Glucoprivic Regulation of Puberty Onset

Honors Thesis
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Abstract

The purpose of our studies was to determine 1) if rats experience hypoglycemia during diet-restriction when puberty is delayed, and 2) if histaminergic pathways mediate glucoprivic-induced delay in puberty onset. Plasma blood glucose was measured in Experiment 1 at 60-min intervals for 4h after a single meal and for 2h before the next meal in diet-restricted (n=6) compared to ad libitum-fed females (n=5). Diet-restricted rats demonstrated decreased blood glucose in pre-meal samples, relative to ad libitum-fed rats. These data suggest that daily hypoglycemia may delay onset of puberty during diet-restriction. They also suggest that diet-restricted rats resemble food deprived rats, at least for a portion of the day, and that an overlap may exist in the mechanism by which diet-restriction and food deprivation suppress reproductive function. In Experiment 2, first estrus was determined during 2DG-induced glucoprivation, in the presence and absence of a histamine antagonist, α-fluoromethylhistidine (FMH), in previously diet-restricted rats with delayed puberty. During a 72h refeeding period, female rats were treated with either saline (sc) + aCSF (200 nl, 3V, n=8), saline + FMH (2.24 μmol/rat/day, 3V, n=8), 2DG (400 mg/kg, every 6h, sc) + aCSF (n=9), or 2DG + FMH (n=11). Rats receiving 2DG/aCSF and 2DG/FMH had delayed first estrus (7.3 ± 1.1 and 6.8 ± 1.4 days, respectively), compared to the control group (4.4 ± 0.3 days). Fewer 2DG/FMH rats had first estrus compared to 2DG/aCSF rats (4/11 vs. 6/9). These data suggest that 2DG-induced glucoprivation suppresses onset of puberty. Reducing brain histamine levels potentiated this effect, suggesting a role for brain histamine in metabolic regulation of reproductive function in the developing animal.

Background

Mammals use oxidizable metabolic fuels for cell maintenance, thermoregulation, and locomotion (57). When these needs are met, the animal can direct energy toward more costly processes, such as growth and reproduction. This is not surprising since it is well known that there is a clear link between diet and reproductive function in the female mammal (3,18). Reproduction, for the female, is especially costly, since it involves ovulation, conception, pregnancy, lactation, and parental care (18). Thus, it may be highly disadvantageous for the female to reproduce when fuel resources are scarce.

When there is a scarcity of available food, reproductive function of a young female is effectively suppressed through the delay of onset of puberty, that is, a delay of the onset of estrous cycling and reproductive fertility. Thus, developing females maintained on a restricted diet direct energy away from reproduction (19, 29), while in the adult female, reproduction is suppressed leading to anestrus and the cessation of estrous-related behavior (39, 56). The body appears to monitor the amount of oxidizable metabolic fuels and a decreased metabolic fuel supply suppresses reproduction by inhibiting the release of gonadotropin-releasing hormone (GnRH) from the hypothalamus (19, 33). This GnRH inhibition causes a decrease in the release of luteinizing hormone (LH) secretion, which prevents the preovulatory LH surge, ovulation, and estrous cycling.

In general, our research investigates the mechanisms by which a mammal is able to monitor the amount of available energy in the form of oxidizable metabolic fuels and to determine the signals which function in partitioning this energy, especially to growth and reproduction. The **specific aims** for the experiments described herein are two-fold:

1. To determine if peripheral glucose availability provides a signal causing delayed puberty when energy availability is low. Specifically, we tested the hypothesis that peripheral glucose levels in growth-restricted rats are lower than those of ad libitum fed rats.

2. To determine if glucoprivation delays onset of puberty in the young female rat through activation of central histamine pathways, as occurs in the adult. Specifically, we tested the hypothesis that there is an increase in central histamine levels due to 2DG-induced glucoprivation, which in turn causes suppression of the reproductive axis, leading to delayed puberty onset.

Puberty and the Growth-Restricted Prepubertal Rat Model

Developmental changes in metabolic state occur in response to changes in nutrient partitioning as an animal grows (12). With growth, less energy is expended for maintenance of basal metabolism per unit of body mass and this energy surplus could be sensed by the brain (12). However, we must consider that multiple sequential signals may be involved during sexual maturation and that a developmental block exists for the proximate metabolic signal to work at

some early stage (12). These signals include photoperiod and social cues in addition to growth and metabolic ones. In attempting to understand how the brain utilizes these signals, specifically the metabolic ones here, it is necessary to attempt to separate out these cues. In order to do so, the growth-restricted prepubertal rat model has been developed. When food is restricted in the prepubertal female rat, a break is put on growth and fuel storage, easily manipulated variables during development in the normally growing animal. Puberty is also delayed in this model, but the other variables necessary for onset of puberty are in place as demonstrated by Bronson (1, 2). Upon refeeding, we have hypothesized that all of the signals from metabolism necessary for puberty onset and first estrus are given in the first 72 hours (18).

We use the prepubertal, growth-restricted female rat as the animal model in our studies. Puberty is delayed by maintaining a body weight of approximately 80-90 g after weaning. Under conditions of restricted food, the rats remain acyclic despite being beyond the age of normal pubertal onset (37.0 ± 1.4 days) (1, 2, 29). In this growth-restricted prepubertal rat model, reproductive hormone levels are low, but can be reinstated to the normal post-pubertal high level by ad libitum feeding (unlimited food) for 72 h (29). In addition, estrous cycles begin within one week and normal pregnancy and litter size ensue if animals are mated at the first estrus after refeeding (1, 2, 29).

Glucose as a Signal of Metabolic Fuel Availability

Although ad libitum feeding for 72 h induces estrous cycles within one week, we are attempting to deduce exactly what signal is detected during that refeeding period. Current research suggests that the key metabolic fuel monitored by the body for regulation of reproduction and puberty is glucose (18, 57). 2-Deoxy-D-glucose (2DG) is a competitive inhibitor of phosphofructokinase (PFK), a key enzyme in glucose metabolism, and it therefore blocks utilization of glucose and causes glucoprivation. 2DG administration suppresses the reproductive axis inducing anestrus in ad-libitum fed adult Syrian hamsters (50) and inhibiting pulsatile LH secretion in adult rats (59). 2DG also causes extended estrous cycle length and decreased reproductive behavior in the adult female (59), and delays onset of puberty in the female rat (10).

Cellular hypoglycemia can be produced experimentally by either 2DG or insulin administration. Both treatments cause a variety of neural and endocrine responses, in addition to suppression of the reproductive axis. Initially, glucoprivation causes an increase in feeding, epinephrine, norepinephrine, glucocorticoids ("stress" hormones), and glucagon (49). In a rat model of hypoglycemia-associated autonomic failure (HAAF), a condition of reduced sensitivity to repeated hypoglycemic episodes, the responses of feeding, plasma epinephrine, norepinephrine and glucagon are severely diminished. Thus, increased appetite and autonomic signs that normally occur during hypoglycemia are reduced (48, 49), but activation of the hypothalamic-pituitary-adrenal (HPA) axis continues to occur with each hypoglycemic episode

(49). Thus it is possible that HPA axis activation, rather than chronic hypoglycemia, is responsible for suppression of reproductive function during 2DG- or insulin-induced glucoprivation. Sanders has also shown that administration of the synthetic glucocorticoid, dexamethsone, produces the same effect as a second 2DG injection, providing evidence for HPA axis involvement in HAAF development (48).

Studies in which the area postrema (AP) of the brain stem has been lesioned provide evidence for a specific role of glucoprivation, rather than activation of the HPA axis, in suppressing reproductive function. AP lesion prevents 2DG-induced anestrus in the hamster (53), insulin-induced hypoglycemic suppression of pulsatile LH release in the rat or sheep (6, 8), and the inhibition of estrous behavior by food deprivation or insulin treatment (37). The 2DG-induced feeding response is also significantly attenuated in rats with AP lesions, however more importantly, activation of the HPA axis induced by 2DG remains intact in rats with AP lesions (11). These results suggest that continued activation of the HPA axis is not directly responsible for suppression of reproductive function. Thus, the chronic effect of glucoprivation on the reproductive axis is most likely due to a lack of available glucose specifically, rather than to activation of other neural and endocrine responses. In addition, these studies suggest that one area of detection of this metabolic signal is the area postrema in the brain stem.

Other metabolic fuels, such as fatty acids, have also been investigated as possible signaling molecules which could regulate the reproductive axis. Studies involving methyl palmoxirate (MP), an inhibitor of fatty acid metabolism, do not demonstrate an increase in cycle

length in the adult (17, 51, 52) or a delay in puberty onset in the young female (16). Since MP inhibits fatty acid metabolism, it causes the animal to increase glucose availability and glucose metabolism. Studies performed with both MP and 2DG in the rat and the hamster show that reproductive suppression is not achieved unless 2DG is also administered, indicating that low glucose availability is the specific signal that shuts off the reproductive axis, rather than a general decrease in oxidizable metabolic fuels (16, 50, 51).

Peripheral vs. Central Glucose Availability

Blood glucose levels at any one time reflect the interaction of two opposing mechanisms (27). Hepatic glycogenolysis and gluconeogenesis and glucose absorption by the intestinal mucosa tend to increase glucose levels, while glucose utilization and insulin action tend to remove glucose from the blood (27). Insulin is additionally important in diverting circulating metabolic fuels away from tissues where they are oxidized and into adipose tissue for storage (37).

Experimental evidence has shown that a deficit in glucose availability to tissues triggers food intake in rats fed ad libitum and determines meal onset and meal size after food deprivation (24). Previous work has also demonstrated that short-term food deprivation in rats produces a fall in the blood glucose level which is largely dependent on time of day (24, 27). Blood glucose levels have also been shown to fall during fasting in adult male rhesus macaques (23). During food deprivation, glucose absorption by the intestinal mucosa would not occur, while glucose

utilization would still be necessary, particularly for the brain, which uses glucose almost

exclusively for energy production.

Our rats are diet-restricted, meaning that they are fed a single meal a day. Therefore, for a

large portion of the day, the rats are essentially food deprived. Thus, we might hypothesize that

blood glucose levels fall as time progresses after the meal. However, it has been shown that

living on a single daily meal for a long period induces general metabolic changes that lead to

saving stored energetic substrates and a better utilization of energy supplying nutrients (27). This

would suggest that the rats might actually be able to maintain normoglycemia, during the entire

time between the single daily meals.

Overall, there are no distinct data that examines glucose levels in diet-restricted

compared to ad libitum fed animals. Thus, our first specific aim is to determine if peripheral

glucose levels in diet-restricted rats are altered from those of continuously fed rats. We

hypothesize that blood glucose levels would fall in the diet-restricted (single meal) rats as time

progresses after the meal, compared to those rats with food available continuously, where

glucose levels would remain higher.

Data on the pattern of glucose availability during growth-restriction and ad libitum

feeding may help in the understanding of metabolic state as it relates to onset of puberty in our

other experiments, since we believe that glucose serves as a central signal of nutritional status

and controls other neuroendocrine responses, such as growth and reproduction. However, it is

possible that peripheral glucose levels are not indicative of brain glucose levels or use. It has

been shown that peripheral glucose levels are maintained when glucose levels are experimentally

altered centrally (5). If this is the case, a change in peripheral glucose levels would not be

necessary to implicate glucose as a central metabolic signal.

Glucose and the Histamine Pathway

Another element of the hypoglycemic response that may play a role in glucose signaling

is the hypothalamic neurotransmitter histamine. Increased activity of hypothalamic histamine, a

neurotransmitter synthesized endogenously from histidine, has been shown to increase secretions

from the adrenal glands through increased corticotropin releasing hormone (CRH) secretion (28).

This increase in CRH secretion induces systemic hyperglycemia by activating sympathetic

outflow from the brain, causing an increase in catecholamine secretion from the adrenal medulla

(34), and also contributes to behavioral adaptations in response to changes in ambient

temperature (46). Glucoprivic challenges increase turnover, synthesis and release of histamine in

the hypothalamus (34, 45, 47). In addition, the involvement of hypothalamic histamine in brain

glycogen metabolism has been examined in the rat under insufficient energy supply. It has been

demonstrated that fasting of rats causes increased activity of hypothalamic histamine which

increases hypothalamic glycogen use as an energy source in order to reduce glucose uptake and

its catabolism (45). These results suggest that the activation of histamine in response to energy

deficit may play an essential role in glucose utilization through glycogenolytic processes in the

hypothalamus (45).

Histaminergic activity, particularly in the ventromedial hypothalamus and paraventricular nucleus, is associated with the regulation of food intake, eliciting feeding when histamine levels are decreased (35, 36, 44, 46) and suppressing feeding when histamine levels are elevated (26). There is a burst of central histamine release following a meal suggesting that increased histaminergic activity may result from eating and lead to satiation (28). Additionally, regulation of the histaminergic system is associated with both dietary-induced anorexia and hyperphagia (28). The highest concentrations of histamine are in the hypothalamus, where histidine decarboxylase synthesizes histamine from histidine and where the histamine receptors are located (28). Activation of the system leads to increased activity of the HPA axis, decreased blood pressure, decreased pain perception, wakefulness, hyperactivity, and decreased body temperature (28), suggesting that histamine has a central role in various homeostatic mechanisms.

Studies investigating the roles of neural histamine often use α-fluoromethylhistidine (FMH), which blocks the conversion of histidine to histamine by inhibiting the rate limiting enzyme histidine decarboxylase. Peripheral or central FMH administration inhibits enzymatic histamine synthesis and results in almost complete depletion of neural histamine (21, 44). Histamine depletion alters the circadian rhythm of the sleep-wakefulness cycle by suppressing the surge of wakefulness during the dark period (21) and the circadian rhythm of feeding by increasing feeding, drinking and ambulatory behavior in the light period, but decreasing the same during the dark period (9). Conversely, increasing histamine levels by administration of an H3

receptor antagonist to specific brain regions (46) or by inhibiting histamine catabolism (26) alters the circadian rhythm of feeding by decreasing food intake.

Earlier studies in our lab (55) have shown that FMH pretreatment restored normal cycles in 2DG-treated adult rats, suggesting that glucoprivation extends estrous cycles in the adult female by increasing brain histamine levels. Since suppression of brain histamine levels reverses the suppressive effects of glucoprivation on estrous cycle length (55), we have hypothesized that in the adult female the glucoprivic emergency produced by 2DG may activate histaminergic systems in the hypothalamus to help prevent an energy deficit by repartitioning available energy (47). If histamine functions in energy partitioning, it may be important in our pubertal model, as growth and onset of reproduction depend on repartitioning of available energy.

Thus, our second specific aim is to determine if delayed onset of puberty in the young female rat due to glucoprivation is mediated through central histamine pathways, as it seems to be in the adult. Specifically, we hypothesize that there is an increase in central histamine due to 2DG-induced glucoprivation, which in turn causes suppression of the reproductive axis, leading to delayed puberty onset.

Materials and Methods

Weanling female Long Evans rats (21 days of age) were obtained from Charles Rivers Laboratories. The rats were caged in groups during acclimation for 1 day, after which they were

individually caged and housed at 23 °C and 40% humidity with a self-watering system on a 14L:10D schedule.

On arrival to the lab, the rats were allowed ad libitum feeding of Purina Rat Chow Formula #5008 until they reached a weight of 80-90 grams. In order to maintain this body weight and to prevent estrous cycling, diets were adjusted in response to weight fluctuations by feeding a single meal of 5.3-7.3 grams daily. Such dieted rats receive approximately 34% of an ad libitum diet (29). Animals were weighed three times a week prior to surgery and daily during the post-surgical and experimental periods. Self-watering was maintained throughout the experiment. Vaginal cytology was performed daily from the day of vaginal opening onward to determine the reproductive status of each rat. This method has been previously validated in growth-restricted prepubertal female rats with delayed puberty (3; 29). Rats were also handled daily (via weighing or checking for vaginal opening) prior to vaginal cytology to acclimate to handling and interacting with humans.

Drugs

The glucose metabolism inhibitor, 2-deoxy-D-glucose (2DG, 400 mg/kg; Sigma-Aldrich), was dissolved in sterile saline (0.9%, 400 mg/ml) and administered subcutaneously (sc). The histidine decarboxylase inhibitor, α-fluoromethylhistidine (FMH, 200 nl/2.24 μmol; Research Biochemicals International, Natick, MA), was dissolved in aCSF (Harvard Apparatus, Holliston, MA) and administered centrally into the third ventricle (icv). Saline was used as the

vehicle for control groups for peripheral (sc) injections and aCSF was used as the vehicle for central (icv) injections.

Experimental Design - Peripheral Blood Glucose Study

Blood glucose levels were measured in diet-restricted rats and during refeeding following diet restriction. Blood samples were collected from growth-restricted rats (n= 6) during the period immediately after the single meal and immediately before the single meal the following day. Blood samples were collected from ad libitum fed animals (n = 5) immediately following onset of refeeding, when the animals were first allowed ad libitum food at the end of diet restriction. This period corresponds to the period after the single meal in the growth restricted group. The ad libitum fed rats were also sampled the following day, corresponding to the period before the single meal in the growth-restricted group.

We collected samples from the tail vein by nicking the tip of the tail and "milking" a blood sample (50 µl per sample) at 60-min intervals for 4 hours beginning an hour after the time of the meal (single meal or onset of refeeding) for a total of 5 samples where possible. The following day, this procedure was repeated for 2 samples, the first one an hour before regular feeding time and the second immediately before the meal (23 h after the first sample). The samples were assayed for glucose using the glucose oxidase method (43).

Experimental Design - Histamine Study

On day 50 (approximately 10 days after surgery), the rats were separated into four

groups: saline (sc)/ aCSF (icv), 2DG (sc)/ saline (icv), 2DG (sc)/ FMH (icv), saline (sc)/ FMH

(icv), such that the mean body weights for the groups were not different. FMH was administered

centrally at a dose of 2.24 µmol/ rat at 1100 h each day, while 2DG was administered

peripherally every 6 h for 72 h (400 mg/kg, sc) beginning at noon on the first day of the 72h

treatment period. The 2DG dose and treatment regimen has been shown to delay puberty onset

during refeeding in the present model (10). The FMH dose and treatment regimen has been

shown to significantly reduce mean levels of histamine when infused in this manner (44). During

the experimental period, all rats were fed a daily "controlled" ad libitum diet of 10.8 g + mean

diet (6.1 to 7.1 g) of the week before treatment. This limited diet is designed to prevent the rats

from acutely increasing food intake to counteract glucoprivation due to 2DG. We have

previously shown that normal puberty onset will occur within one week when growth-restricted

prepubertal rats are fed this diet for 3 days (10, 29). Remaining food was collected and weighed

daily during the treatment period in order to measure daily and cumulative food consumption.

After the 72 h treatment regimen, rats were returned to a restricted diet (mean level of previous

food-restricted daily intake initially) and then manipulated as previously described until the end

of the study (approximately day 65).

Intraventricular Cannulation

Approximately 10 days prior to the histamine experimental period (days 38 through 40),

the rats underwent surgery to implant an ICV cannula for central FMH injections and were

allowed to recover before the experimental period (at least 10 days, as described previously, 42).

The stainless steel cannula (13 mm, 26 gauge) was placed in the third ventricle approximately in

the region flanked by the paraventricular nuclei (PVN) of the hypothalamus: (AP: -1.2 mm from

Bregma, DV: -6.4 mm from dura, 0 mm from midline). An obturator and cap were inserted into

the cannula at the end of surgery to keep the cannula clean and patent.

Cannula Placement Verification

At the end of the experimental period, the animals were euthanized and perfused with

phosphate buffered saline (approximately 300 ml) followed by 10% paraformaldehyde

(approximately 300 ml). Brilliant blue dye (200 nl) was then injected into the cannula and after 1

min the brain was removed from the cranium. The brain was then post fixed in 10%

paraformaldehyde for 4 hours and cryoprotected overnight in 30% sucrose. To check for dye

presence as an indication of appropriate cannula placement within the third ventricle, the brains

were sectioned at 40 μm, mounted on slides, and observed via light microscopy.

Statistical Analyses

Growth rates before and during treatment and daily food intake prior to treatment were compared using separate regression analyses (GraphPad Prism version 2.0, San Diego, CA). Daily food intake during the 72h treatment period was compared using analysis of variance (ANOVA) with time as the repeated measure (Statview 5.0.1, Cary, NC). Total food intake and day of first estrus were compared using a single factor ANOVA (Statview 5.0.1, Cary, NC). A modification of the Fisher exact test for comparing more than two variables was used to compare the percentage of animals achieving first estrus between the groups (62). Hourly changes in blood glucose from initial sample were compared using analysis of variance (ANOVA) with time as the repeated measure (Statview 5.0.1, Cary, NC). In all analyses, $p < 0.05$ will be considered significant and data are presented as mean ± standard error of the mean.

Results

Experiment 1 - Peripheral Blood Glucose Study

Before the treatment period, both the growth-restricted (GR) and ad libitum fed (AL) animals maintained similar body weights (Day -1: GR: 93.1 ± 1.0 g; AL: 93.36 ± 3.0 g) and growth was minimal (r^2: GR: 0.002; AL: 0.005, Figure 1). After refeeding, the ad libitum fed rats grew significantly more than the growth-restricted animals (Day 3: GR: 95.0 ± 1.3 g; AL: 118.3 ± 3.1 g, Figure 1).
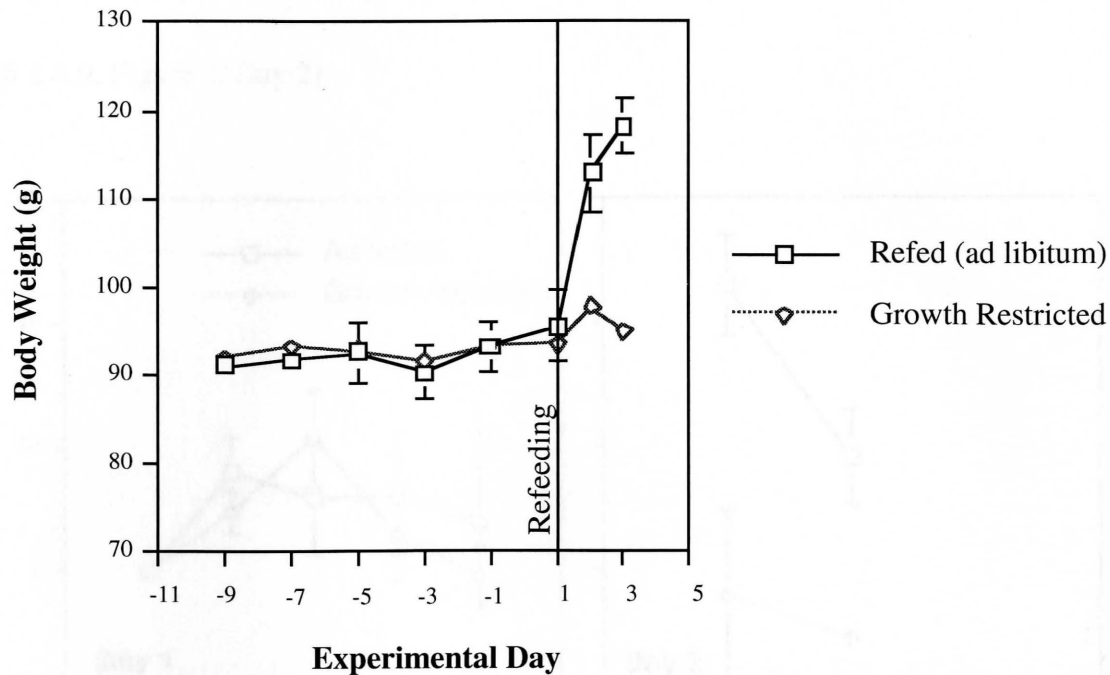
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Figure 1. Experiment 1 body weights for ad libitum fed (open square) and diet-restricted (open diamond) prepubertal rats. Vertical line denotes onset of refeeding for the ad libitum group.

Prior to the treatment period, both groups were maintained on a restricted diet, 6.1 ± 0.2 g daily. Upon refeeding, the ad libitum fed animals were provided with unlimited food, while the growth-restricted animals were maintained on the restricted diet, 6.1 ± 0.3 g/day.

Blood glucose levels were similar between the growth-restricted and ad libitum fed animals during the time just after the single daily meal or refeeding, respectively (blood glucose 60 minutes *after* feeding: GR: 96.4 ± 4.0 mg/dl; AL: 94.4 ± 4.7 mg/dl). Both groups initially demonstrated an increase in blood glucose levels (shown as change from initial value in Figure 2, Day 1). However, blood glucose levels 1 hour *before* the next meal were significantly decreased

in the growth-restricted rats compared to the ad libitum-fed animals (GR: 88.5 ± 7.0 mg/dl; AL: 115.8 ± 4.9, Figure 2, Day 2).
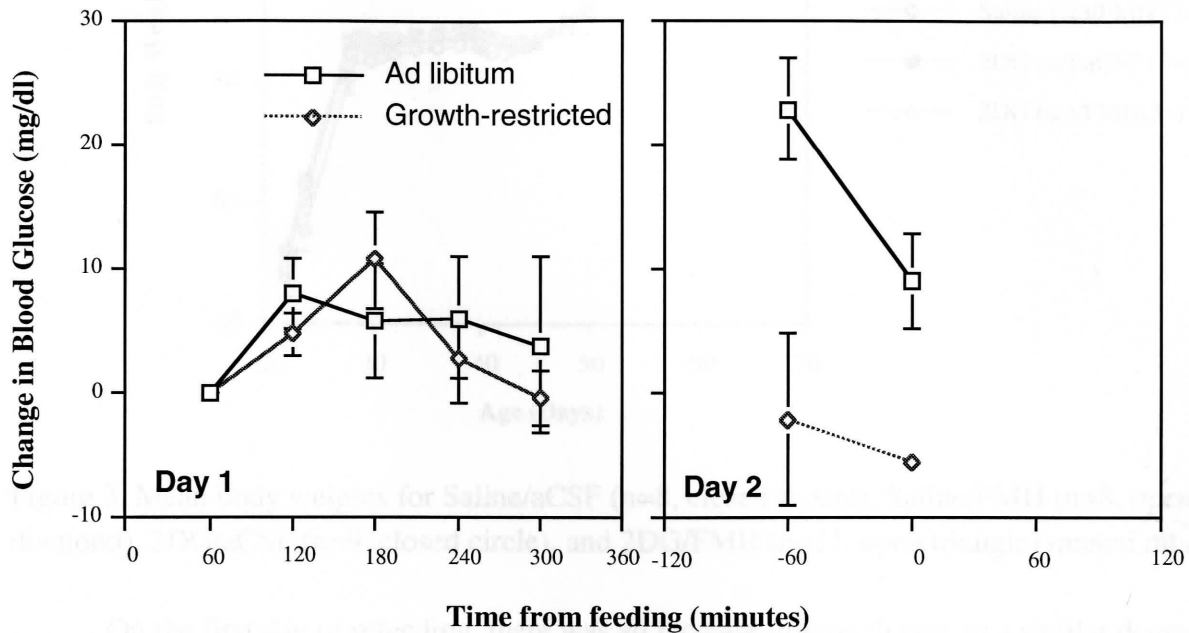

Figure 2. Change in blood glucose levels in ad libitum-fed (open square) and growth-restricted (open diamond) rats. Day 1 and Day 2 correspond to the day of refeeding for the ad libitum animals.

Experiment 2 - Histamine Study

Before the treatment period, all groups maintained similar body weights and growth rates (saline/aCSF: 93.0 ± 0.71 g, n = 8; saline/FMH: 92.2 ± 1.51 g, n=8; 2DG/aCSF: 93.1 ± 0.66 g, n=9; 2DG/FMH: 92.1 ± 0.88 g, Figure 3).
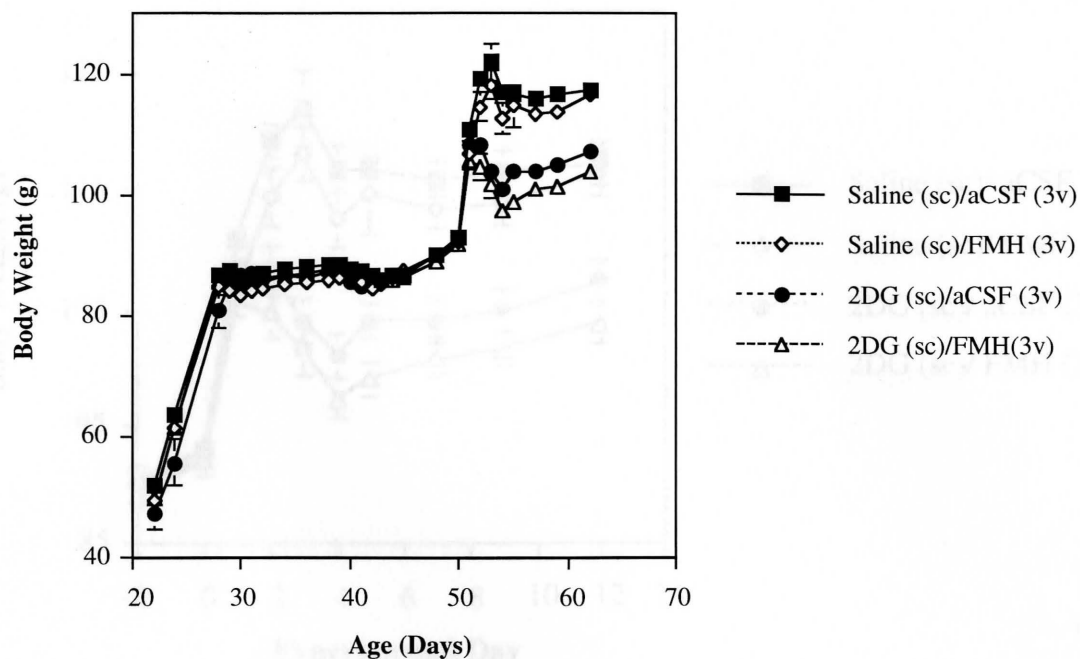
Figure 3. Mean body weights for Saline/aCSF (n=8, closed square), Saline/FMH (n=8, open diamond), 2DG/aCSF (n=9, closed circle), and 2DG/FMH (n=11, open triangle) treated rats.

On the first day of refeeding, there was an increase in growth rate, to a similar degree, in all groups. However, the 2DG/aCSF and 2DG/FMH rats gained significantly less weight overall compared to the saline treated groups (Day 3 body weight: saline/aCSF: 122.0 ± 3.2 g; saline/FMH: 118.2 ± 2.3 g; 2DG/aCSF: 104.1 ± 1.9 g; 2DG/FMH: 101.8 ± 2.2 g). There was no significant difference in body weight gain during treatment between the two 2DG treated groups (Figure 4).
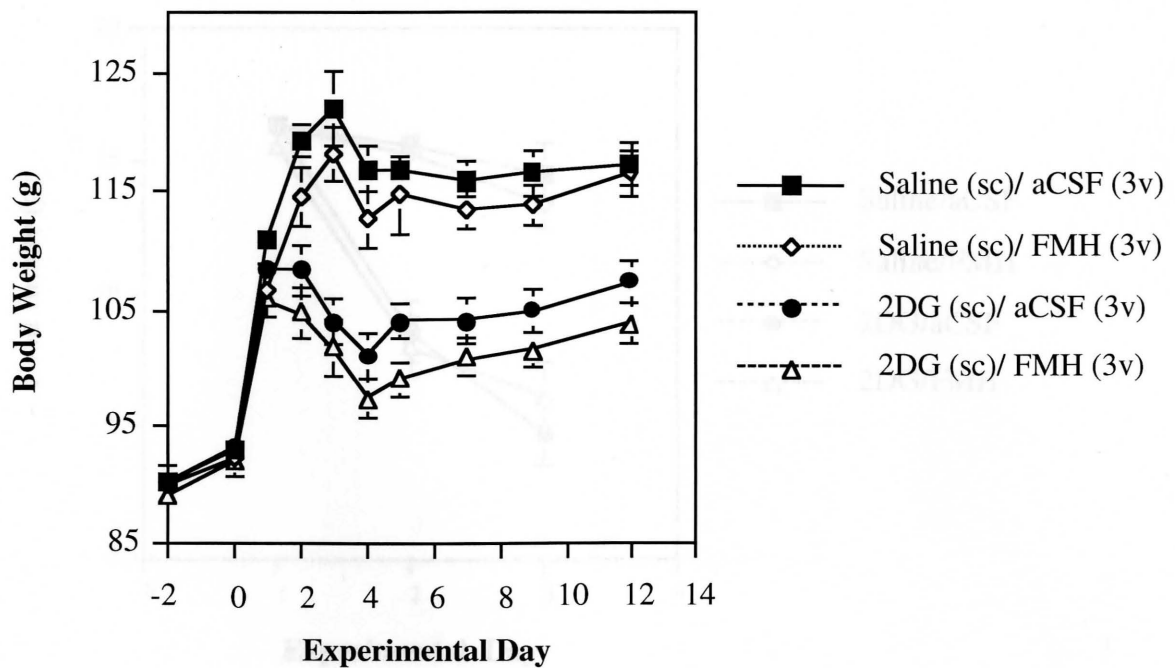
Figure 4. Mean body weights for Saline/aCSF (n=8, closed square), Saline/FMH (n=8, open diamond), 2DG/aCSF (n=9, closed circle), and 2DG/FMH (n=11, open triangle) treated rats during the experimental period.

Prior to treatment, all rats were maintained on a restricted diet (5.1 to 7.3 g food daily). During treatment, all rats were provided with their mean diet one week before treatment + 10.2 g food (total 16.2 ± 0.2 g food per day of treatment). Rats treated with 2DG ate less than saline-treated animals, irrespective of FMH treatment (total 72h food intake: 2DG/aCSF: 29.86 ± 2.39 g; 2DG/FMH: 29.87 ± 2.12 g; saline/aCSF: 46.68 ± 1.52 g; saline/FMH: 45.20 ± 1.50 g). Food intake for each day was also lower in the 2DG-treated rats than the saline-treated groups, with food intake decreasing as treatment progresses (Figure 5). There were no significant differences between the 2DG/aCSF and 2DG/FMH groups in terms of food intake on individual days.
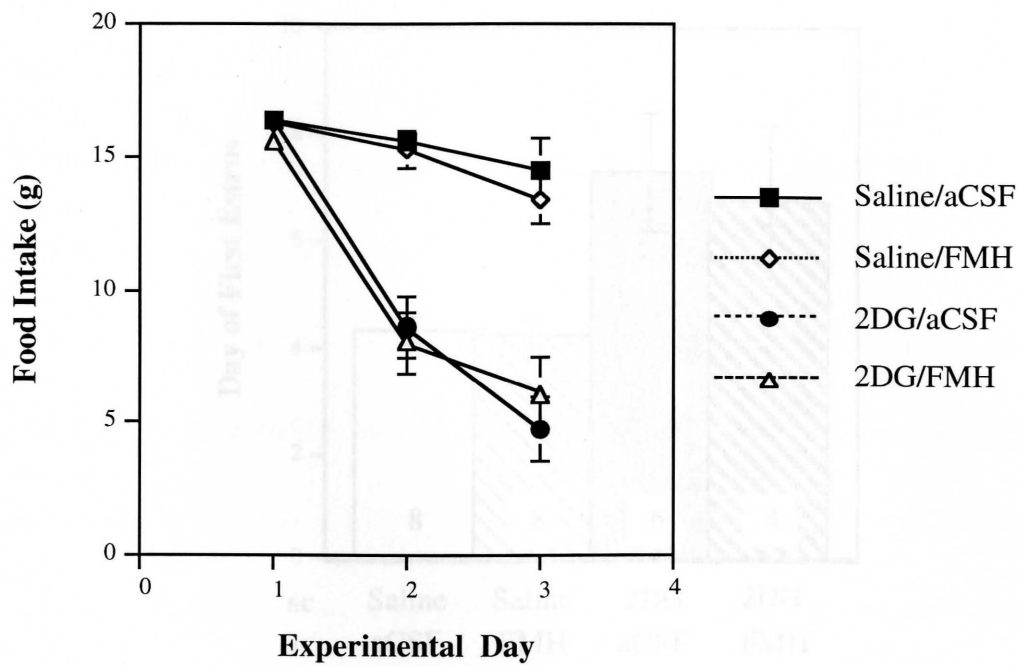
Figure 5. Daily food intake during treatment for the Saline/aCSF (n=8, closed square), Saline/FMH (n=8, open diamond), 2DG/aCSF (n=9, closed circle), and 2DG/FMH (n=11, open triangle) treated rats.

All animals in the saline/aCSF and saline/FMH groups achieved first estrus within the first 4-5 days after refeeding (4.38 ± 0.263 and 4.25 ± 0.25 days, respectively, Figures 6 & 7). The 2DG/aCSF and 2DG/FMH groups achieved first estrus significantly later than the saline-treated animals (7.33 ± 1.085 and 6.75 ± 1.436 days, respectively, Figure 6). In addition, significantly fewer 2DG-treated rats achieved first estrus compared to the saline-treated rats, with a tendency for even fewer to achieve first estrus with FMH treatment (2DG/aCSF: 6/9; 2DG/FMH: 4/11; $p < 0.1$, Figure 7).
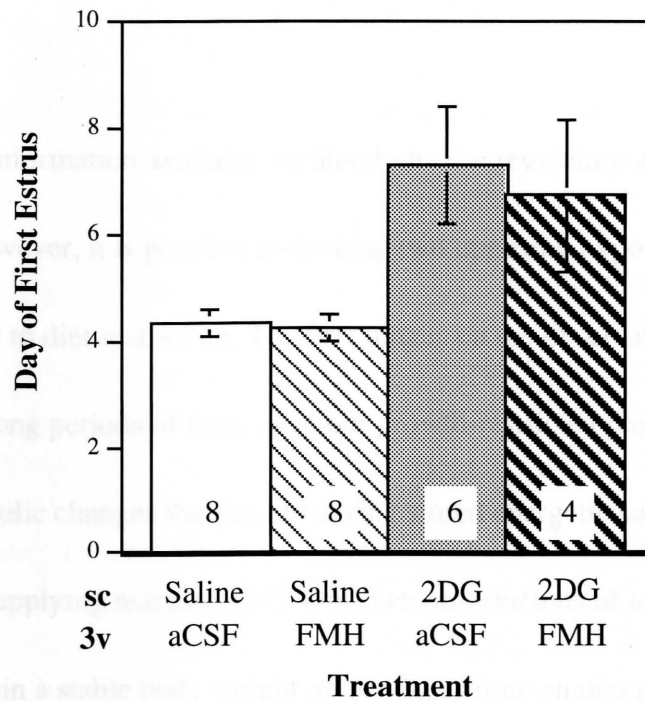
Figure 6. Day of first estrus for those rats achieving first estrus (numbers reflected on the bars) following saline/aCSF, saline/FMH, 2DG/aCSF, or 2DG/FMH treatment.
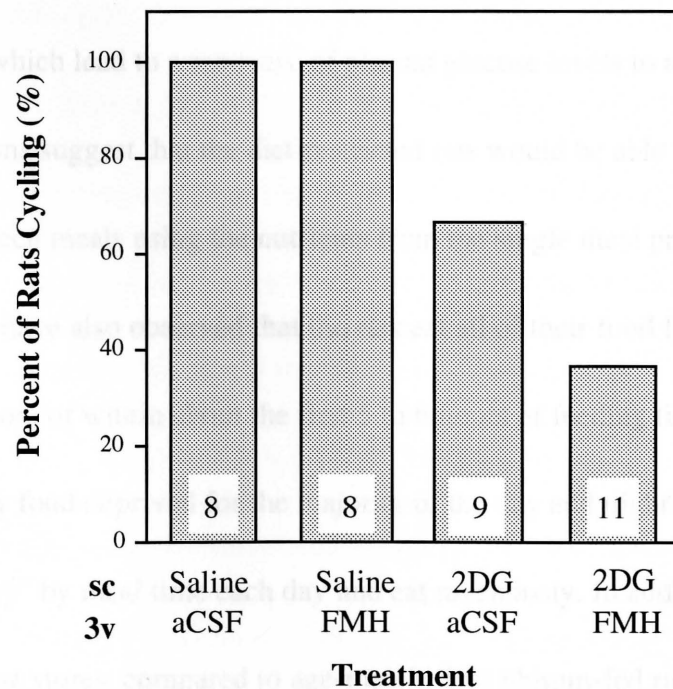

Figure 7. Percentage of rats achieving first estrus after saline/aCSF, saline/FMH, 2DG/aCSF, or 2DG/FMH treatment. Numbers within the bars reflect total number of rats in the group.

Discussion

There is little information available on blood glucose regulation and homeostasis in diet-restricted animals. However, it is possible to develop two opposing hypotheses as to how blood glucose levels respond to diet-restriction. The first hinges on the observation that living on a single daily meal for long periods of time, as in our growth-restricted prepubertal rat model, induces general metabolic changes that lead to saving stored energetic substrates and a better utilization of energy supplying nutrients (27). Our own observations of animals requiring less food per day to maintain a stable body weight of 80-90g as time on diet restriction increases supports this hypothesis. In addition, short-term hypoglycemia generates a counterregulatory response characterized by release of counterregulatory hormones and activation of endogenous glucose production, which lead to a recovery of plasma glucose levels to a specific set point (13, 40). These observations suggest that the diet-restricted rats would be able to maintain normoglycemia between meals using the nutrients from the single meal provided daily.

However, we have also observed that the rats eat all of their food from the single daily meal by early afternoon, or within about the first 5 to 6 hours of feeding time. This means that the rats are essentially food deprived for the majority of the day and night (18-19 hours). Our young rats are "hungry" by meal time each day and eat ravenously. In addition, they have extremely low body fat stores, compared to age-matched ad libitum-fed rats, with which they might supplement their daily nutrient intake (personal observations). Thus, we might expect a

period of peripheral hypoglycemia each day during the time when our animals are food deprived. Low blood glucose levels have been observed in food deprived primates, sheep, rats, and fish (14, 23, 24, 58, 60). Hypoglycemia has been induced by only 3h of food deprivation in adult male rats that have more than adequate body fat stores. In addition, subsequent food intake is highly correlated with the level of fasting-induced hypoglycemia in such rats (24).

We observed similar blood glucose levels during the first 4h period following the single meal or refeeding for both diet-restricted and ad libitum-fed rats. However, blood glucose levels decreased significantly in the diet-restricted rats when sampled before the next single meal, as compared to the ad libitum fed animals. These results support the hypothesis that diet-restricted rats experience daily hypoglycemia between meals and suggest an overlap may exist in the mechanism by which diet-restriction and food deprivation suppress reproductive function.

In fasted animals, there is a decrease in LH pulse frequency with food deprivation. In adult rats, this does not become significant until 6h after onset of fasting (22), probably due to the availability of stored fuels in such animals that would blunt the ensuing energy crisis initially. In contrast, in the diet-restricted rat model that we use, LH secretion can be modulated by food intake on an almost hour-by-hour basis (4), perhaps reflecting the lack of stored fuels in these rats. Our results suggest a mechanism of action by which diet restriction modulates LH levels. Bronson measured circulating LH levels in female rats fed a single daily meal and LH pulses were only observed for approximately 4h after the meal (4). This 4h period of elevated LH secretion corresponds to the period when our diet restricted rats have only recently finished their

single meal and to the period when they demonstrate normoglycemia, relative to ad libitum-fed

rats (our current results). Frequent blood samples taken 10, 20, and 26 hours after single meal

feeding demonstrated no LH pulses and low mean LH levels (4). These samples, especially the

sample at 20h, correspond to the period in our diet-restricted rats when we measured

hypoglycemia. Our diet restricted model is similar to that used by Bronson (4), and the

combination of data from our two labs provide more support for the hypothesis of glucose

importance as a signal for energy partitioning to reproduction.

Thus, we propose that daily bouts of hypoglycemia may delay onset of puberty during

diet restriction. A follow up study might be to provide diet-restricted prepubertal rats with a

glucose supplement in addition to their daily food ration, in an attempt to reverse the

hypoglycemia and determine if puberty is then able to occur.

A decrease in available energy supply, specifically from glucose metabolism, can be

mimicked by administration of 2-deoxy-D-glucose (2DG), a competitive inhibitor of glucose

utilization, and its administration produces similar results to food deprivation. Reproduction is

effectively suppressed by 2DG-induced glucoprivation, as measured by estrous cycle length and

decreased reproductive behavior in hamsters (51), and estrous cycle length in the female rat (56).

A large body of data suggests that sensors monitoring glucose availability and regulating both

feeding behavior and reproductive function are located in the brain stem (11, 32, 37, 53).

Glucoprivation decreases LH pulse frequency, which reflects hypothalamic GnRH pulse

frequency, in rats and sheep after 2DG administration (5, 33). Infusion of 2DG into the fourth

ventricle suppresses pulsatile LH secretion in the rat (32), implying that glucoprivation in the brain stem alone is sufficient to suppress reproductive function. Lesion of the area postrema, located in the dorsomedial hindbrain, restores LH pulses in rats during acute insulin-induced glucoprivation (6) and estrous cycles and estrous behavior in hamsters during 2DG- or insulin-induced glucoprivation (37, 53). These data suggest a role for neurons located in the area postrema of the brain stem in regulating reproductive function during periods of cellular glucoprivation.

An increase in brain histamine turnover has been measured during both 2DG- and insulin-induced glucoprivation (34), suggesting an inverse relationship between energy availability and brain histamine activity. If central histamine levels are decreased prior to 2DG-induced glucoprivation, fewer extended estrous cycles are observed in the adult female rat (55). This suggests that glucoprivation decreases reproductive function in the adult, at least in part, by increasing brain histamine levels. Histamine has also been implicated in the control of thermoregulation, cardiovascular regulation, motor activity, feeding and drinking (45, 54). Thus, histamine may be an important component of central energy partitioning pathways. Based on these considerations, our initial hypothesis was that increasing brain histamine levels during chronic glucoprivation signals a low energy emergency state which in turn causes the animal to decrease reproductive function, locomotion, and core body temperature to conserve energy expenditure for more vital processes such as thermoregulation and cell survival. Specifically, in our experiment, we would have expected that preventing this increase in brain histamine levels

with FMH administration would prevent the low energy emergency signal from glucoprivation

and the animals treated with 2DG and FMH would grow and achieve first estrus, similar to the

saline treated animals.

In the present experiment, 2DG-induced glucoprivation caused a delayed first estrus, as

we have previously observed (10); providing further support for the hypothesis that variations in

glucose availability may provide a signal to the reproductive axis that times onset of puberty.

However, our observation that first estrus was delayed by glucoprivation, regardless of FMH

treatment, was unexpected. In addition, there was a trend for fewer 2DG/FMH rats to achieve

first estrus compared to the 2DG treated rats (4/11 vs. 6/9). This suggests that instead of

reversing the 2DG-induced glucoprivic effect, reducing brain histamine levels may have even

potentiated the glucoprivic effect.

There are several hypotheses that might account for our unexpected results. Histamine

neurons have been shown to regulate glucose metabolism in the brain. Sakata has proposed that

rats may use hypothalamic glycogen as an energy source through activation of hypothalamic

histamine in cases of insufficient energy supply, such as during starvation and relative

glucoprivation (47). Glycogenolysis in the brain may therefore be regulated by neuronal

histamine and activation of histamine in response to an energy deficit may play an essential role

in glucose utilization through glycogenolytic processes in the hypothalamus (47).

Thus, when glucoprivation is induced in an animal by treatment with 2DG and brain

histamine levels are allowed to adjust normally, histamine levels are elevated. It is possible that

the increased histamine levels increase activation of glycogenolysis and that this supplies enough

energy to provide a delayed signal for reproduction, once the glucoprivic challenge is terminated.

In this regard, the delay in first estrus in our 2DG-treated rats is about 3 days, or about the length

of the glucoprivic treatment period. It is possible that the suppression of histamine-induced

glycogenolysis leads to an even greater energetic emergency, in animals treated with 2DG and

FMH. Thus, the reproductive system is less likely to be activated, as we observed, and even

fewer animals achieve first estrus in this group. Perhaps in prepubertal females an increase in

brain histamine is facilitatory if energy reserves are compromised at the onset of puberty.

One future experiment to address this question might be to measure central glucose and

glycogen levels during the glucoprivic challenge, both with and without histamine suppression.

A decrease in central glucose and glycogen levels, when histamine is blocked, would indicate

even less energy availability than during a glucoprivic challenge alone and provide insight into

how decreasing brain histamine actually potentiates the glucoprivic effect on reproductive axis

activation.

Studies of feeding behavior in adult mammals have demonstrated a relationship between

hypothalamic histamine and the adiposity signaling hormone, leptin. Leptin administration

increases histamine turnover (31, 61) and hypothalamic neuronal histamine plays an essential

role in leptin-induced feeding suppression (30, 61). Evidence also exists to suggest that leptin

acts as a signal triggering puberty (7), especially when previous energy reserves have been low

(15). Normal prepubertal mice injected with leptin grew at a slower rate than controls but they

achieved vaginal opening and first estrus up to 9 days earlier than the controls and showed earlier

maturation of the reproductive tract (7). Leptin levels rise dramatically during realimentation in

previously diet-restricted prepubertal rats, simultaneous with activation of the reproductive axis

and first estrus (15). In addition, central leptin infusion in food-restricted prepubertal female rats

led to a significant decrease in body weight compared to controls, but vaginal opening occurred

in 8/9 rats, representing induction of the process of sexual maturation and confirmed by increases

in ovarian and uterine weights, at the expense of whole body growth (15). Thus, rising plasma

levels of leptin in the prepubertal period appear to represent a signal to the brain indicating that

the young animal is metabolically ready to go through the process of reproductive system

activation (15).

This leptin signal may function through increased central glucose availability, due to

leptin-induced brain histamine release. As previously discussed, increased brain histamine levels

cause increased glycogenolysis that could provide energy for GnRH activation and onset of

puberty.

However, during 2DG-induced glucoprivation, the increased glucose availability from

the leptin-histamine pathway is not able to be used while 2DG is present, due to competition for

the glycolytic enzyme, hexokinase. Therefore, additional energy, in the form of increased

glucose, provides a signal for reproductive onset that can be utilized once the glucoprivic

challenge is removed.

Without the increase in histamine, as in those rats treated with FMH, this leptin-histamine pathway is blocked and no additional glucose is provided as leptin levels increase. Therefore, even after the glucoprivic challenge is removed, there is no additional energy to signal reproductive activation and the signal for puberty onset is not given. This could be one explanation as to why fewer of the 2DG/FMH-treated rats achieved first estrus, compared to the 2DG/aCSF rats.

One future experiment might be to measure plasma leptin levels and see if there is a difference between the 2DG/aCSF and 2DG/FMH groups in terms of leptin levels. We would expect leptin levels to increase during realimentation in all groups. Therefore, a more appropriate way to investigate this interaction would be to actually replace histamine, either during glucoprivation and refeeding or immediately after. This elevation of histamine during treatment should counter the decrease in histamine levels due to treatment by the antagonist, FMH, and the animals should demonstrate the glucoprivic effect alone. That is, they would experience delayed onset of puberty and more animals should actually achieve first estrus. Histamine given after the period of glucoprivation and refeeding could be used to determine the timeline for histamine importance in puberty onset. If the signal for reproductive onset given by the leptin increase at refeeding is dependent on increased histamine levels, then animals provided with histamine after the glucoprivic challenge is removed should also achieve a delayed first estrus.

During glucoprivation, we also observed a decrease in food intake over the course of the 3-day treatment period. Food restriction or decreased total calories, if severe enough, has been

show to delay onset of puberty in our model (29). However, we have shown that the decrease in

food intake and therefore calories due to 2DG-treatment in our model is not sufficient to delay

onset of puberty (unpublished data). This suggests that 2DG does not suppress reproductive

function through a reduction in caloric intake. Thus, the signal for suppression of puberty onset

is not a reduction in total calories, but rather is a glucoprivic signal. A decrease in food intake

may seem an unusual response to glucoprivation, but the strategy may be employed as a way to

conserve energy by decreasing locomotion and therefore feeding as a consequence (3). When fed

their single daily meal, our rats remove their food from their bowls and store, or hide, the food

throughout their cages. The rats must then forage, or move around the cage, to find their food

and eat it. Since they do forage for their food, locomotion is a prerequisite for feeding. A similar

strategy of decreased locomotion and feeding has been noted in peripubertal house mice who

grow and reach puberty at the expected time when required to run about 15 miles per night for

their food, but only if the environmental temperature is held quite high (23 °C). If the

temperature is lowered to 9 °C, then these young females will not run for their food, but rather

will remain in their nest and not work for sufficient food for normal growth or reproductive

development (38). Thus, with energy in short supply, the observed suppression of reproduction is

logical, but may also be accompanied by a decrease in locomotion and feeding.

Decreasing food intake has also been shown to suppress reproductive ability in many

mammalian species, including humans, such as during anorexia nervosa, in undernourished

women, and in female athletes (18). The mechanism of this suppression may involve conditions

of hypoglycemia in these women. In general, a decrease in food intake, whether voluntary or

involuntary, causes a decrease in the level of oxidizable metabolic fuel available. Under these

conditions, energy is shunted away from the nonessential processes of growth and reproduction,

toward thermoregulation, cellular maintenance, and locomotion. Further reductions in metabolic

fuel availability will next compromise locomotion and thermoregulation. As mentioned above,

histamine has been shown to regulate thermoregulation, locomotion, feeding behavior, and brain

glycogen stores (9, 21, 25, 26, 28, 36, 44, 45, 46). Our experiments have provided a new twist to

a role for histamine in energy partitioning in the developing animal.

In summary, we have provided further support for the importance of glucose availability

in metabolic regulation of reproduction, with evidence that hypoglycemia and glucoprivation

suppress onset of puberty. Reducing brain histamine levels may potentiate this glucoprivic effect,

and if so suggests an important role for brain histamine in the metabolic regulation of

reproductive function in the developing animal.

References

1. Bronson FH. Effect of food manipulation on the GnRH-LH-estradiol axis of young female rats. *American Journal of Physiology* 254(4 pt. 2): R616-21, 1988.

2. Bronson FH. Food-restricted, prepubertal, female rats: Rapid recovery of luteinizing hormone pulsing with full recovery of pubertal development with gonadotropin-releasing hormone. *Endocrinology* 118: 2483-2487, 1986.

3. Bronson FH. *Mammalian Reproductive Biology.* Chicago: the University of Chicago Press, 1989.

4. Bronson FH, and Heideman PD. Short-term hormonal responses to food intake in peripubertal female rats. *American Journal of Physiology* 259: R25-R31, 1990.

5. Bucholtz DC, Vidwans NM, Herbosa CG, Schillo KK, and Foster DL. Metabolic interfaces between growth and reproduction. V. Pulsatile luteinizing hormone secretion is dependent on glucose availability. *Endocrinology.* 137(2): 601-607, 1996.

6. Cates PS, and O'Bryne KT. The area postrema mediates insulin hypoglycaemia-induced suppression of pulsatile LH secretion on the female rat. *Brain Research* 853: 151-155, 2000.

7. Chehab FF, Mounzih K, Lu R, and Lim ME. Early onset of reproductive function in normal female mice treated with leptin. *Science* 275: 88-90, 1997.

8. Clarke IJ, Horton RJE, and Doughton BW. Investigation of the mechanism by which insulin-induced hypoglycemia decreases luteinizing hormone secretion in ovariectomized ewes. *Endocrinology* 127: 1470-1476, 1990.

9. Doi T, Sakata T, Yoshimatsu H, Machidori H, Kurokawa M, Jayasekara LALW, Niki N. Hypothalamic neuronal histamine regulates feeding circadian rhythm in rats. *Brain Research* 641: 311-318, 1994.

10. Dougherty EJ, Chartisanthian K, Kitchens KG, Vassallo MA, and I'Anson H. Determination of the effect of glucoprivation on the onset of puberty in female rats. *Biology of Reproduction* 58(suppl. 1): 213, 1998.

11. Edmonds BK and Edwards GL. Dorsomedial hindbrain participation in glucoprivic feeding response to 2DG but not 2DG-induced hyperglycemia or activation of the HPA axis. *Brain Research* 801: 21-28, 1998.

12. Foster DL and Nagatani S. Physiological perspectives on leptin as a regulator of reproduction: Role in timing puberty. *Biology of Reproduction* 60: 205-215, 1999.

13. Gabriely I, Hawkins M, Vilcu C, Rossetti L, and Shamoon H. Fructose amplifies counterregulatory responses to hypoglycemia in humans. *Diabetes* 51: 893-900, 2002.

14. Gillis TE and Ballantyne JS. The effects of starvation on plasma free amino acid and glucose concentrations in lake sturgeon. *Journal of Fish Biology* 49: 1306-1316, 1996.

15. Gruaz NM, Lalaoui M, Pierroz DD, Englaro P, Sizonenko PC, Blum WF, and Aubert ML. Chronic administration of leptin into the lateral ventricle induces sexual maturation in severely food restricted female rats. *Journal of Neuroendocrinology* 10: 627-633, 1998.

16. Howenstein MJ, Meyers EA, Russell KA, and I'Anson H. Lipoprivation and onset of puberty in the female rat. *Soc. Neurosci. Abstr.* 26(2): 1450, 2000.

17. I'Anson H. Does reduction in fatty acid availability suppress estrous cycles in adult female rats? *Biology of Reproduction.* 60(suppl. 1): 121, 1999.

18. I'Anson H, Foster DL, Foxcroft GR, and Booth PJ. Nutrition and Reproduction. *Oxford Reviews of Reproductive Biology.* 13: 239-311, 1991.

19. I'Anson H, Manning JM, Herbosa CG, Pelt J, Friedman CR, Wood RI, Bucholtz DC, and Foster DL. Central inhibition of gonadotropin-releasing hormone secretion in the growth-restricted hypogonadotropic female sheep. *Endocrinology* 141(2): 520-527, 2000.

20. Itoh Y, Oishi R, Nishibori M, and Saeki K. Characterization of histamine release from the rat hypothalamus as measured by in vivo microdialysis. *Journal of Neurochemistry* 56: 769-774, 1991.

21. Itowi N, Yamatodani A, Kiyono S, Hiraiwa ML, and Wada H. Effect of histamine depletion on the circadian amplitude of the sleep-wakefulness cycle. *Physiology and Behavior* 49: 643-646, 1991.

22. Komatsu H, Miyamura A, Shibano T, and Ota K. Chronological study on changes in luteinizing hormone release during fasting and refeeding in ovariectomized estrogen-primed rats. *Journal of Animal Science* 71: 239-249, 2000.

23. Lado-Abeal J, Veldhuis JD, and Norman RL. Glucose relays information regarding nutritional status to the neural circuits that control the somatotropic, corticotropic, and gonadotropic axes in adult male rhesus macaques. *Endocrinology* 143(2): 403-410, 2002.

24. Larue-Achagiotis C, and Le Magnen J. Feeding rate and responses to food deprivation as a function of fasting-induced hypoglycemia. *Behavioral Neuroscience* 99(6): 1176-1180, 1985.

25. Lecklin A, Jarvikyla M, and Tuomisto L. The effect of metoprine on glucoprivic feeding induced by 2-Deoxy-D-glucose. *Pharmacology Biochemistry and Behavior* 49: 853-857, 1994.

26. Lecklin A, and Tuomisto L. Feed intake after inhibition of histamine catabolism. *Agents and Actions* 50: 216-219, 1990.

27. Lima FB, Hell NS, and Timo-Iaria C. Carbohydrate metabolism and food intake in food-restricted rats. Relationship between the metabolic events during the meal and the degree of food intake. *Physiology and Behavior* 35:695-700, 1985.

28. Mercer LP. Histamine and the neuroregulation of food intake. *Nutrition* 13: 581-582, 1997.

29. Messer NA, and I'Anson H. The nature of the metabolic signal that triggers the onset of puberty in female rats. *Physiology and Behavior* 68: 377-382, 2000.

30. Morimoto T, Yamamoto Y, Mobarakeh JI, Yanai K, Watanabe T, Watanabe T, and Yamatodani A. Involvement of the histaminergic system in leptin-induced suppression of food intake. *Physiology and Behavior* 67(5): 679-683, 1999.

31. Morimoto T, Yamamoto Y, and Yamatodani A. Leptin facilitates histamine release from the hypothalamus in rats. *Brain Research* 868: 367-369, 2000.

32. Murahashi K, Bucholtz DC, Nagatani S, Tsukahara S, Foster DL, and Maeda KI. Suppression of luteinizing hormone pulses by restriction of glucose availability is mediated by sensors in the brain stem. *Endocrinology* 137(4): 1171-1176, 1996.

33. Nagatani S, Bucholtz DC, Murahashi K, Estacio MAC, Tsukamura H, Foster DL, and Maeda K. Reduction of glucose availability suppresses pulsatile luteinizing hormone release in female and male rats. *Endocrinology* 137: 166-1170, 1996.

34. Oohara A, Yoshimatsu H, Kurokawa M, Oishi R, Saeki K, Sakata T. Neuronal glucoprivation enhances hypothalamic histamine turnover in rats. *Journal of Neurochemistry* 63: 677-682, 1994.

35. Ookuma K, Sakata T, Fukagawa K, Yoshimatsu H, Kurokawa M, Machidori H, and Fujimoto K. Neuronal histamine in the hypothalamus suppresses food intake in rats. *Brain Research* 628: 235-242, 1993.

36. Ookuma K, Yoshimatsu H, Sakata T, Fujimoto K, and Fugakawa K. Hypothalamic sites of neuronal histamine action on food intake by rats. *Brain Research* 490: 268-275, 1989.

37. Panicker AK, and Wade GN. Insulin-induced repartitioning of metabolic fuels inhibits hamster estrous behavior: role of area postrema. *American Journal of Physiology* 274: R1094-1098, 1998.

38. Perrigo G, and Bronson FH. Behavioral and physiological responses of female house mice to foraging variations. *Physiology and Behavior* 334:437-440, 1985.

39. Quigley K, Goya R, and Meites J. Rejuvenating effects of 10-week underfeeding period on estrous cycles in young and old rats. *Neurobiology of Aging* 8: 225-232, 1987.

40. Randall D, Burggren W, and French K. *Eckert Animal Physiology, Ed. 5*. New York: W. H. Freedman and Company, 2002.

41. Ritter S, Bugarith K, and Dinh TT. Immunotoxic destruction of distinct catecholamine subgroups produces selective impairment of glucoregulatory responses and neuronal activation. *Journal of Comparative Neurology* 432: 197-216, 2001.

42. Ritter, S, Dinh, TT, and Zhang, Y. Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. *Brain Research* 856: 37-47, 2000.

43. Saifer A, and Gerstenfeld S. The photometric microdetermination of blood glucose with glucose oxidase. *Journal of Laboratory Clinical Medicine* 51: 445-460, 1958.

44. Sakata T, Fukagawa K, Ookuma K, Fujimoto K, Yoshimatsu H, Yamatodani A, and Wada H. Hypothalamic neuronal histamine modulates ad libitum feeding by rats. *Brain Research* 537: 303-306, 1990.

45. Sakata T, Kurokawa M, Oohara A, and Yoshimatsu H. A physiological role of brain histamine during energy deficiency. *Brain Research Bulletin* 35(2): 135-139, 1994.

46. Sakata T, Ookuma K, Fujimoto K, Fukagawa K, and Yoshimatsu H. Histaminergic control of energy balance in rats. *Brain Research Bulletin* 27: 371-375, 1991.

47. Sakata T, Tamari Y, Kang M, and Yoshimatsu H. 2-Deoxy-D-glucose suppresses food intake through activation of hypothalamic histamine in rats. *American Journal of Physiology* 267: R616-R618, 1994.

48. Sanders NM, and Ritter S. Acute 2DG-induced glucoprivation or dexamethasone abolishes 2DG-induced glucoregulatory responses to subsequent glucoprivation. *Diabetes* 50: 2831-2836, 2001.

49. Sanders NM, and Ritter S. Repeated 2-Deoxy-D-glucose-induced glucoprivation attenuates FOS expression and glucoregulatory responses during subsequent glucoprivation. *Diabetes* 49: 1865-1874, 2000.

50. Schneider JE, Friedenson DG, Hall AJ, and Wade GN. Glucoprivation induces anestrus and lipoprivation may induce hibernation in Syrian hamsters. *American Journal of Physiology* 264: R573-R577, 1993.

51. Schneider JE, Goldman MD, Leo NA, and Rose. Central vs. peripheral metabolic control of estrous cycles in Syrian hamsters. II. Glucoprivation. *American Journal of Physiology* 272(1 Pt 2): R406-12,1997.

52. Schneider JE, Hall AJ, and Wade GN. Central vs. peripheral metabolic control of estrous cycles in Syrian hamsters. I. Lipoprivation. *American Journal of Physiology* 272(1 Pt 2): R400-5, 1997.

53. Schneider JE, and Zhu Y. Caudal brain stem plays a role in metabolic control of estrous cycles in Syrian hamsters. *Brain Res.* 661: 70-74, 1994.

54. Schwartz JC, Arrang JM, Garbarg M, Pollard H, and Ruat M. Histminergic transmission in the mammalian brain. *Physiological Reviews* 71(1): 1-51, 1991.

55. Starer CA, and I'Anson H. Does glucoprivation extend estrous cycles in the adult female rat by increasing brain histamine levels? *Soc. Neurosci. Abstr.* 25(1): 417, 1999.

56. Vassallo M, Chartisanthian K, Dougherty EJ, Kitchens KG, Markham JA, and I'Anson H. Does a reduction in glucose availability suppress estrous cycles in adult female rats? *Biology of Reproduction* 58(suppl. 1): 209, 1998.

57. Wade GN, Schneider JE, and Li H. Control of fertility by metabolic cues. *American Journal of Physiology* 270: E1-E19, 1996.

58. Wang J, Akabayashi A, Yu HJ, Dourmashkin J, Alexander JT, Silva I, Lighter J, and Leibowitz SF. Hypothalamic galanin: control by signals of fat metabolism. *Brain Research* 804: 7-20, 1998.

59. Weidenfield J, Corcos AP, Wohlman A, and Feldman S. Characterization of the 2-deoxyglucose effect on the adrenocortical axis. *Endocrinology* 134: 1924-1931, 1994.

60. Wronska D, Niezgoda J, Sechman A, and Bobek S. Food deprivation suppresses stress-induced rise in catabolic hormones with a concomitant tendency to potentiate the increment of blood glucose. *Physiology and Behavior* 48: 531-537, 1990.

61. Yoshimatsu H, Itateyama E, Kondou S, Tajima D, Himeno K, Hidaka S, Kurokawa M, and Sakata T. Hypothalamic neuronal histamine as a target of leptin in feeding behavior. *Diabetes* 48: 2286-2291, 1999.

62. Zar, JH. *Biostatistical Analysis*, 2nd Ed. Englewood Cliffs, NJ: Prentice-Hall, 1984.