2000

Neuropeptide Y, leptin and selected hormones in hypergravity exposed rats

Megan M. Moran
San Jose State University

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI®

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

NEUROPEPTIDE Y, LEPTIN AND SELECTED HORMONES IN HYPERGRAVITY EXPOSED RATS.

A Thesis Presented to

The Faculty of the Department of Biological Sciences

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Sciences

by

Megan M. Moran

June 2000

APPROVED FOR THE DEPARTMENT OF BIOLOGICAL SCIENCES

Dr. Daniel C. Holley (Graduate Advisor)

Dr. Michael Sneary (Graduate Committee Member)

Dr. Charles E. Wade (Senior Scientist, NASA Ames Research Center)

APPROVED FOR THE UNIVERSITY

ABSTRACT

NEUROPEPTIDE Y, LEPTIN AND SELECTED HORMONES IN HYPERGRAVITY EXPOSED RATS.

Exposure to hypergravity causes a reduction in circulating leptin, and in body and fat mass. However, food intake remains similar to controls. This study, conducted in two experiments, examined plasma neuropeptide Y (NPY) in the presence of hypergravity induced low leptin and euphagia. Experiment 1 rats were centrifuged at 2, 1.5, or remained at 1 G. Experiment 2 rats were centrifuged at 1.5, 1.25, or remained at 1 G. Plasma NPY concentration (ng/ml) was similar between the 2.0 (13.1 ±2.5), 1.5 (13.6 ±1.4), and 1.0 (15.7 ±1.9) G groups of experiment 1. There were no differences in NPY between the 1.5 (9.7 ±1.4), 1.25 (8.2 ±0.7), and 1.0 (8.5 ±1.0) G groups of experiment 2. Significant differences in NPY were found between experiments 1 and 2 ($p \leq 0.05$). During hypergravity exposure when plasma leptin is low, food intake is maintained by an alternate peripheral feedback signal, which acts to sustain NPY.

ACKNOWLEDGMENTS

This study was performed in the laboratory of Dr. Charles Wade at NASA-Ames Research Center. I would like to thank Fang Yuan, Tom Wang, Lisa Baer, and Heather Jonasson for their efforts with the data collection. In addition I would like to thank Rudy Ortiz for his assistance with the radioimmunoassays and Mari Eyestone for analyzing the samples. An extra special thank you goes to Dr. Daniel Holley (SJSU) and Dr. Michael Sneary (SJSU) for their support and critical review of the manuscript and to Dr. Wade for his support and guidance throughout this effort. This work was supported by NASA grant #121-10-30.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

Introduction

Neuropeptide Y (NPY) is a 36 amino acid polypeptide that is localized in sympathetic ganglia, sympathetic postganglionic fibers, epinephrine (E) and norepinephrine (NE) containing cells of the adrenal medulla, platelets, the GI neural plexus and within the central nervous system (8, 9, 10, 11, 13, 14, 40, 42). Peripherally, NPY has been implicated in vasoconstriction, stimulation of cardiac contraction, and reduction of catecholamine release from chromaffin cells (12, 15, 16, 17, 27). Centrally, NPY is produced and released within the arcuate and paraventricular nuclei of the hypothalamus respectively. Although other central mediators of food intake have been found, including agouti related protein (AgRP) and α-melanocyte stimulating hormone (α-MSH), NPY is considered the most potent stimulus of food intake (1). Recently the *ob* gene was cloned from adipose tissue and its end-product leptin was implicated as an inhibitor of NPY production and release (2).

Leptin modulates hypothalamic neuropeptide-Y production in a negative-feedback loop to decrease arcuate nucleus NPY production and consequently to decrease food consumption (25, 26). In non-obese subjects the amount of leptin in the blood is a function of fat mass (3, 4, 5, 6, 7). Although the leptin-NPY axis influences food intake, there are other neural and hormonal factors which regulate food intake, and the role the leptin-neuropeptide Y axis plays in the control of food intake remains unclear.

Centrifugation is a unique tool for studying physiological control of body weight. During centrifugation, body weight is a function of the mass of the subject and the magnitude of the horizontal force vector that is produced by the speed of rotation and the

distance of the subject from the axis of rotation. Consequently, body weight can be increased immediately without manipulating food intake and/or activity. In a 1G (terrestrial) environment, increases in food intake that exceed the amount of energy that is lost through exercise and/or activity cause body weight and fat to increase (18). Hypergravity increases body weight and causes a transient reduction in food intake and body mass (24, 23). However, within seven days of the onset of centrifugation, food intake (per g/body mass) and rate of body mass gain are similar to 1.0 G controls (23). Despite the increased body weight, rats exposed to hypergravity have less fat than 1.0 G controls (19, 20, 21). In addition, the loss of fat is paralleled by a reduction in plasma leptin (39). The reduction in plasma leptin should cause an increase in NPY production and hyperphagia. However, rats exposed to hypergravity do not exhibit hyperphagia, and therefore NPY levels should not change. The absence of hyperphagia in the presence of low leptin levels suggests that an alternated peripheral signal is working to inhibit NPY over-production. This would lessen the importance of the leptin-NPY axis in the regulation of food intake. The focus of the present study was to examine the response of circulating NPY to low plasma leptin levels following 14-days of centrifugation at graded G loads ranging from 1.0 to 2.0 G.

Methods

Before initiation of these studies, approval was received from the Institutional Animal Care and Use Committee (IACUC) at the National Aeronautics and Space Administration (NASA) Ames Research Center. The centrifuge can accommodate two different G-loads at a time; thus the study was conducted in two experiments (1 & 2). Both experiments were conducted at NASA Ames Research Center and conform to NASA's *Animal Users Guide* and the National Research Council guidelines for animal experimentation. Approval was received from the San Jose State University IACUC, for the measurement of NPY in stored plasma samples that were collected during the experiments, which are described below.

Study Design (Experiment 1)

The experiment was conducted using 1.5 month-old, male Sprague-Dawley derived albino rats (Simonsen Laboratories, Gilroy, CA). Rats were received at the Animal Care Facility (ACF) at NASA Ames Research Center. Upon receipt from the vendor, each rat was weighed and housed (1 rat/cage) in standard vivarium cages for a three-day acclimation period. The objective of the acclimation period was to familiarize the rats with the new housing environment. Food (Ralston Purina #5012, St. Louis, MO) and water were provided *ad libitum*. Rats were maintained on a 12:12 hour light dark cycle (06:00 on:18:00 off), which was maintained throughout the study. The acclimation period was followed by surgery.

Each rat was implanted with a telemeter to measure core temperature and activity (Data Sciences International, St Paul, MN). These data are not presented. Rats were anesthetized with a 2-3% isoflurane, 98-99 % oxygen mixture. The fur from the abdomen was shaved and the skin was wiped with betadine. A ventral incision was made along the midline, and the telemeter placed in the peritoneal cavity. The peritoneal wall was closed with 4-0 ethilon sutures (Ethicon, Inc, Somerville, NJ) and skin closed with skin clips. Rats were given a mixture of Tylenol and codeine (2.4 mg codeine/ml water and 24 mg, Tylenol/ml water) in their water bottles for three days, and individually housed for seven days. Following the seven-day recovery period rats were brought from the ACF to the 24'-Centrifuge Facility at NASA Ames Research Center.

Once the rats were brought to the centrifuge they underwent a seven-day baseline data collection period. Before the initiation of the baseline period, rats were randomly selected and placed into one of three groups; 2.0, 1.5, or were stationary 1 G controls (n=8 rats/group). Rats in the centrifuge groups were housed on-board the centrifuge. Note that the centrifuge was not spinning during the baseline period. Control rats were housed in off-board cabs, which were in the same room as the centrifuge. Each rat was housed individually in a metabolic cage (cage dimensions: length-width-height, 23" - 14" - 13'). Food and water were provided on the side of the cage to prevent contamination in the urine and feces. Rats were fed the same diet described above, however it was powdered. The powdered diet prevents contamination of feces and urine, as the rats eat the powdered diet in the food cups and not on the grid cage floor. Water bottle lix-its were modified to prevent dripping during the starting and stopping of the centrifuge.

Room temperature was maintained at 23 ± 2°C. Daily data collection and animal health checks occurred at 08:00 and lasted for 45 minutes. Data collection consisted of weighing each animal, and their food and water. Intake was measured by taking the difference in food and water weight from one day to the next day. Urine was collected daily (see description below).

A 14-day test period followed the baseline period. Rats were centrifuged at either 2.0 (12 ft. 21.1 RPM) G, or 1.5 (8ft, 21.1 RPM) G. Control rats remained at 1 G. Animal husbandry was the same as described above, and daily data collection and animal health checks followed the same schedule.

Study Design (Experiment 2)

The design was similar to experiment 1 with the exception of the centrifuge groups. Rats were centrifuged at either 1.25 G (8 ft, 16.06 RPM), 1.5 (12 ft. 16.06 RPM) G, or were stationary 1 G controls (n=8 rats/group). Centrifugation has both a linear and rotational component. Thus, to account for physiological changes that may be due to the rotation component, the 1.5 G groups from experiments 1 and 2 were centrifuged at the same G load, however the radius and rate of rotation were different.

Urine Collection

Daily urine samples were collected from each rat. In each cage, urine was passed through a funnel, filtered by a urine and fecal separator, and collected into 30-ml conical tubes. To minimize evaporation, 1 ml of decahydronapthylene oil (Fisher Scientific,

Pittsburgh PA) was added to each tube. At the end of the 24-hr collection period the tubes were brought to the lab, the samples were weighed (scale was adjusted for the weight of the tube and 1 ml of oil), the oil was removed, and the samples were centrifuged. The samples were frozen at –20 °C. Urine catecholamine analysis was performed on pooled samples that were collected from each rat on days 11 to 14 of experiment 1 and 2. Sample analysis was performed by high pressure liquid chromatography (HPLC) ((DIONEX, Santa Clara CA). Catecholamine excretion rates were the product of the concentration multiplied by the mean volume excreted during a 24-hr period.

Dissections

Dissections were performed on each rat at day 14 of the test period of both experiments 1 and 2. The rats were anesthetized with isoflurane and killed by decapitation. Prior to decapitation, blood was collected by cardiac puncture, kept on ice, centrifuged, and frozen at –20 °C for further analysis. Bilateral epididymal fat pads were collected, and weighed. Previous data collected in our laboratory has shown a positive correlation between epididymal fat pad weight and total body fat in rats (R^2 =0.797, n=12, y=0.9044x + 0.0891). In addition, this technique has been proven a successful indicator of percent body fat in other rodents (32).

Plasma Leptin and Corticosterone

Commercial radioimmunoassay (RIA) kits were used to measure plasma leptin (ALPCO, Windham NH), and corticosterone (ICN Biomedicals, Costa Mesa CA). For leptin, intra-assay coefficient of variability (CV) was less than 5% and inter-assay CV was less than 8 %. The sensitivity of the plasma leptin assay was 0.6 pg/ml. Corticosterone was measured with a double antibody RIA. The sensitivity of the corticosterone assay was 12.5 pg/ml at the lowest standard level, intra-assay CV was 7.1 %, and the inter-assay CV was 6.5 %.

Measurement of plasma neuropeptide-Y

Samples were extracted using octadecyl (C-18) extraction columns (E & K Scientific, Campbell CA) and assayed by enzyme linked immunosorbent assays (ELISA, Peninsula Laboratories, San Carlos, CA). The assay is based on competitive binding in which unlabeled NPY, biotin labeled NPY, and antibody are placed in wells and mixed. The antibodies bind to the treated walls of the wells. Both the labeled and unlabeled NPY competed for binding sites on the antibodies. After an incubation period, the unbound NPY is washed out of the wells, so that only bound NPY remains. Strepavidin-conjugated Horseradish Peroxidase (SA-HRP) is added and allowed to bind to the biotinylated NPY. The excess SA-HRP is washed out of the well and 3,3',5,5'-Tetramethyl Benzidine Dihydrochloride (TMB) is added to the wells. The TMB reacts with the bound HRP to produce a colored byproduct. The color intensity is proportional

to the quantity of biotinylated NPY. Thus the more non-biotinylated (unknown) NPY the lighter the color of the product. The color intensity will be measured on a 96-well microplate reader with a wavelength of 450 nm (Peninsula Laboratories, San Carlos, CA).

Performance characteristics of the assay were as follows. Rat plasma containing an unknown concentration of NPY, was pooled and aliquoted into ten 100 ul samples. The ten samples included five non-spike samples and five spiked samples. The mean observed final concentration of the non-spiked samples was 0.83 ng/ml. The spiked samples included 75 ul of the non-spike pool (0.63 ng) and 25 ul of the 25 ng/ml standard. There are 0.63 ng of NPY in 25 ul of the 25 ng/ml standard which would make the expected concentration of the spiked samples 1.26 ng/ml. Samples were extracted and assayed in duplicate. The observed, mean concentration of the spiked samples was 1.43 ng/ml, thus the recovery was calculated at 113 %. The standard deviation of the spiked samples was 0.57, which was due to one sample. If this sample was removed from the calculations the spiked recovery was 94 %. Note that the results of the spiked recovery test included both the extraction and the assay.

The sensitivity of the assays were tested by taking the mean coefficient of variation (% CV) of the 0 ng/ml standards which were run in duplicate in each assay. The % CV from each assay were grouped together and the mean was calculated. This number was doubled and subtracted from 100 %, and then multiplied by the mean optical density of the 0 ng/ml standards to provide the sensitivity of each assay. The mean was taken from the sensitivity of each assay, which provided the total assay sensitivity of 0.37

ng/ml. Note that the data from Peninsula Labs has shown a sensitivity of the NPY of 0.04-0.06 ng/ml. The difference between the two results may have been a high % CV of the 0 ng/ml standard in two of the four assays that were used to determine NPY. However, although the sensitivity of the assays was high, all of the collected data fell above 0.37 ng/ml, and are reliable points.

Intra-assay % CV was determined for the assays by calculating the mean (0.83 ng/ml) and the standard deviation (0.02) of the optical density of the non-spiked samples. From these data, intra-assay % CV was 2.9 %. Similar intra-assay % CV results were determined by the company, at < 5 %. Inter-assay % CV was 27 %. This was determined by taking the mean of the % CV the 1.25, 0.625, 0.313, and 0.156 ng/ml standards from each assay. These standards were selected, as they were the points which demonstrated the most separation on each standard curve, and to minimize the contribution of interfering factors, which would skew the results on the extreme ends of the curves. The inter-assay % CV reported by Peninsula Labs was 14 %.

Parallelism tests were run to determine the reliability of diluted samples, which were too high in their original concentration to fall on the standard curve. The test was performed on three different samples at their original concentrations and dilutions of 75, 50, and 25 %. The samples were assayed in duplicate, and mean values were plotted for a total of 12 points (Fig. 1). The observed values were plotted against the expected values and a significant correlation was observed $R^2 = 0.9139$. Thus, diluted samples which fell between a minimum concentration of 0.58 ng/ml and 4.8 ng/ml were reliable.

Figure 1

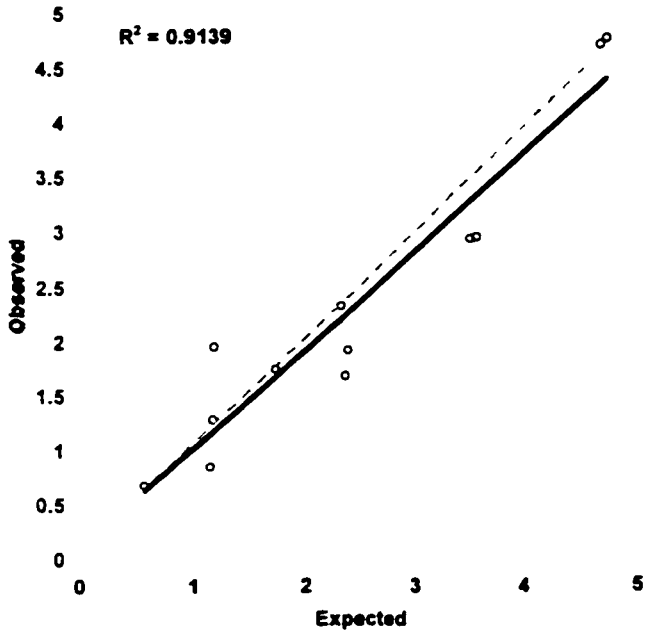

Figure 1 Parallelism test between three different plasma samples at four different concentrations of 100 (original concentration), 75, 50, and 25 % dilutions. Comparison of observed values to expected values. Dashed line represents line of identity.

Statistics. All statistics were performed by using the Statistica software program (Statsoft, version 4.1, Tulsa OK). Data were compared by analysis of variance (ANOVA). If a significant difference ($P \leq 0.05$) was found by ANOVA, a Newman-Keuls post hoc test was performed. To account for differences due to rotation, data from 1.5 G groups were compared by ANOVA.

RESULTS

The body weight, food, fat, leptin, catecholamine, and corticosterone data have been submitted for publication (22). Data are presented in the thesis for clarification of this NPY results and completeness of the study.

Food Consumption. In experiment 1, baseline food consumption was not different between the 1.0, 1.5, and 2.0 G groups (Table 1). During the first seven days of the centrifugation period, both the 1.5 and 2.0 G groups ate significantly less than controls. These changes were transient. There were no significant differences between groups on days eight through fourteen.

During experiment 2, food consumption followed a pattern similar to experiment 1 (Table 1). Baseline food consumption was similar between the controls, 1.25 and 1.5 G groups. During the first week of the test period food consumption was lower in the 1.25 and 1.5 G groups than the controls. These changes were transient. After eight days, food intake returned to control values.

Body Mass. During the baseline period of experiment 1, there were no differences in body mass between the 1.0, 1.5, and 2.0 G groups (Table 1). Within the first seven days of centrifugation, body mass was reduced significantly in both the 1.5 and 2.0 G groups from controls. From day eight to day fourteen, the body mass of the centrifuged groups remained lower than controls. However, the rate of growth was similar at 4 ±1.2, 5 ±0.6, and 4 ± 0.7 g/day in the 1.0, 1.5, and 2.0 G groups respectively.

During the baseline period of experiment 2, body mass was also similar between each of the three groups. However, body mass was significantly lower in the 1.25 and 1.5 G groups for the first week of the test period, and was not significantly different from controls after eight days.

Table 1. Food Intake and Body mass during experiments 1 and 2

Group	Food Consumption (g food /100 g body mass)			Body Mass (g)		
Experiment 1	Baseline	Early	Late	Baseline	Early	Late
1.0 G	10.0 ±0.2	8.9 ±0.1	8.0 ±0.1	246 ±2	280 ±3	314 ±4
1.5 G	9.6 ±0.1	7.1 ±0.1*	8.0 ±0.1	246 ±2	250 ±3*	283 ±3*
2.0 G	9.5±0.2	7.1 ±0.2*	7.7 ±0.2	247 ±2	246 ±3*	273 ±3*
Experiment 2						
1.0 G	9.8 ±0.2	8.6 ±0.1	7.4 ±0.1	238 ±3	268 ±6	301 ±10
1.25 G	9.5 ±0.1	6.9 ±0.1*	7.6 ±0.2	236 ±2	241 ±3*	279 ±3
1.5 G	9.4 ±0.1	6.9 ±0.1*	7.8 ±0.1	240 ±2	246 ±3*	283 ±4

Comparison of food intake and body mass during the baseline (three days prior to the start of the centrifuge), early (days one to seven of centrifugation), and late (days eight to fourteen of centrifugation) of experiments 1 and 2. Values are group means ± se. Comparisons were made within each experiment and * denotes a significant ($p \leq 0.05$) difference from 1 G controls.

Epididymal Fat Pad and Plasma Leptin. In experiment 1, there were no significant differences in fat pad weights between the 1.0 and 1.5 G groups (Fig. 2A). Centrifugation at 2.0 G however, resulted in a 21 % reduction in fat pad weight. Fat pad weight was 14 % less in the 2.0 than the 1.5 G group. Plasma leptin levels were 45 and 63 % lower in the 1.5 and 2.0 G groups respectively, than the controls (Fig. 2C). The 1.5

and 2.0 G groups had similar plasma leptin levels (Fig. 2C). In addition significant correlations were observed between epididymal fat mass and plasma leptin (R^2 =0.8768).

In experiment 2, the epididymal fat pad weights of 1.25 and 1.5 G groups were reduced by 14 and 19 % respectively (Fig. 2B). There were no differences between the 1.25 and 1.5 G groups (Fig. 2B). Plasma leptin followed a similar pattern. Centrifugation at 1.25 and 1.5 G reduced plasma leptin levels by 46 and by 45 % respectively (Fig. 2D). No significant differences in leptin levels were observed between the centrifuge groups (Fig. 2D). In addition significant correlations were observed between epididymal fat mass and plasma leptin (R^2 =0.8245).

Figure 2

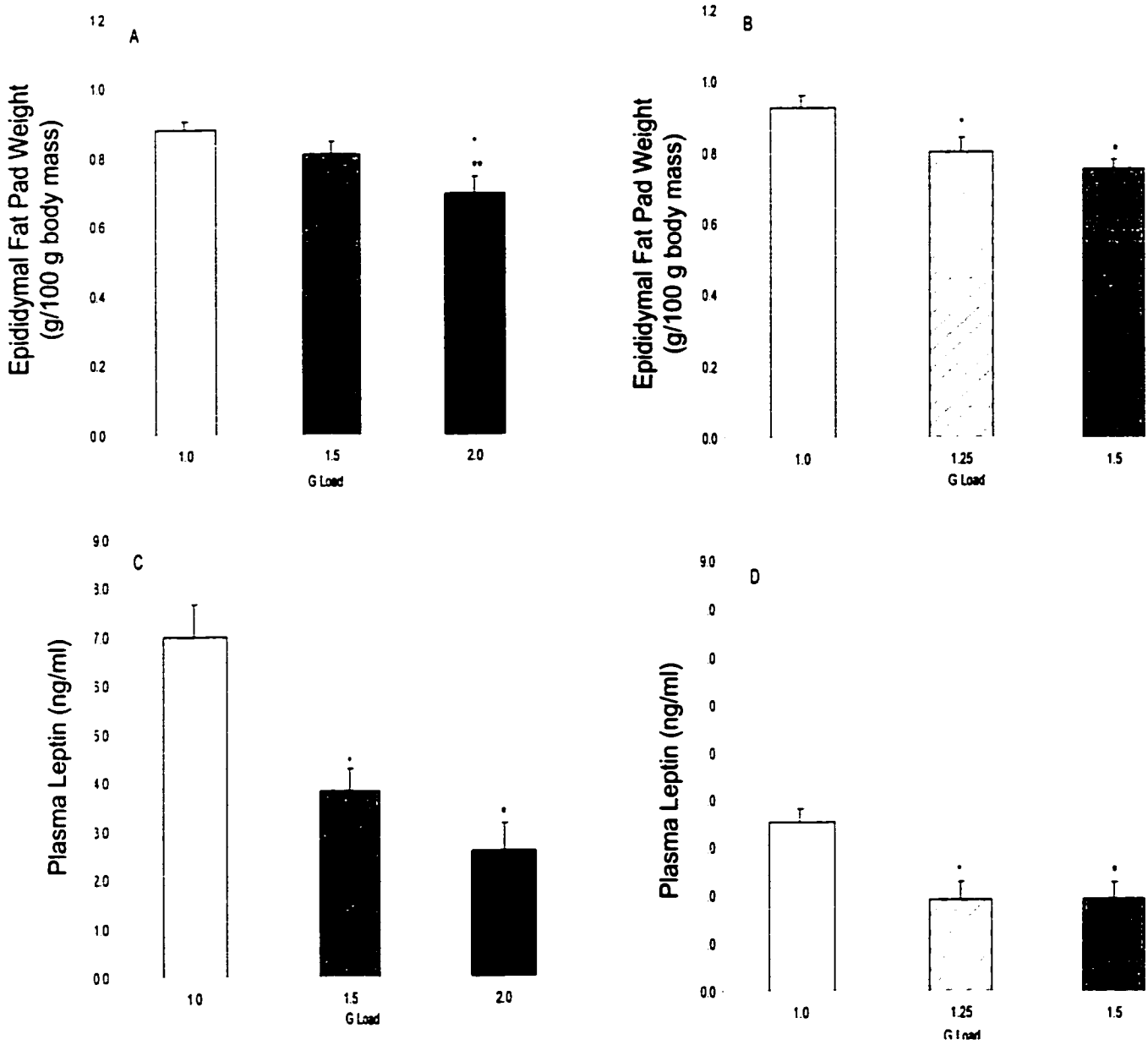

Figure 2. A) Comparison of epididymal fat pad weight between 1 (open bars), 1.5 (gray bars), and 2.0 G (black bars) on day 14 of experiment 1. B) Comparison of epididymal fat pad weight between 1 (open bars), 1.25 (slashed bars), and 1.5 G (gray bars) on day 14 of experiment 2. C) Comparison of mean plasma leptin concentration between 1 (open bars), 1.5 (gray bars), and 2.0 G (black bars) on day 14 of experiment 1. D) Comparison of mean plasma leptin concentration between 1 (open bars), 1.25 (slashed bars), and 1.5 G (gray bars) on day 14 of experiment 2. Values are group means + SE. * Denotes a significant difference from 1 G controls. ** Denotes a significant difference from 1.5 G rats in experiment 1.

Plasma Corticosterone. No differences were found between any of the groups in plasma corticosterone in either experiment (Table 2).

Table 2 Comparison of plasma Corticosterone

Group	Corticosterone (ng/ml)
Experiment 1	
1	135 ±27.7
1.5	113 ±26.4
2	196 ±40.6
Experiment 2	
1	112 ±21
1.25	96 ±23
1.5	99 ±28

Comparison of plasma corticosterone levels following 14 days of centrifugation to 1 G controls. Comparisons were made within each experiment and are group means ± se.

Urinary Catecholamines. In experiment 1, urinary norepinephrine and epinephrine were significantly higher in both the 1.5 and 2.0 G groups than the 1.0 G controls (Fig. 3A). In addition, urinary norepinephrine and epinephrine were significantly higher in the 2.0 G compared with the 1.5 G group (Fig. 3A).

In experiment 2, there were no differences in urinary epinephrine between the 1.0, 1.25, and 1.5 G groups (Fig. 3B). There were no differences in urinary norepinephrine levels between the 1.25 G group and controls (Fig. 3B). The 1.25 G and 1.5 G groups exhibited similar urinary norepinephrine levels, however there was a significant difference between the 1.5 G and control groups (Fig. 3B).

Figure 3

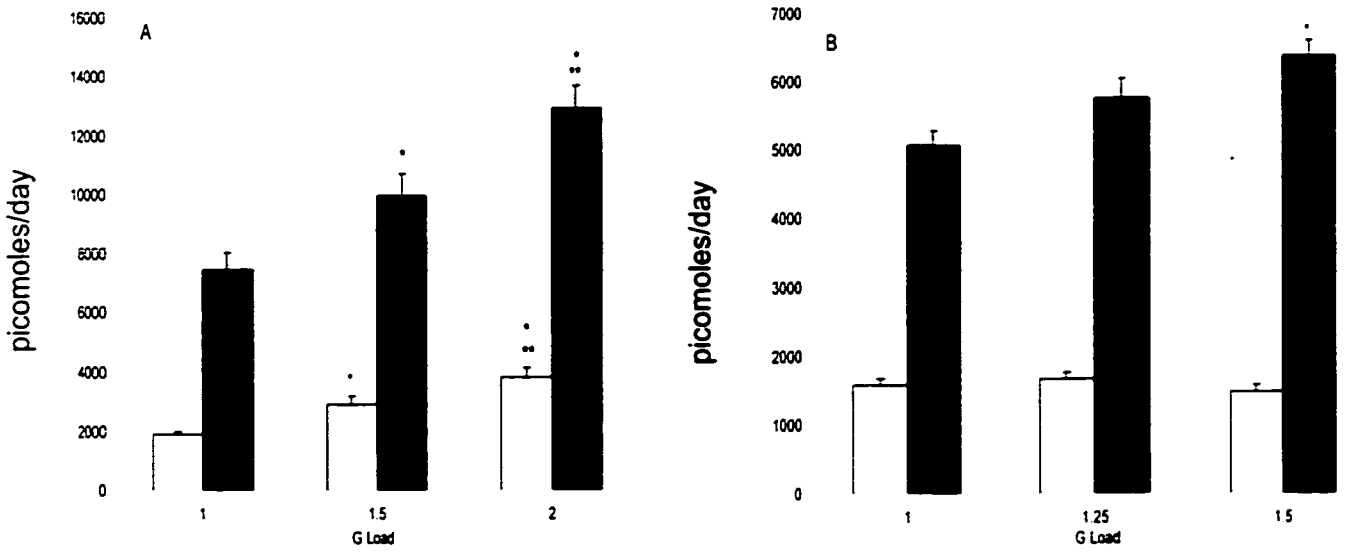

Figure 3. Comparison of 24-hour urinary epinephrine (open bars) and norepinephrine (closed bars) excretion from experiment 1 A) and experiment 2 B). Values are pooled samples collected from each rat on days 11-14 of both studies and are group means +se.

Plasma NPY. There were no differences between the groups in plasma NPY in experiment 1 or 2 (Figures 4A and 4B). However there were differences between the 1.0 G controls of experiment 1 and the 1.5, 1.25, and 1.0 G controls of experiment 2 ($p \leq 0.05$).

Figure 4

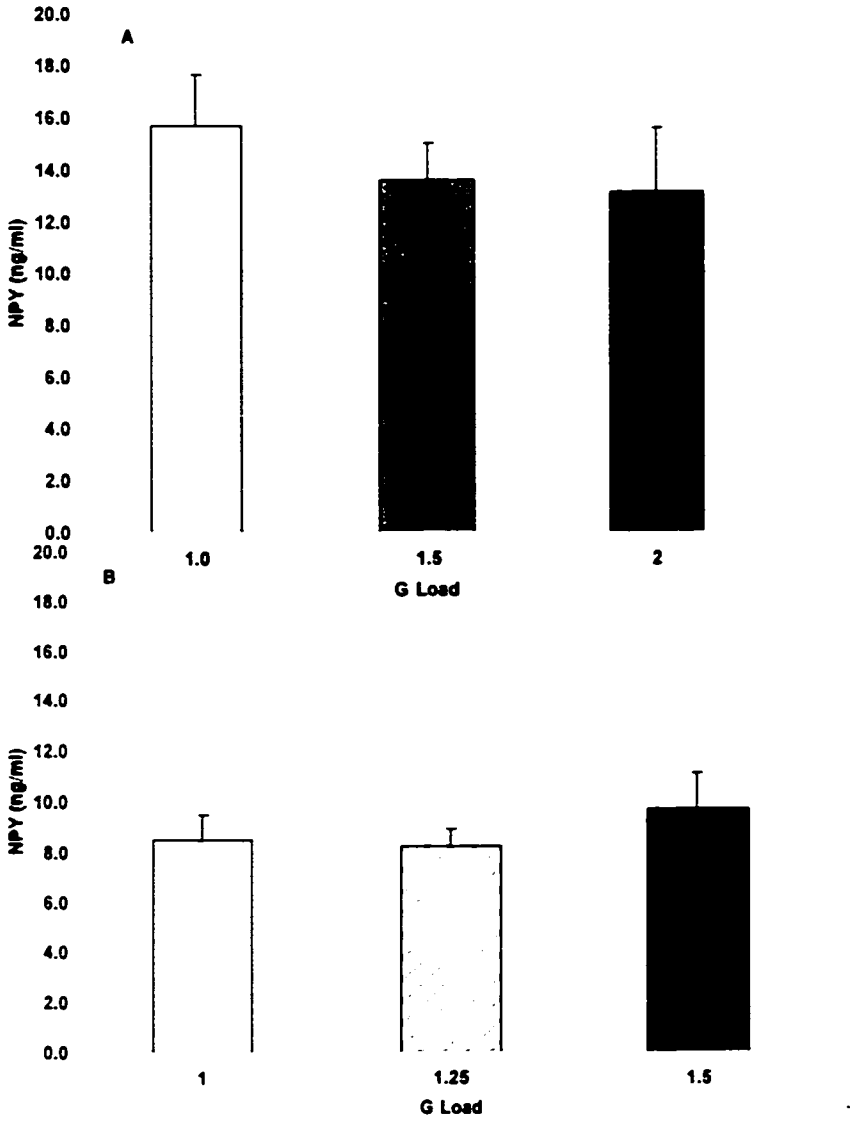

Figure 4. A) Comparison of plasma NPY between 1 (open bars), 1.5 (gray bars), and 2.0 G (black bars) on day 14 of experiment 1. B) Comparison of plasma NPY between 1 (open bars), 1.25 (slashed bars), and 1.5 G (gray bars) of experiment 2.

Rotational Responses. To evaluate the rotational effects of centrifugation, the two 1.5 G groups were compared. The difference between the groups was in the speed of rotation and distance of the rats from the axis of rotation. In experiment 1, the 1.5 G group was centrifuged at a rate of 21.1 RPM, and was housed 8 ft from the axis. In experiment 2, the 1.5 G group was centrifuged at a rate of 16.06 RPM, and was housed 12 ft from the axis. No differences were found in the following parameters; body mass, food consumption, body fat, plasma NPY or corticosterone. Differences were found in plasma leptin, urinary epinephrine and norepinephrine. It is likely that these were population differences, as the change from 1.0 G controls was similar and both 1.5 G groups.

Discussion

The literature suggests that under normal and non-pathological conditions, the leptin-NPY axis is essential for long-term regulation of food intake. Deficiencies in leptin that are due to mutations in the protein coding gene or in leptin's receptor prevent the negative feedback signal from being sensed in the hypothalamus (41). Consequently NPY increases and hyperphagia is observed. Exposure to hypergravity produces a reduction in plasma leptin. Rats exposed to hypergravity of different G loads lose fat and consequently leptin in a dose dependent manner (22). However, despite the low leptin levels, rats do not exhibit hyperphagia. This is presumably a result of normal NPY levels, as fluctuation in NPY would cause changes in food intake. The focus of the present study was to examine the response of NPY in the presence of low plasma leptin levels.

Neuropeptide Y is ubiquitous and therefore it is difficult to discern whether or not circulating levels are indicative of appetite control mechanisms. Previous data suggests that in laboratory rodents NPY is co-released with NE during stimulation of sympathetic nerves (27). However, Pernow et al showed that sympathetic nerve stimulation in dog gracilis muscle at 0.59, 2.0, and 6.9 Hz caused a significant overflow of NE into the vasculature. However a detectable overflow of NPY was observed only at an intensity of 6.9 Hz (29). In the present study, rats in the 1.5 and 2.0 G groups in experiment 1 and 2, exhibited a significant increase in urinary NE from 1.0 G controls, however the magnitude of the increase may not have been enough to evoke a similar release of NPY. Furthermore, the amount of NE released was dependent upon G load. If NPY were co-

released with NE it would follow a similar release pattern. Others have suggested that NPY is released in rats exposed to stressors such as foot shock and immobilization. However, NE increased while NPY levels remained constant (28). In addition, a stress response was not evident in the centrifuged animals, as plasma corticosterone levels remained similar to controls (Table 2). Thus a direct relationship between circulation NPY and increased sympathetic tone remains illusive.

Cells of the adrenal medulla are possible sources of NPY (42). NPY has been associated with ganglion nerves, adrenergic chromaffin tissue, and in the superficial adrenal cortex. The relationship between adrenal stimulation and NPY is unclear. For example, rats given chlorisondamine (a nicotinic antagonist of the nerves controlling the adrenal medulla), exhibited a significant decrease in circulating NE and E levels. However, circulating NPY levels increased (30). Moreover, adrenalectomized rats exhibit a reduction in plasma corticosterone and epinephrine and no change in plasma NPY (31). The pattern of E release in experiment 1 should show a corresponding release pattern of NPY, if NPY was derived from the adrenal medulla. This was not the case as NPY remained similar in each group. Furthermore there were no differences in E in experiment 2. These data suggest that extra-hypothalamic sources of NPY are minimum contributors to circulating NPY levels. Thus it is appropriate to consider that changes in circulating NPY would be indicative of hypothalamic release.

The similar NPY levels between each G load were expected, as food intake did not change. However, it is important to note that both Agouti Related Protein (AgRP) and α-Melanocyte Stimulating Hormone (α-MSH) are central modulators of appetite and

if NPY had changed both AgRP and α-MSH would be suspect. This was not the case. It is more likely that an alternate peripheral signal was active which suppressed the constitutive expression of NPY and prevented hyperphagia. These data indicate that the leptin-NPY system may not play a significant role in modulating appetite during exposure to hypergravity. Similarly, Bing et al showed that to sustain thermogenesis, cold-exposed rats increase sympathetic outflow to brown adipose, lose weight but experience compensatory hyperphagia. However, they did not observe any changes in hypothalamic NPY (33). It is likely that another central neurohormone controls appetite under these conditions. Similarly, when leptin levels are low, it is likely that another peripheral hormonal signal may overcome the deficiency in plasma leptin.

Other peripheral modulators of food intake include glucose, insulin, glucocortcoids, and catecholamines. For example, insulin and leptin work synergistically to inhibit food intake (34). There were no differences between the groups in plasma insulin, or glucose (data not shown). If these peripheral signals were going to compensate for a reduction in plasma leptin, it is likely that significant changes would have been observed. Glucocorticoids prevent leptin induced inhibition of food intake, and reverse decreases in food intake that are observed when exogenous leptin is provided (35, 36, 37). Less information is available regarding glucocorticoid mediated control of appetite in the absence of leptin. In addition, there were no differences in plasma corticosterone between centrifuge and control groups of experiments 1 and 2. Thus it is unlikely the corticosterone played a role in maintaining food intake. Catecholamines released in the periphery may have prevented hyperphagia, as both E and NE act

centrally to reduce food intake (38). It remains unclear what hormone is maintaining NPY in the absence of leptin and consequently preventing hyperphagia. In conclusion these data emphasize the complexity of the physiological circuitry which regulates food intake. Furthermore, future obesity research should be directed towards examining the hormonal control of appetite on a large scale as another peripheral signal may overcome effect of another.

References

1. Jequier, E, and Tappy, L. Regulation of body weight in humans. *Physiological Reviews* 79(2): 451-480, 1999.

2. Zhang, Y, Proenca, R, Maffei, M, Barone, M, Leopold, L, and Friedman, JM. Positional cloning of the mouse *ob* gene and its human homologue. *Nature* 372: 425-432, 1994.

3. Considine, RV, Considine, EL, Williams, CJ, Nyce, MR, Magosin, SA, Bauer, TL, Rosato, EL, Colberg, J, and Caro JF. Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. *J Clin Invest* 95: 2986-2988, 1995.

4. Considine, RV, Sinha, MK, Heiman, ML, Kriauchunas, A, Stephens, TW, Nyce, MR, Ohannesian, JP, Marco, CC, McKee, LJ, Bauer, TL, and Caro, JF. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *New England J Med* 334: 292-295, 1996.

5. Hamilton, BS, Paglia, D, Kwan, YM, and Deitel, M. Increased obese mRNA expression in omental fat cells from massively obese humans. *Nature Med* 1: 953-956, 1995.

6. Klein, S, Coppack, SW, Mohamed-Ali, V, and Landt, M. Adipose tissue leptin production and plasma leptin kinetics in humans. *Diabetes* 45: 984-987, 1996.

7. Maffei, M, Halaas, J, Ravussin, E, Pratley, RE, Lee, GH, Zhang, Y, Fei, H, Kim, S, Lallone, R, and Ranganatha, S. Leptin levels in human and rodent: measurements of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1(11): 1155-1161, 1995.

8. Tatemoto, K, Carlquist, M, and V Mutt. Neuropeptide-Y a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* 296(5858):659-60, 1982.

9. Allen, JM, Bircham, PMM, Bloom, SR, and Edwards, AV. Release of neuropeptide Y in response to splanchnic nerve stimulation in the conscious calf. *J Physiol* 357: 401-408, 1984.

10. Adrian, TE, Allen, JM, Bloom, SR, Ghatei, MA, Rossor, MN, Roberts, GW, Crow, TJ, Tatemoto K, and Polak JM. Neuropeptide Y distribution in human brain. *Nature* 306: 584-586, 1983.

11. Lundberg, JM, Franco-Cereceda, A, Hemsen, A, Lacroix, JS, and Pernow, J. Pharmacology of noradrenaline and neuropeptide tyrosine (NPY)-mediated sympathetic cotransmission. *Fundam Clin Pharmacol* 4: 373-391, 1990.

12. Malmstrom, RE, and Lundberg, JM. Neuropeptide Y in sympathetic nerves-evidence for Y_1 receptor mediated vascular control. In Neuropeptide Y and Drug Development. Academic Press, San Diego, 1997, p41-55.

13. Lundberg, JM, Terenius, L, Hokefelt, T, and Goldstein, M. High levels of neuropeptide y in peripheral noradrenergic neurons in various mammals including man. *Neurosci Lett* 42: 167-172, 1983.

14. Lundbeg, JM, Terenius, L, Hokfelt, T, Martling CR, Tatemoto, K, Mutt, V, Polak, J, Bloom, S, and Goldstein, M. NeuropeptideY (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. *Acta Physiol Scand* 116: 477-480, 1982.

15. Lundberg, JM, and Tatemoto, K. Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistance to α-adrenoreceptor blockade. *Acta Physio Scand* 116: 393-402, 1982.

16. Lundberg, JM, Hua, X-Y, and Franco-Cereceda, A. Effects of neuropeptide Y (NPY) on mechanical activity and neurotransmission in the heart, vas deferens and urinary bladder of the guinea-pig. *Acta Physiol Scand* 121: 325-332, 1984.

17. Higuchi, H, Costa, E, and Yang, HYT. Neuropeptide Y inhibits the nicotine-mediated release of catecholamines from bovine adrenal chromaffin cells. *J Pharmacol Exp Therap* 244(2): 468-74, 1988.

18. Leibel, RL, Rosenbaum, M, and Hirsch, J. Changes in energy expenditure resulting from altered body weight. *New England J Med* 332: 621-628, 1995.

19. Keil, LC. Changes in growth and body composition of mice exposed to chronic centrifugation. *Growth* 33: 83-88, 1969.

20. Oyama, J, and Platt WT. Effects of prolonged centrifugation on growth and organ development of rats. *Am J Physiol* 209: 611-615, 1965.

21. Pitts, GC, Bull, LS, and Oyama J. Effect of chronic centrifugation on body composition in the rat. *Am J Physiol* 223:1044-1048, 1972.

22. Moran, MM, Stein, TP, and Wade, CE. Peripheral signals of food intake in response to low leptin levels induced by centrifugation. *Submitted to J Appl Physiol (in review)*, May 18, 2000.

23. Katovich, MJ, and Smith, AH. Body mass, composition, and food intake in rabbits during altered acceleration fields. *J Appl Physiol: Respirat Environ Exercise Physiol* 45(1): 51-55, 1978.

24. Ortiz, RM, and Wade, CE. Water balance in rats exposed to chronic centrifugation. *J Appl Physio l* 89: *(in press)*.

25. Schwartz, MW, Baskin, DG, Bukowski, TR, Kuijper, JL, Foster, D, Lasser, G, Prunkard, DE, Porte, D Jr, Woods, SC, Seeley, RJ, Weigle, DS. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. *Diabetes* 45: 531-535, 1996.

26. Erikson, JC, Hollopeter, G, and Palmiter, RD. Attenuation of the obesity syndrome of *ob/ob* mice by the loss of neuropeptide Y. *Science* 274: 1704-1707, 1996.

27. Lundberg, JM, Torssell, L, Sollevi, A, Pernow, J, Theodorsson Norheim, E, Anggard, A, Hamberger, B. Neuropeptide Y and sympathetic vascular control in man. *Regul Pept* 13(1):41-52, 1985.

28. Zukowska-Grojec, Z, Konarska, M, and McCarty, R. Differential plasma catecholamine and neuropeptide Y responses to acute stress in rats. *Life Sciences* 42: 1615-1624, 1988.

29. Pernow, J, Schwieler, J, Kahan, T, Hjemdahl, P, Oberle, J, Wallin, BG, Lundberg, JM. Influence of sympathetic discharge pattern on norepinephrine and neuropeptide Y release. *Am J Physiol* 257(3 Pt 2): H866-72, 1989.

30. Laborie, C, Bernet, F, Dutriez-Casteloot, I, Lesage, J, Dupouy, JP. Effect of cholinergic blockade on glucocorticoid regulation of NPY and catecholamines in the rat adrenal gland. *Neuroendocrinology* A66(2): 98-105, 1997.

31. Bernet F, Dedieu, JF, Laborie, C, Montel, V, Dupouy, JP. Circulating neuropeptide Y (NPY) and catecholamines in rat under resting and stress conditions. Arguments for extra-adrenal origin of NPY, adrenal and extra-adrenal sources of catecholamines. *Neurosci Lett* 250(1): 45-8, 1998.

32. Eisen, EJ, and Leatherwood JM. Predicting percent fat in mice. *Growth* 45(2): 100-107, 1981.

33. Bing, C, Frankish, HM, Pickavance, L, Wang, Q, Hopkins, DFC, Stock, MJ, and Williams, G. Hyperphagia in cold-exposed rats is accompanied by decreased plasma leptin but unchanged hypothalamic NPY. *Am J Physiol* 274(43): R62-R68, 1998.

34. Mizuno, TM, Bergen, H, Funabashi, T, Kleopoulos, SP, Zhong, YG, Bauman, WA, Mobbs, CV. Obese gene expression: reduction by fasting and stimulation by insulin and glucose in lean mice, and persistent elevation in acquired (diet-induced) and genetic (yellow agouti) obesity. *Proc Natl Acad Sci U S A* 93(8): 3434-8, 1996.

35. Dallman, MF, Strack, AM, Akana, SF, Bradbury, MJ, Hanson, ES, Scribner, KA, and Smith, M. *Frontiers in Neuroendocrinology*, New York, Raven, 1993, p 303-347.

36. Tempel DL, Leibowitz SF. Adrenal steroid receptors: interactions with brain neuropeptide systems in relation to nutrient intake and metabolism. *J Neuroendocrinol* 6(5): 479-501, 1994.

37. Solano JM, Jacobson L. Glucocorticoids reverse leptin effects on food intake and body fat in mice without increasing NPY mRNA. *Am J Physiol* 277(4 Pt 1): E708-16, 1999.

38. Bray, GA, York, DA. The MONA LISA hypothesis in the time of leptin. *Recent Prog Horm Res* 53:95-117; discussion 117-8, 1998.

39. Warren, LE, Horwitz, BA, and Fuller CA. Gravity and body mass regulation. *J Grav Physiol* 4(2): 89-92, 1997.

40. Wang, YF, Mao, YK, Xiao, Q, Daniel, EE, Borkowski, KR, and McDonald, TJ. The distribution of NPY-containing nerves and the catecholamine contents of canine enteric nerve plexuses. *Peptides* 18(2): 221-34, 1997.

41. Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, et al The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 377(6549): 530-2, 1995.

42. Maubert, E, Tramu, G, Croix, D, Beauvillain, JC, and Dupouy, JP. Co-localization of vasoactive intestinal polypeptide and neuropeptide Y immunoreactivities in the nerve fibers of the rat adrenal gland. *Neurosci Lett* 113: 121-126, 1990.

43. Wade, CE, Hannon, JP, Bossone, CA, Hunt, MM, Loveday, JA, Coppes, RI Jr, Gildengorin, VL. Neuroendocrine responses to hypertonic saline/dextran resuscitation following hemorrhage. *Circ Shock* 35(1): 37-43, 1991.