe into the stomach through a curved stainless steel feeding needle (Lafeber Company, Hendersonville, NC). Additional blood samples were taken from each animal 10, 20, 40, 60, 90, 120 and 180 minutes after glucose administration.

The ability of exogenous insulin to lower plasma glucose levels was determined by injecting insulin (20 mU in 0.9% NaCl/100 gms body wt) into the caudal vein. Blood was taken before (0 minute) and 10, 20, 30, 40 and 50 minutes after insulin administration.

Results

Experiment 1: As shown in figure 1 daily thermopulses were generally ineffective in changing metabolic indices with several noteworthy exceptions. Temperature pulses given 16-hours after light onset (TP-16) produced especially interesting effects in that they reduced body weight gains by 26%, retroperitoneal fat stores by 21% and plasma insulin concentrations by 50%, compared with controls. Plasma glucose concentrations were not
altered. On the other hand, thermopulses at light onset (TP-0) increased retroperitoneal fat stores even though body weight gains were reduced, compared with controls. Plasma insulin and glucose levels were unaffected. Thermopulses given 12 hours after light onset (TP-12) promoted gains in body weight but did not alter either retroperitoneal fat stores or plasma insulin and glucose concentrations. Thermopulses at the other times of day (4, 8, and 20 hours after light onset) did not change any of the parameters measured (Figure-1).

**Experiment 2:** TP-16 treatment produced results similar to those obtained by similar TP-16 treatment in experiment 1. This treatment reduced the gains in body weight and decreased retroperitoneal fat stores by 18 and 32%, respectively, compared with controls. It also reduced plasma insulin concentrations from 2.12 ± 0.20 (controls) to 1.47 ± 0.08 (TP-16) ng/ml without affecting plasma glucose levels. On the other hand, TP-0 treatment did not alter significantly any of these parameters (Table 1). Differences in the plasma insulin levels between the TP-0 and TP-16 control groups are probably due to circadian variations of plasma insulin levels (de Souza and Meier, 1987) since the 2 groups were bled at different times of day (0 and 16 hours after light onset).

**Experiment 3:** TP-0 treatment did not alter body weight gain, retroperitoneal fat weights, food consumption and plasma concentrations of insulin, glucose, cholesterol and triglycerides compared with controls. However, TP-16 treatment decreased body weight gains, retroperitoneal fat weights (by 28%) and food consumption (by 16%). TP-16 treatment also reduced plasma insulin (by 25%), cholesterol (by 25%) and triglyceride concentrations (by 43%). Plasma glucose concentrations were unchanged. Oxygen consumption was not altered by either temperature treatment (Table 2). As in experiment 2, differences between the 2 control groups are probably due in part to sampling at different times of day (12 hours after the last thermopulse for each treatment group).

TP-0 treatment did not alter oral glucose tolerance compared with controls (Figure-}
2a). Although plasma glucose levels appeared to be higher in the 5 experimental animals used to test for glucose tolerance than in controls, the plasma glucose changes over the duration of the glucose test were similar. Furthermore when values of all of the TP-0 treated animals are considered the basal plasma glucose levels were found to be similar to control levels (Table 2). Plasma insulin changes resulting from the glucose administration were also similar in the TP-0 animals and their respective controls (Figure-2b).

However, the effects of TP-16 treatment on glucose tolerance were substantial. Although plasma glucose levels (basal and after oral glucose administration) were comparable in the TP-16 group and their controls (Figure-3a), plasma insulin levels were not (Figure-3b). Basal plasma insulin levels were 25% lower in the TP-16 group. Furthermore, increases in plasma insulin in response to oral glucose administration were less in the TP-16 animals at each time period during the test. The area under the curve for the TP-16 treated animals was 29% less than that for controls.

The hypoglycemic effect of exogenous insulin did not differ between TP-0 animals and their respective controls (Figure-4a). However, the hypoglycemic effect was enhanced by TP-16 treatment. Exogenous insulin administration induced a greater decrease in plasma glucose levels compared with controls over the entire duration (180 minutes) of the test period (Figure-4b). Although plasma glucose concentrations of controls rapidly returned to near normal levels 40-50 minutes after insulin administration the plasma glucose levels of the TP-16 treated animals were still strongly depressed (30% less) at that time.
Table 1. Effects of daily thermopulses at one of 2 times of day on body weight gains, retroperitoneal fat levels and plasma insulin and glucose levels in male Holtzman rats

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TREATMENT</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TP-0</td>
<td>TP-16</td>
</tr>
<tr>
<td>B.W. GAIN</td>
<td>CONT</td>
<td>10.9±0.4</td>
</tr>
<tr>
<td>(% increase)</td>
<td>EXPT</td>
<td>10.0±0.4</td>
</tr>
<tr>
<td>INSULIN</td>
<td>CONT</td>
<td>1.52±0.37</td>
</tr>
<tr>
<td>(ng/ml)</td>
<td>EXPT</td>
<td>1.62±0.19</td>
</tr>
<tr>
<td>GLUCOSE</td>
<td>CONT</td>
<td>154.4±5.2</td>
</tr>
<tr>
<td>(mg/dl)</td>
<td>EXPT</td>
<td>158.9±5.1</td>
</tr>
<tr>
<td>RETROPERITONEAL</td>
<td>CONT</td>
<td>2.10±0.10</td>
</tr>
<tr>
<td>FAT (gms%bw)</td>
<td>EXPT</td>
<td>2.14±0.13</td>
</tr>
</tbody>
</table>

1. Treatment consisted of 14 days of daily 2 hour thermopulses administered 0 (TP-0) or 16 (TP-16) hours after light onset.

2. Mean ± S.E.M.

3. Means with dissimilar lettered superscripts differ by Student’s t-test (p < 0.05).
Table 2. Effects of daily thermopulses at one of 2 time of day on several hormonal and metabolic parameters in male Holtzman rats.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>TREATMENT(^1)</th>
<th>TP-0</th>
<th>TP-16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>TP-0</strong></td>
<td><strong>TP-16</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>CONT</em></td>
<td><em>EXPT</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>EXPT</em></td>
<td><em>CONT</em></td>
</tr>
<tr>
<td>B.W. GAIN (% increase)</td>
<td>CONT</td>
<td>8.3±1.2 (10)(^2)</td>
<td>10.8±0.8 (11)(^{a,3})</td>
</tr>
<tr>
<td></td>
<td>EXPT</td>
<td>6.8±0.1 (12)(^{a,3})</td>
<td>5.4±0.9 (11)(^b)</td>
</tr>
<tr>
<td>FOOD CONSUMP (gms/day %bw)</td>
<td>CONT</td>
<td>5.47±0.06 (16)(^a)</td>
<td>5.47±0.06 (16)(^a)</td>
</tr>
<tr>
<td></td>
<td>EXPT</td>
<td>5.53±0.15 (12)(^a)</td>
<td>4.66±0.11 (10)(^b)</td>
</tr>
<tr>
<td>O₂ CONSUMP (mls/min %bw)</td>
<td>CONT</td>
<td>1.64±0.06 (6)</td>
<td>1.37±0.08 (6)</td>
</tr>
<tr>
<td></td>
<td>EXPT</td>
<td>1.52±0.10 (6)</td>
<td>1.31±0.08 (6)</td>
</tr>
<tr>
<td>INSULIN (ng/ml)</td>
<td>CONT</td>
<td>2.12±0.10 (10)</td>
<td>3.13±0.19 (10)(^a)</td>
</tr>
<tr>
<td></td>
<td>EXPT</td>
<td>2.20±0.19 (11)</td>
<td>2.34±0.21 (8)(^b)</td>
</tr>
<tr>
<td>GLUCOSE (mg/dl)</td>
<td>CONT</td>
<td>139.6±2.5 (8)</td>
<td>150.1±3.1 (10)</td>
</tr>
<tr>
<td></td>
<td>EXPT</td>
<td>147.6±4.1 (10)</td>
<td>144.8±3.6 (9)</td>
</tr>
<tr>
<td>TRIGLYCERIDE (mg/dl)</td>
<td>CONT</td>
<td>144.0±10.2 (8)</td>
<td>128.2±10.4 (10)(^a)</td>
</tr>
<tr>
<td></td>
<td>EXPT</td>
<td>135.9±10.2 (10)</td>
<td>72.6±8.4 (9)(^b)</td>
</tr>
<tr>
<td>CHOLESTEROL (mg/dl)</td>
<td>CONT</td>
<td>43.0±2.8 (9)</td>
<td>46.5±2.6 (10)(^a)</td>
</tr>
<tr>
<td></td>
<td>EXPT</td>
<td>38.6±2.6 (10)</td>
<td>34.9±3.2 (9)(^b)</td>
</tr>
<tr>
<td>RETROPERITONEAL FAT (gms%bw)</td>
<td>CONT</td>
<td>2.37±0.12 (10)</td>
<td>2.77±0.1 (11)(^a)</td>
</tr>
<tr>
<td></td>
<td>EXPT</td>
<td>2.61±0.18 (11)</td>
<td>1.99±0.13 (9)(^b)</td>
</tr>
</tbody>
</table>

1. Treatment consisted of 14 days of daily 2 hour thermopulses administered 0 (TP-0) or 16 (TP-16) hours after light onset.

2. Mean ± S.E.M.

3. Means with dissimilar lettered superscripts differ by Student’s t-test (p < 0.05).
**Figure 1:** Effects of 2-hour thermopulses provided daily for 14 days at one of 6 times of day on body weight gains, retroperitoneal fat stores and plasma insulin and glucose concentrations. Results are plotted as means ± SEM. * p<0.05.
Figure 2: Plasma glucose (A) and insulin (B) levels during an oral glucose tolerance test in TP-0 treated animals (X----X) and their controls (X—X). Results are plotted as mean ± SEM.
ORAL GLUCOSE TOLERANCE TEST

PLASMA GLUCOSE (mg/dl)

TIME AFTER GLUCOSE ADMINISTRATION (mins)

PLASMA INSULIN (ng/ml)
Figure 3: Plasma glucose (A) and insulin (B) levels during an oral glucose tolerance test in TP-16 treated animals (X···X) and their controls (X—X). Results are plotted as mean ± SEM. * p<0.05.
ORAL GLUCOSE TOLERANCE TEST

![Graph A: Plasma Glucose (mg/dl)]

- Plasma Glucose: 250, 226, 200, 175, 150, 125, 100, 75, 50, 25, 0
- Time After Glucose Administration (mins): 0, 10, 20, 40, 60, 90, 120, 180

![Graph B: Plasma Insulin (ng/ml)]

- Plasma Insulin: 7, 6, 5, 4, 3, 2, 1, 0
- Time After Glucose Administration (mins): 0, 10, 20, 40, 60, 90, 120, 180

* denotes significant differences.
Figure 4: Plasma glucose levels during an insulin intolerance test in (A) TP-0 treated animals (X-----X) and their controls (X—X) and in (B) TP-16 treated animals (X····X) and their controls (X—X). Results are plotted as means ± SEM. * p<0.05.
INSULIN INTOLERANCE TEST

GLUCOSE (% decrease)

TIME AFTER INSULIN ADMINISTRATION (mins)
Discussion

The results indicate that temperature pulse treatment has a time of day dependent effect on several metabolic and endocrine parameters in the rat. A 2-hour interval of warm temperature provided daily from 16 to 18 hours after light onset (TP-16) was the only time when the thermopulses consistently decreased retroperitoneal fat stores and plasma concentrations of triglyceride, cholesterol and insulin. Temperature pulses at 0, 4, 8, 12 and 20 hours after light onset were largely ineffective.

A decrease in plasma insulin without a concomitant increase in plasma glucose levels (Table 2) suggests that TP-16 treatment may have increased insulin sensitivity, ie, decreased tissue insulin resistance. The decreased levels of insulin, an important mammalian lipogenic hormone, could in turn account in part for the decrease in retroperitoneal fat stores and plasma concentrations of triglyceride and cholesterol (Olefsky et al., 1974; Golay et al., 1986; Stern and Haffner, 1986). The possibility that timed thermopulses reduce insulin resistance was tested directly in experiment 3. TP-16 treatment dramatically altered both the insulin response to an oral glucose load (OGTT) and the hypoglycemic response to exogenous insulin administration. Blood glucose was reduced to normal levels with a lower concentration of endogenous insulin in TP-16 treated animals than in controls (Figure-3). Apparently, the thermopulses enhanced insulin sensitivity. Furthermore, insulin administration lowered plasma glucose levels to a greater extent and for a longer duration in the TP-16 treated animals than in controls (Figure-4b). On the other hand, TP-0 treatment did not alter sensitivity to insulin.

Food consumption, while unchanged in TP-0 treated animals, was decreased (16%) by the TP-16 treatment (Table 2). This reduction in caloric intake could conceivably account for the observed increases in insulin sensitivity. Rats maintained on a severely restricted diet (55% less calories for 8-9 weeks) exhibit similar changes (Reaven et al., 1983). Calorie restriction also decreased body weight gains and plasma insulin levels. Decreased plasma insulin levels, without concomitant increases in plasma glucose levels,
were attributed to increased insulin sensitivity. Calorie restriction also decreased steady state plasma insulin and glucose levels during a glucose clamp procedure (Reaven et al., 1983). Similar increases in insulin sensitivity were also observed in rats after a 48-hour period of starvation (Berger et al., 1975; Goodman and Ruderman, 1979). Because these restrictions on caloric intake are much more severe, it seems probable that properly timed thermopulses enhance insulin sensitivity by other means as well.

Exercise has also produced results similar to those produced by TP-16 treatment in both rats (Berger et al., 1979; Galbo, et al., 1977) and humans (Bjorntorp et al., 1972, 1977; Lohmann et al., 1978). The authors attributed these results to enhanced skeletal muscle sensitivity to insulin (Mondon et al., 1980). However exercise, unlike temperature pulsing and calorie restriction, enhanced food consumption to compensate in part for the increased energy expenditure associated with exercising (Mondon et al., 1980). It is noteworthy that the metabolic and physiological changes produced by exercise apparently depended on the time of day when exercise occurred. No changes in insulin levels occurred in rats forced to exercise at a time of day that did not correspond with their normal activity cycle (Crew et al., 1969; Deb and Martin, 1975; Owens et al., 1977).

Several environmental stimuli provided daily have been demonstrated to produce variable physiological and metabolic changes depending on the time of day the stimuli were administered. Daily intervals of food availability have time-dependent effects on growth, body fat stores and reproductive indices in fish (Spieler et al., 1977). Time-dependent effects of daily mechanical stimuli and disturbances have been observed in species of all major vertebrate classes (Meier et al., 1973; Horseman et al., 1976; Meier and Horseman, 1977). Daily intervals of increased ambient temperature have variable effects on body fat stores in fish and lizards as a function of the time of day the thermopulses are administered (Ferrell and Meier, 1981; Weld and Meier, 1983). These temperature studies were recently extended to an endothermic vertebrate. Daily thermopulses altered retroperitoneal fat stores in hamsters as a function of the time of day the pulses were
administered (Waldrop and Meier 1986).

It has been hypothesized that the daily photoperiod entrains a circadian rhythm of thermoresponsiveness. If an appropriate thermal stimulus coincides with the responsive phase, metabolic and physiological changes are induced (Ferrell and Meier 1981; Weld and Meier 1983). The environmental stimuli (light and temperature changes) are thought to set the phase relationships of 2 or more circadian neuroendocrine pacemakers and their multiple circadian expressions. This could include phase changes between two plasma hormonal rhythms and between the circadian rhythms of a hormone stimulus and the target tissue responsiveness to the stimulus. Changes in the temporal relations between such rhythms would alter the net effects of the stimuli. Numerous instances of physiological and metabolic changes resulting from altered relationships of circadian rhythms (ie, temporal synergisms) have been described (Meier 1975; Meier et al., 1978).

Temporal interactions of prolactin and corticosteroids are of special interest. The phase relationship between these two hormones differ in lean and fat animals (Meier 1972, 1984; Meier et al., 1981). Furthermore, timed daily injections of these hormones for 1-2 weeks in a temporal relation that simulates the daily peaks of the hormones found in the plasma of fat and lean animals produce the appropriate increases and decreases in body fat stores (Meier and Martin, 1971; Meier et al., 1971; Meier and Burns 1976; Cincotta et al., 1989). The rhythms of corticosteroids and prolactin are thought to be expressions of two neural oscillations that also control other neural and hormonal rhythms. In addition to their direct effects on lipid metabolism, the hormonal injections are thought to alter the phase relationship of the neural oscillators and their multiple circadian expressions, thereby altering metabolism (Meier, 1984; Meier and Russo, 1984).

Recent studies in hamsters and rats provide further evidence that daily injections of prolactin and corticosteroids given in specific temporal relationships are capable of reducing as well as increasing body fat stores. The administrations of prolactin and corticosteroids in a 0-hour relationship in hamsters and a 4-hour relationship in rats
decrease body fat stores in obese animals. Furthermore, decreases in body fat stores were accompanied by smaller gains in body weight and decreased plasma insulin levels without concomitant increases in plasma glucose. A decrease in plasma insulin without a change in plasma glucose level was attributed to increased tissue sensitivity to insulin. This conclusion was supported by findings of greater hypoglycemic responses to exogenous insulin administration in the hormone-treated animals. The treatment promoted metabolic and physiological conditions that were equivalent to those found in naturally lean/nonobese animals of the same species. The effects were also long lasting in that they persisted for several months after termination of the treatment (Cincotta et al., 1989; Cincotta and Meier, in preparation).

Obesity, hyperinsulinemia and increased insulin resistance are correlates of type-II diabetes. High levels of plasma cholesterol and triglycerides are often associated with obesity and may result in atherosclerosis. Because TP-16 treatment decreases body fat, plasma insulin levels and insulin resistance in normal healthy rats, it is hoped that temperature pulse treatment might also alleviate the above mentioned pathologies associated with type-II diabetes in humans. Additionally the reduction in levels of plasma triglycerides and cholesterol could help deter pathological states associated with the high circulating levels of these plasma lipid metabolites.
Bibliography


necessary distinction. Metabolism: Clinical and Experimental 27 (Suppl. 2): 1893.


Chapter 4

Daily Intervals of Heat Exposure Alter the Phases of Plasma Hormonal Circadian Rhythms in the Holtzman Rat.
Abstract

The effects of timed daily increases in ambient temperature (thermopulses) (from $22 \pm 1^\circ C$ to $40 \pm 1^\circ C$ for 2 hours) on circadian rhythms of hormones involved in glucose and lipid metabolism were tested in male Holtzman rats (3-4 months old) exposed to 12-hour daily photoperiods. The thermopulses were administered for 14 days either at light onset (TP-0) or 16 hours after light onset (TP-16). Body weight gains and food consumption were monitored during the experiment. Retroperitoneal fat weights and plasma concentrations of insulin, glucose, glucagon, corticosterone and prolactin were monitored every 4 hours during a 24-hour period commencing 24 hours after the last thermopulse. TP-0 treatment did not alter any of the parameters tested. Conversely, TP-16 treatment obliterated the circadian rhythms of insulin and corticosterone present in the controls (nonthermopulsed) and decreased body weight gains, retroperitoneal fat stores, food consumption and the baseline levels of plasma insulin and corticosterone. The present study supports a role for circadian neuroendocrine interactions in the reduction of fat stores induced by properly timed daily thermopulses.
**Introduction**

Several daily environmental cues induce variable physiological and metabolic changes (e.g., body fat stores) in representative species of all the major vertebrate classes as a function of the time of day these stimuli are provided. These stimuli include brief periods of handling and mechanical agitation (Meier et al., 1973; Horseman et al., 1976; Weld and Meier, 1984), periodic availability of food (Spieler et al., 1977) and intervals of increased ambient temperature (Noeske and Meier, 1977, 1983; Ferrell and Meier, 1981; Weld and Meier, 1983). Alterations of body fat stores in Syrian hamsters (Waldrop and Meier, 1985) and rats (de Souza and Meier, in prep) by timed daily increases in ambient temperature (thermopulses) are of special interest in that they occur in endothermic species.

Meier and coworkers have proposed that several physiological and metabolic parameters are regulated by the temporal interaction of circadian rhythms. Timed daily environmental stimuli that induce physiological and metabolic changes are thought to do so by altering the temporal interaction between circadian rhythms (Meier et al., 1973; Meier and Russo, 1984). Hence it was hypothesized that the physiological and metabolic changes induced by daily thermopulses administered 16 hours after light onset (de Souza and Meier, in prep) may also result from altered temporal relationships between circadian rhythms. This hypothesis is examine in the present study by comparing hormonal and behavioral rhythms in rats treated by timed thermopulses that do and that do not alter metabolic conditions.

**Materials and Methods**

Male Holtzman rats (2-3 months old) were maintained on 12-hour daily photoperiods (LD 12:12) for at least 2 weeks prior to experimentation. The animals were held at 22 ± 1°C and had free access to food (Purina Rodent Chow) and water. Daily temperature increases (thermopulses) were administered for 14 days by raising the
ambient temperature in the temperature chambers from 22 to 40 ± 1°C for 2 hours. The 18°C elevation was achieved within 20 minutes of thermopulse onset. Ambient temperature returned to normal room temperature within 15 minutes of thermopulse offset. Control animals were maintained constantly at 22 ± 1°C. Thermopulses were administered at either 0 or 16 hours after light onset in order to allow for a comparison between thermopulse treatments that alter (TP-16) and do not alter (TP-0) the physiological and metabolic parameters studied (de Souza and Meier, in preparation).

Body weights were measured before and after treatment to calculate percent changes. Food consumption was measured during the last 2 days of experimental treatment and expressed as a percent of the body weight. At the end of the 14-day treatment period animals were anesthetized with sodium pentobarbital (65mg/kg body weight) for collection of blood samples. Blood was collected from the orbital sinus into chilled heparinized glass tubes and maintained on ice until it was centrifuged and the plasma harvested for storage at -20°C. The animals were then sacrificed with an overdose of the anesthetic, and the weight of the retroperitoneal fat stores were determined and expressed as a percent of the body weight.

Plasma insulin, prolactin, corticosterone and glucagon were assayed by radioimmunoassays utilizing second antibodies in the precipitation step. Materials and methodology for the prolactin assay were kindly supplied by the National Hormone and Pituitary Program, NIDDK, NIH and the University of Maryland School of Medicine. NIDDK-rPRL-RP-3 was used as the standard. Commercially available kits and reagents were used to assay for corticosterone (ICN Biomed. Inc., Carson, CA) and glucagon (Linco, St. Louis, MO). Plasma glucose concentrations were determined enzymatically using commercially available kits (Sigma, St. Louis MO).

Data are represented as means ± standard errors of the means (SEM). The mean baseline value represents the mean of all samples taken during a 24-hour sampling period. Variations among treatment groups were examined by Analysis of Variance (ANOVA) and
the Waller-Duncan k ratio t-test was used as a post ANOVA test. Student's t-test was used to examine differences between experimental groups. The a priori alpha value was set at $p<0.05$.

**Experiment 1:** Thirty-two animals were thermopulsed 16 hours after light onset (TP-16) and 36 animals acted as controls (nonthermopulsed). Blood sampling commenced on the fifteenth day, 16 hours after light onset, (i.e., 24 hours after the last thermopulse). The first subgroups (5-6 animals in each group) of treated and control animals were bled 16 hours after light onset. The other 5 paired subgroups were bled every 4 hours thereafter (20, 0, 4, 6 and 10 hours after light onset). Animals were then sacrificed to remove and weigh retroperitoneal fat.

**Experiment 2:** This experiment was similar to Experiment 1 except that the daily thermopulse was administered at light onset (TP-0). Blood sampling was taken every 4 hours from 6 paired experimental and control subgroups (5 animals per subgroup) commencing on the fifteenth day, 0 hours after light onset (i.e., 24 hours after the last thermopulse).

**Experiment 3:** This experiment was a repetition of Experiment 1. Animals were thermopulsed 16 hours after light onset and blood collections commenced 24 hours after the last thermopulse, (i.e., 16 hours after light onset). The experimental and control groups each contained a total of 30 animals.

**Experiment 4:** Temperature sensitive transmitters (Model V, Mini-Mitter, Sunriver, Oregon) were calibrated and implanted into the abdominal cavity of 8 rats. After an 8-day post operative period animals were placed in swing bottom cages. Prior to thermopulse treatment body temperatures were monitored every 4 hours for a period of 3 days. The
rats were then thermopulsed at either 0 (4 animals) or 16 (4 animals) hours after light onset for 14 days. Body temperature was monitored every 4 hours during the 2-day period before and immediately after the treatment ended. Locomotor activity was also monitored using swing bottom cages. Activity data was collected and digitized through a serial port as a TTL zero or one response using an Atari computer, beginning 10 days before the first thermopulse and ending 10 days after the last thermopulse.

Results

Experiment 1: Daily thermopulses given 16 hours after light onset (TP-16) produced pronounced metabolic and hormonal effects. TP-16 treatment reduced body weight gains, retroperitoneal fat weights and food consumption, compared with controls (Student's t-test). Additionally, mean baseline levels of plasma insulin and corticosterone were 29 and 37% lower, respectively, in the TP-16 group than in controls (Student's t-test). Plasma glucose levels were unchanged. Mean baseline concentrations of plasma prolactin and glucagon were also unaltered (Table 1).

Plasma insulin, corticosterone and prolactin concentrations of the controls varied during a 24-hour period (ANOVA). Peak levels of plasma insulin, corticosterone and prolactin occurred 20-0 (24) hours, 8 hours, and 4-8 hours after light onset, respectively (Waller-Duncan). However, plasma insulin and corticosterone levels did not vary during the 24-hour period in the TP-16 treated animals. The greatest reductions of plasma insulin and corticosterone in the thermopulsed rats occurred at the times when control levels peaked. Plasma prolactin concentrations were comparable in control and treated animals at all time periods except at light onset when they were greater in the treated animals (Figure-1).

Experiment 2: Daily thermopulses at light onset (TP-0) did not alter metabolic and hormonal indices. No changes were observed in body weight gains, retroperitoneal fat
weights and food consumption; and the mean baseline concentrations of glucose, insulin, corticosterone, prolactin and glucagon were also unaltered, compared with controls (Table 1).

Plasma insulin, corticosterone, prolactin and glucagon levels varied during a 24-hour period (ANOVA). These 24-hour variations were coincident in controls and TP-0 treated animals. Peak levels of plasma insulin, corticosterone and prolactin occurred 20 hours, 12 hours and 8-12 hours after light onset, respectively, in both groups (Waller-Duncan) (Figure-2). Plasma glucagon levels peaked 8-12 hours after light onset (Waller-Duncan).

**Experiment 3:** TP-16 treatment produced results similar to those produced by TP-16 treatment in Experiment 1. The treatment reduced body weight gains, retroperitoneal fat weights and food consumption, compared with controls (Student's t-test). TP-16 treatment also decreased mean baseline concentrations of plasma insulin and corticosterone levels by 31 and 23%, respectively (Student's t-test), compared with controls. Mean baseline concentrations of plasma glucose, prolactin and glucagon were unaltered (Table 1).

Plasma insulin, corticosterone and prolactin levels varied during a 24-hour period (ANOVA) in controls with peak levels occurring at 0, 8-12 and 4-12 hours after light onset, respectively (Waller). However, plasma insulin and corticosterone concentrations did not vary during the 24-hour period in the TP-16 treated animals. The greatest reductions of plasma insulin and corticosterone occurred at times corresponding to the peak levels in controls. Plasma prolactin levels were comparable in control and treated animals at all times tested (Figure-3).

**Experiment 4:** Neither of the 2 treatments, TP-0 or TP-16, altered the baseline or circadian rhythms of body temperatures. Body temperatures peaked 12-16 hours after light onset (early during the scotophase) when measured before, during and after the
treatment period. Circadian rhythms of locomotor activity were also unaltered by either TP-0 or TP-16 treatments. Activity was maximal during the scotophase with sporadic bouts of activity during the photophase.
Table 1. Effects of daily thermopulses at one of 2 times of day on several hormonal and metabolic parameters in male Holtzman rats.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>CONT</th>
<th>TP-16 (Expt 1)</th>
<th>TP-0 (Expt 2)</th>
<th>TP-16 (Expt 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BODY WEIGHT GAIN (% increase)</td>
<td></td>
<td>29.9±0.8 (36)*</td>
<td>39.8±1.1 (30)</td>
<td>37.2±0.7 (32)*</td>
</tr>
<tr>
<td></td>
<td>EXPT</td>
<td>22.8±0.6 (32)b</td>
<td>37.5±1.1 (29)</td>
<td>27.8±0.7 (32)b</td>
</tr>
<tr>
<td>FOOD CONSUMPTION (gms/day %bw)</td>
<td></td>
<td>6.67±0.14 (12)*</td>
<td>6.74±0.08 (18)</td>
<td>7.16±0.33 (12)*</td>
</tr>
<tr>
<td></td>
<td>EXPT</td>
<td>5.79±0.10 (12)b</td>
<td>6.62±0.12 (18)</td>
<td>5.98±0.26 (12)b</td>
</tr>
<tr>
<td>RETROPERITONEAL FAT (gms/bw)</td>
<td></td>
<td>2.99±0.03 (36)*</td>
<td>1.91±0.07 (32)</td>
<td>1.98±0.05 (32)*</td>
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<tr>
<td></td>
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<td>1.67±0.04 (32)b</td>
<td>1.82±0.04 (32)</td>
<td>1.46±0.03 (32)b</td>
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<tr>
<td>INSULIN (ng/ml)</td>
<td></td>
<td>2.45±0.16 (33)*</td>
<td>1.97±0.17 (30)</td>
<td>2.17±0.18 (30)*</td>
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<tr>
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<td>2.05±0.14 (30)</td>
<td>1.51±0.07 (30)b</td>
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<tr>
<td>GLUCOSE (mg/dl)</td>
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<td>155.5±1.9 (33)</td>
<td>161.4±2.3 (30)</td>
<td>163.7±2.2 (30)</td>
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<tr>
<td></td>
<td>EXPT</td>
<td>159.3±2.1 (31)</td>
<td>157.3±1.9 (30)</td>
<td>164.3±1.0 (30)</td>
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<tr>
<td>GLUCAGON (pg/ml)</td>
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<td>165.2±3.5 (33)</td>
<td>142.1±5.1 (30)</td>
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<tr>
<td></td>
<td>EXPT</td>
<td>165.4±4.9 (31)</td>
<td>140.0±4.0 (30)</td>
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<tr>
<td>CORTICOSTERONE (ng/ml)</td>
<td></td>
<td>146.1±19.4 (31)*</td>
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<td>150.3±5.9 (31)*</td>
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<tr>
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<tr>
<td>PROLACTIN (ng/ml)</td>
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<td>53.4±7.9 (33)</td>
<td>67.4±8.2 (30)</td>
<td>65.5±5.3 (30)</td>
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<td></td>
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<td>70.9±5.8 (27)</td>
<td>65.7±6.4 (30)</td>
<td>66.3±5.0 (30)</td>
</tr>
</tbody>
</table>

1 Treatment consisted of 14 days of daily 2-hour thermopulses administered 0 (TP-0) or 16 (TP-16) hours after light onset.

2 Mean ± S.E.M.

3 Means with dissimilar lettered superscripts differ by Student's t-test (p < 0.05).
Figure 1: Circadian rhythms of plasma insulin, corticosterone and prolactin in TP-16 treated animals (Expt 1) (X···X) and their controls (X—X). Results are plotted as means ± SEM. Means within a treatment group with dissimilar lettered superscripts differ by ANOVA and Waller-Duncan tests. ** denotes means significantly different from controls at that time period (Student's t-test).
Figure 2: Circadian rhythms of plasma insulin, corticosterone and prolactin in TP-0 treated animals (Expt 2) (X—-—X) and their controls (X—-X). Results are plotted as means ± SEM. Means within a treatment group with dissimilar lettered superscripts differ by ANOVA and Waller-Duncan tests.
Figure 3: Circadian rhythms of plasma insulin, corticosterone and prolactin in TP-16 treated animals (Expt 3) (X- - - X) and their controls (X——X). Results are plotted as means ± SEM. Means within a treatment group with dissimilar lettered superscripts differ by ANOVA and Waller-Duncan tests. ** denotes means significantly different from controls at that time period (Student's t-test).
**Discussion**

As noted previously (de Souza and Meier, in preparation) thermopulses given 16 hours after light onset had marked physiological effects. The TP-16 treatment consistently reduced body weight gains by 25% and decreased retroperitoneal fat stores by 15-25%. Furthermore, baseline levels of plasma insulin and corticosterone were also decreased dramatically (30% and 23-37% respectively) (Table 1). A decreased plasma insulin level without a concomitant increase in plasma glucose level (Table 1) is probably the result of decreased tissue resistance to insulin demonstrated in rats by this methodology (de Souza and Meier, in preparation).

Insulin is an important mammalian lipogenic hormone and hyperinsulinemia is often associated with obesity as in type II (noninsulin dependent) diabetes. A reduction in circulating insulin often leads to a reduction of obesity (Jeanrenaud, 1978; Berstein et al., 1981; DeFronzo, 1982). Although, insulin is an important lipogenic hormone, it's ability to stimulate hepatic lipogenesis in the hamster varies markedly during a day (Cincotta and Meier, 1984, 1985; Martin et al., 1990). Lipogenesis and fat deposition are maximal if the phases of the circadian rhythms of plasma insulin and of lipogenic responsiveness to the hormone coincide. Other relationships between the phases of these 2 rhythms are associated with decreasing levels of lipogenesis and fat deposition (de Souza and Meier, 1987). Numerous instances of this concept termed "Temporal Synergism" wherein physiological and metabolic changes result from altered relationships of circadian rhythms have been described (Meier, 1972, 1975; Meier et al., 1978). If maximal lipogenic responsiveness to insulin occurs near light onset in the rat, as it does in the Syrian hamster (Cincotta and Meier, 1984), high levels of insulin at this time would result in maximal fat production. In fact, high insulin levels do occur near light onset in controls (Figures 1 and 3) and the reduction of plasma insulin induced by TP-16 treatment that also reduces body fat is largely restricted to times near light onset (Figures 1 and 3).

Conversely, TP-0 treatment does not alter the circadian rhythm of plasma insulin and has
no effect on body fat stores.

Corticosteroids as well as insulin have also been implicated with the obese state. Diabetic animals of several mammalian species, including humans, have elevated concentrations of plasma cortisol during a 24-hour period with exaggerated peak levels (Saito and Bray, 1983; Cameron et al., 1987; Oster et al., 1988). These diabetics are also generally obese, a correlate of type-II diabetes. Contrariwise, TP-16 treatment which reduced body fat stores also resulted in lower levels of plasma corticosterone with the greatest reduction occurring at normal peak times.

The reduction in body fat stores induced by TP-16 treatment could be attributed to a reduction in food consumption (Table 1). However, the regulation of body fat stores ought not be summarized in such a simplistic manner. Although food consumption is a contributory factor for the amount of body fat it is not necessarily the primary determinant under ad libitum conditions. Several lines of evidence point to a secondary role of food consumption in regulation of body fat stores. Timed daily injections of prolactin can increase body fat stores in both fed and fasted fish (Lee and Meier, 1967). Seasonal variations in body fat stores in the Syrian hamster (Bartness and Wade, 1984) are not accompanied by changes in total intake of food consumption (de Souza and Meier, 1987). Additionally, properly timed bromocriptine treatment of hamsters reduces body fat levels without reducing food consumption (Cincotta and Meier, 1987).

Meier and co-workers have proposed that several physiological conditions including body fat stores are regulated by temporal interactions of circadian rhythms. Injections of prolactin and cortisol can markedly increase or decrease body fat stores depending on their temporal relationships. This pioneering research has been extended to include representative species of all the vertebrate classes (Meier and Martin, 1971; Meier et al., 1971; Meier and Burns, 1976; Meier et al., 1978; Cincotta et al., 1989). Recently, prolonged reversion of type-II diabetic like symptoms including obesity were achieved in several rodent species by appropriately timed daily injections of prolactin and cortisol (Cincotta et
al., 1989, Cincotta and Meier, submitted). Circadian variations of these two hormones are thought to be the expressions of two circadian neural oscillators. Injections of these 2 hormones via feedback mechanisms reset the controlling oscillators and their multiple neural and hormonal circadian expressions. Similar alterations in physiological conditions can also be produced by properly timed daily injections of neurotransmitter precursors, namely DOPA (precursor for dopamine) and 5-HTP (precursor for serotonin) (Miller and Meier, 1983; Emata et al., 1985; Wilson and Meier, 1989).

Several environmental stimuli provided daily have also been demonstrated to produce variable physiological and metabolic changes depending on the time of day the stimuli are provided. These stimuli include brief periods of handling and mechanical agitation (Meier et al., 1973; Horseman et al., 1976; Weld and Meier, 1984), periodic availability of food (Spieler et al., 1977) and daily intervals of increased ambient temperature (Ferrell and Meier, 1981; Noeske and Meier, 1983; Weld and Meier, 1984). It may be argued that the daily thermopulses mediated their physiological and metabolic effects by altering the metabolism of these ectothermic species (fish and lizards). However, thermopulses produce similar effects in endothermic rodents (Waldrop and Meier, 1985; de Souza and Meier, in preparation). Daily thermopulses at 16 hours after light onset decreased retroperitoneal fat without altering O_2 consumption (de Souza and Meier, in preparation) or basal body temperature (Experiment 4). Hence, it is our belief that the effects of thermopulses are mediated by neuroendocrine pathways, perhaps via serotonergic afferents from the raphe nucleus (Hellon, 1981).

It has been hypothesized that the daily photoperiod entrains a circadian rhythm of thermoresponsiveness. Coincidence of an appropriate thermal stimulus with a responsive phase results in metabolic and physiological changes (Ferrell and Meier, 1981; Noeske and Meier, 1983; Weld and Meier, 1983). The environmental stimuli (photoperiod and thermopulse in the present study) are thought to set the phase relationships of two or more circadian neural pacemakers and their multiple circadian expressions. This could include
phase changes between plasma hormone rhythms and between circadian rhythms of a hormone stimulus and the target tissue responsiveness to the stimulus. The results of the experiments herein support this postulate in that thermopulse treatment at the appropriate time of day (TP-16) alters some rhythms, viz, insulin and corticosterone, and not other rhythms, viz, prolactin (Figure 3), body temperature and locomotor activity. These circadian rhythms may be expressions of two or more circadian neural oscillators that control metabolic and physiological conditions as a function of their phase relationships which in turn is determined by daily photoperiods and thermopulses.
Bibliography


Cincotta, A.H. and Meier, A.H. Long lasting inhibition of aging induced fattening, hyperinsulinemia, and insulin resistance in male Sprague-Dawley rats by properly timed injections of corticosterone and prolactin. (Submitted).


Summary

The results herein demonstrate an important role for plasma circadian insulin rhythms in the control of lipid metabolism in both seasonal and nonseasonal animals i.e., Syrian hamsters and Holtzman rats, respectively.

1. Seasonal changes in body fat stores, especially prominent in the female hamster, are accompanied by changes in the mean basal concentration and the circadian rhythm of plasma insulin. Female scotosensitive hamsters held on short daily photoperiods have the greatest amount of body fat stores (Bartness and Wade, 1984) and a basal level of plasma insulin that is 4 times greater than that found in hamsters at other seasons (de Souza and Meier, 1987). Furthermore, the greatest concentration of plasma insulin in fat hamsters occurs at light onset, a time when hepatic lipogenic response to insulin is also maximal (Cincotta and Meier, 1984, 1985). Lower fat stores in both males and females at other seasons and photoperiodic regimes were correlated with lower basal insulin levels. In addition, daily insulin peaks occurred at times other than light onset. Contrary to popular expectation, the seasonal high levels of body fat stores in females were not accompanied by increased food consumption.

2. Circadian rhythms of plasma insulin concentration are probably not a result of endogenous rhythms of B-cell secretion or responsiveness since B-cells do not respond in vitro to constant or pulsatile glucose stimulation in a cyclic manner. On the contrary, in the presence of a constant glucose stimulus insulin secretion wanes. Exposing B-cells to pulses of the hexose helped prevent this decrease but did not reveal a circadian rhythmicity.

3. Only thermopulses administered 16 hours after light onset (TP-16) consistently decreased body fat stores and reduced body weight gains and food consumption when compared with controls (nonthermopulsed). Plasma insulin concentrations were also decreased and this decrease may be attributed to an increase in glucose tolerance and insulin sensitivity as indicated by results from oral glucose and insulin tolerance tests.
Thermopulses at other times of day were largely ineffective respecting the parameters monitored. Since both $O_2$ consumption and body temperature were unchanged by thermopulses (TP-16) the changes were probably not a result of altered metabolic rates. It is likely that the effects are mediated by the neuroendocrine system. Inasmuch as circadian hormonal rhythms may be considered expressions of two or more circadian neural pacemaker oscillators, it seems probable that thermopulses mediate their effects by altering the phase of at least one of these oscillators. Alterations in the rhythms of insulin and corticosterone with no changes in the circadian rhythms of prolactin, body temperature and locomotor activity support this postulate (figure 1). Hence, these studies collectively infer a role for circadian temporal neuroendocrine interactions in mediating the changes of lipid and carbohydrate metabolism produced by properly timed daily thermopulses.
Figure 1: Proposed neuroendocrine model by which thermopulses alter circadian rhythms: Metabolic conditions may be altered by an interaction of daily photocycles and thermopulses. Thermopulses alter the circadian rhythms of plasma insulin and corticosterone by way of a secondary pacemaker. Circadian rhythms of prolactin, body temperature and locomotor activity are controlled by another secondary pacemaker that is coupled with a primary pacemaker set by the daily photoperiod. A temporal synergism of the secondary pacemakers and their multiple circadian neural and hormonal rhythms regulate metabolic conditions.
PHOTOPERIOD

<table>
<thead>
<tr>
<th>PRIMARY PACEMAKER</th>
<th>THERMOPULSE</th>
<th>TIMED FEEDING</th>
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SCN

<table>
<thead>
<tr>
<th>SECONDARY PACEMAKER</th>
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</thead>
</table>

PROLACTIN
BODY TEMP
ACTIVITY

INSULIN
CORTICOSTERONE
Bibliography


Vita

Christopher de Souza was born in Calcutta, India on November 2, 1959. He graduated with a B.Sc. in zoology and biochemistry in May 1981 from St. Xavier's College followed by a M.Sc. in endocrinology in May 1983 from the Institute of Sciences. In the fall of 1983 he entered Louisiana State University to begin work on his Masters in physiology which he completed in August, 1985. He subsequently started work on his Ph.D. in physiology.
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Christopher J. de Souza

Major Field: Physiology

Title of Dissertation: Timed Daily Thermopulses Alter the Circadian Hormonal Regulation of Glucose and Lipid Metabolism

Approved:

[Signatures and names]

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures and names]

Date of Examination:

March 11, 1991