Hormonal Modulation of Food Intake in Response to Low Leptin Levels Induced by Hypergravity

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A loss in fat mass is a common response to centrifugation and it results in low circulating leptin concentrations. However, rats adapted to hypergravity are euphagic. The focus of this study was to examine leptin and other peripheral signals of energy balance in the presence of a hypergravity-induced loss of fat mass and euphagia. Male Sprague-Dawley rats were centrifuged for 14 days at gravity levels of 1.25, 1.5, or 2 G, or they remained stationary at 1 G. Urinary catecholamines, urinary corticosterone, food intake, and body mass were measured on Days 11 to 14. Plasma hormones and epididymal fat pad mass were measured on Day 14. Mean body mass of the 1.25, 1.5, and 2 G groups were significantly ($P < 0.05$) lower than controls, and no differences were found in food intake (g/day/100 g body mass) between the hypergravity groups and controls. Epididymal fat mass was 14%, 14%, and 21% lower than controls in the 1.25, 1.5, and 2.0 G groups, respectively. Plasma leptin was significantly reduced from controls by 46%, 45%, and 65% in the 1.25, 1.5, and 2 G groups, respectively. Plasma insulin was significantly lower in the 1.25, 1.5, and 2.0 G groups than controls by 35%, 38%, and 33%. No differences were found between controls and hypergravity groups in urinary corticosterone. Mean urinary epinephrine was significantly higher in the 1.5 and 2.0 G groups than in controls. Mean urinary norepinephrine was significantly higher in the 1.25, 1.5 and 2.0 G groups than in controls. Significant correlations were found between G load and body mass, fat mass, leptin, urinary epinephrine, and norepinephrine. During hypergravity exposure, maintenance of food intake is the result of a complex relationship between multiple pathways, which abates the importance of leptin as a primary signal. [Exp Biol Med Vol. 226(8):740–745, 2001]

Key words: centrifugation; catecholamines; body mass regulation; epididymal fat

The recently discovered hormone leptin has become a focal point of obesity research. In brief, when leptin levels are elevated, neuropeptide Y (NPY) and agouti-related protein (AgRP) production are inhibited, and at the same time, alpha-melanocyte-stimulating hormone (α-MSH) production increases, causing satiety (1, 2). When leptin levels are low, NPY and AgRP increase, inhibiting α-MSH from binding, and food intake increases (3). Thus, when the satiety is not perceived by the hypothalamus due to low leptin levels or an inability to receive leptin's signal, hyperphagia and obesity ensue (4). Despite these findings, control of food intake remains a complex process and is a function of many peripheral hormonal signals that are integrated within the hypothalamus. Although leptin acts to monitor the status of body energy reserves, the magnitude of its importance in regulating body weight and composition remains unclear.

Centrifugation is a unique tool for studying body weight. To understand how centrifugation can be used to study regulation of body weight, it is important to define the difference between body weight and body mass: ($F = m \times a$) where F = body weight, m = body mass, and a = acceleration due to gravity. In a 1 G (terrestrial) environment, body weight is a function of the mass of the subject and the force of gravity (1 G), and thus, body weight is equal to body mass. During centrifugation at 2 G (hypergravity), 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. Thus, at 2 G, body weight is doubled. In addition, centrifugation allows body weight to be increased immediately without manipulating food intake and/or activity. At 1 G, increases in food intake exceeding the amount of energy that is expended through exercise and/or activity causes body weight and fat mass to increase. However, hypergravity exposure results in a reduction in fat mass and transient reduction in food intake (5–7). The reduction in fat mass was expected to cause a concomitant reduction in circulating leptin and hyperphagia. The pur-

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pose of the present study was to examine the interaction of 14 days of hypergravity and low leptin levels on other peripheral modulators of food intake, including glucose, insulin, corticosterone, epinephrine, and norepinephrine.

Materials and 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 study conforms to NASA's *Animal Users Guide* and the National Research Council guidelines for animal experimentation.

Study Design. The experiment was conducted using 1.5-month-old, male Sprague-Dawley-derived albino rats (Simonsen Laboratories, Gilroy, CA). Upon receipt from the vendor, each rat was weighed and housed (1 rat/cage) in standard vivarium cages for a 3-day acclimation period. The acclimation period was followed by surgery in which a temperature and activity telemeter was placed intraperitoneally (data not shown). Surgery was followed by a 7-day surgical recovery period, a 7-day baseline data collection period, and a 14-day test period of either centrifugation at 2.0 G or a stationary 1.0 G environment. The centrifuge can accommodate two different G-loads at a time; thus, the study was conducted in two experiments and data from similar groups were combined. Rats were centrifuged at 1.25 (n = 8), 1.5 (n = 16), 2.0 (n = 8), or remained stationary as 1 G controls (n = 16). Throughout the study, the rats were maintained on a12:12-hr light:dark cycle (0600 hr on:1800 hr off). Room temperature was maintained at 23° ± 2°C. Animals were fed a powdered diet (Purina Rat Chow no. 5012). Food and water were provided *ad libitum*. The centrifuge was stopped daily at 0800 hr for data collection and animal health checks that lasted for 45 min.

During the baseline and test periods, rats were housed (1 rat/cage) in metabolic cages (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. Water bottle lix-its were modified to prevent dripping during the starting and stopping of the centrifuge. Control rats were housed in the same room as the centrifuge rats.

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 laboratory, the samples were weighed (scale tared for the weight of the oil), the oil was removed, and the samples were centrifuged, aliquoted, and frozen at –20°C. Corticosterone was extracted from the urine (8) and was assayed using double antibody radioimmunoassay (RIA) kits (ICN Biomedicals, Costa Mesa, CA). The sensitivity of the corticosterone assay was 12.5 pg/ml at the lowest standard level, intraassay variability was 7.1%, and the interassay variability was 6.5%. Urine catecholamine analysis was performed on pooled samples that were collected daily from each rat on Days 11 to 14 of the experiment. The urine was acidified after collection. Sample analysis was performed by HPLC (DIONEX, Santa Clara, CA). Catecholamine and corticosterone excretion rates were the product of the concentration multiplied by the mean volume excreted during a 24-hr period. To correct for possible differences between the groups in glomerular filtration rates, urine data were expressed relative to 24-hr urine creatinine excretions. Urine creatinine was measured by clinical chemistry analyzer (Cobas Mira-L, Roche Diagnostic Systems, Inc., Branchburg, NJ).

Dissection. A dissection was performed during the lights-on period of the lighting cycle on Day 14 of the test period. Rats are nocturnal and consume most of their food during the dark period of the lighting cycle (9–11). The dissection started 3 hr after the lights went on, and 6 hr transpired between the dissection of the first rat and the last rat. The dissection order was planned to prevent rats from the same group being dissected simultaneously. Thus, any effect of digestion on plasma hormones would have been similar between the groups. The rats were anesthetized with isoflurane and were sacrificed by decapitation. Prior to decapitation, blood was collected by cardiac puncture, kept on ice, centrifuged, and frozen for further analysis. Bilateral epididymal fat pads were collected and weighed. Epididymal fat pad weights were used as an index of total body fat, as previous data collected in our laboratory has shown a high correlation between epididymal fat pad weight and total body fat in rats (r = 0.893, n = 29, $P < 0.001$). In addition, this technique has been proven a successful indicator of percent body fat in other rodents (12).

Plasma Hormones. Plasma glucose was measure with an automated clinical chemistry analyzer (Cobas Mira-L, Roche Diagnostic Systems, Inc.). Commercial RIA kits were used to measure plasma leptin (ALPCO, Windham, NH) and insulin (Diagnostic Products, Inc., Los Angeles, CA). For leptin, intraassay variability was less than 5% and interassay variability was less than 8%. The sensitivity of the plasma leptin assay was 0.6 pg/ml. The sensitivity of the insulin assay was 9.3 pM/L and both the intra- and interassay variability were less than 10%.

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 among experiments, data were expressed as covariants of the controls from the original experiment. When significant differences were observed between hypergravity and control groups, linear regression analyses were performed using mean data (13).

Results

Body Mass. There were no differences in body mass between the groups during the baseline period (Table 1).

Table I. Body Mass, Food Intake, and Fat Pad Mass

	1 G	1.25 G	1.5 G	2 G
Number of animals (*n*)	16	8	16	8
Baseline body mass[a] (g)	242 ± 2.2	236 ± 2.0	243 ± 1.4	242 ± 2.2
Final body mass[b] (g)	314 ± 6.0	287 ± 3.9[f]	290 ± 2.6[f]	279 ± 3.2[f]
Final body weight[c] (g)	314 ± 6.0	358 ± 4.8[g]	435 ± 3.9[g]	558 ± 6.4[g]
Food intake[d] (g/body mass $^{2/3}$)	0.53 ± 0.01	0.52 ± 0.01	0.54 ± 0.01	0.52 ± 0.01
Epididymal fat pads[e] (g/100 g body mass)	0.91 ± 0.02	0.80 ± 0.04[f]	0.78 ± 0.02[f]	0.69 ± 0.05[f,h]

[a] Mean body mass from last 3 days of the baseline period. Note that when the acceleration due to gravity (*A*) is equal to 1 G, body weight (*F*) is equal to body mass ($F = MA$).
[b] Days 11–14, body mass mean values ± SE.
[c] Days 11–14, body mass multiplied by G level. Values are group means ± SE.
[d] Days 11–14, food intake mean values ± SE.
[e] Day 14, epididymal fat pad mass mean values ± SE.
[f] <1.0 G.
[g] >1.0 G.
[h] <1.25 G.
Significance $P \leq 0.05$.

However, during Days 11 through 14, body mass was significantly lower in the hypergravity groups than controls (Table I). When covariant body mass data were plotted as a function of G load, a significant linear and negative correlation was observed ($r = 0.9657$; Fig. 1A).

Food Consumption. Due to the differences in body mass between the groups, food intake was compared relative to body mass scaled to the two-thirds power. No differences were found in food intake on Days 11 through 14 of the experiment between the 1.25, 1.5. 2.0, and 1.0 G groups (Table I).

Epididymal Fat Pad, and Plasma Leptin. Fat pads were compared relative to body mass. Relative epididymal fat pad mass was significantly less in the 1.25, 1.5, and 2.0 G groups than in controls (Table I). Plasma leptin followed a similar response, as levels were lower in the 1.25, 1.5, and 2.0 G groups than in the controls (Table II). A positive exponential correlation between plasma leptin and epididymal fat pad mass was observed ($r = 0.628$; Fig. 2).

Both epididymal fat pad mass and plasma leptin were plotted as covariant data against G load. Epididymal fat pad mass was negatively correlated with G load ($r = 0.905$; Fig. 1B). Similarly, a significant negative correlation was observed between plasma leptin and G load ($r = 0.857$; Fig. 1C).

Plasma Analysis. Similar plasma glucose levels were found in each of the centrifuge groups and controls (Table II). However, plasma insulin was significantly lower in the 1.25, 1.5, and 2.0 G groups than in controls (Table II). Despite the lower insulin levels in each of the centrifuge groups, a positive linear correlation with G load was not observed.

Urinary Corticosterone. During the last 4 days of hypergravity, 24-hr urinary corticosterone levels were compared relative to 24-hr creatinine excretions. Similar corticosterone levels were observed in each of the three hypergravity groups and the 1.0 G controls (Table II).

Urinary Catecholamines. Urinary catecholamines were also compared relative to creatinine excretion rates. Epinephrine levels were significantly elevated above controls in both the 1.5 and 2.0 G groups (Table II). Greater differences were observed in 24-hr urinary norepinephrine levels, as the 1.25, 1.5, and 2.0 G norepinephrine levels were greater than control norepinephrine levels. In addition, urinary norepinephrine was significantly higher in the 2.0 G group than the 1.25 and 1.5 G groups (Table II). Urinary epinephrine and norepinephrine levels were plotted as covariants against G load, and significant positive linear correlations were observed. Both epinephrine ($r = 0.968$; Fig. 2D) and norepinephrine ($r = 0.977$; Fig. 2E) levels increased with gravity level.

Discussion

Leptin is a product of adipocytes, and circulating levels are a function of fat mass (14). The reduction in circulating leptin with increased gravity was expected, as fat loss is a common response to centrifugation (7, 15). A reduction in circulating leptin would be expected to cause hyperphagia, as leptin has an inhibitory effect on food intake. This was not the case, as rats exposed to hypergravity with free access to food remained euphagic. We recognize that food intake is the result of a complex interaction of different peripheral hormonal signals. Therefore, we examined selected peripheral hormones in the presence of low leptin levels and determined how these hormones respond at different gravitational loads.

To reiterate the importance and uniqueness of increased gravity as a tool for studying control of energy balance, one must consider that body weight is increased immediately without manipulating food intake and/or activity. In addition, one must be able to differentiate between mass and weight. In a 1 G environment, body weight can be used interchangeably with body mass. However, when hypergravity is applied to a system, body mass and body weight are distinct. Body weight on the centrifuge is a function of the mass of the subject and the increase G load that is applied by centrifugation. Thus, for the purpose of this study, body mass is used in place of body weight.

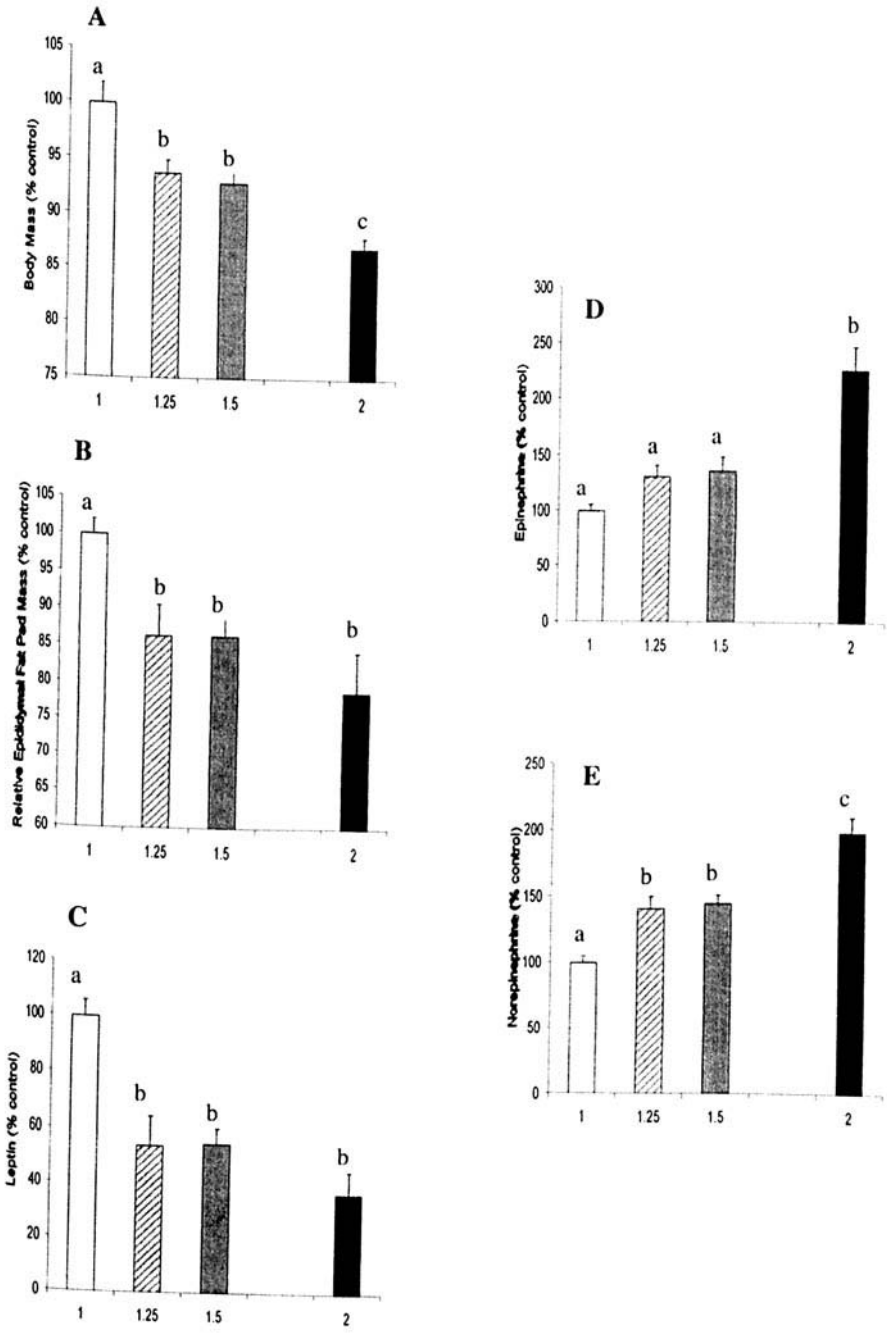

Figure 1. Regression analysis comparing G load. (A) Body mass. (B) Epididymal fat pad mass. (C) Plasma leptin. (D) Urine epinephrine. (E) Urine norepinephrine. Values are covariants. Significant statistical differences are by letters (a, b, or c).

Transient decreases in food intake and body mass were observed and expected in each of the centrifuge groups (16–19). However, following acclimation, centrifuged rats gained mass at a rate similar to controls (data not shown), but body mass remained lower than controls for the remainder of the experiment. We expect that the loss of body mass was due to a loss of body fat and that lipolysis was triggered by a rise in circulating catecholamines and corticosterone. Centrifugation at 2 G evokes a stress response that results in a transient elevation in circulating corticosterone, which returns to control levels after 5 days (8). Furthermore, similar studies have shown that the enzymes involved in fat storage and nonshivering thermogenesis are unaffected by chronic 2 G exposure (15).

Catecholamines play significant roles in regulating energy intake. Urinary norepinephrine levels in the 1.25, 1.5, and 2.0 G groups were significantly higher than in controls. The actions of norepinephrine in the modulation of food intake are 2-fold. At the peripheral level, norepinephrine binds with adipocyte β3-adrenergic receptors (β3-AR) and inhibits leptin gene expression. Despite the inhibition of leptin expression, norepinephrine inhibits food intake by a pathway that is independent of leptin (20, 21). Similarly, epinephrine causes a reduction in food intake (22). Significant correlations were found between G load and urinary catecholamine levels (Fig. 1, D and E). It is possible that the increases in both norepinephrine and epinephrine prevented hyperphagia when plasma leptin was reduced, and empha-

Table II. Plasma and Urinary Hormones

	1 G	1.25 G	1.5 G	2.0 G
Plasma Leptin (ng/ml)[a]	5.2 ± 0.6	1.9 ± 0.4[b]	2.9 ± 0.4[b]	2.6 ± 0.6[b]
Plasma Glucose (mg/dl)[a]	168 ± 4.0	195 ± 2.8	179 ± 4.0	160 ± 2.8
Plasma Insulin (picoMoles/L)[a]	81.8 ± 6.5	59.6 ± 5.0[b]	51.7 ± 2.9[b]	48.1 ± 5.0[b]
Urinary Corticosterone[c] (ng/mg creatinine)	26.4 ± 2.9	25.9 ± 2.5	26.8 ± 2.1	25.3 ± 1.9
Urinary Epinephrine[c] (pmol/mg creatinine)	133 ± 7	185 ± 14	179 ± 15[d]	286 ± 27[d]
Urinary norepinephrine[c] (pmol/mg creatinine)	467 ± 22[e]	632 ± 41[e]	681 ± 33[d,e]	961 ± 56[d]

[a] Day 14, plasma hormones values are group means ± SE.
[b] <1.0 G.
[c] Days 11–14, mean 24-hr urinary corticosterone and catecholamines. Data were corrected for creatinine and are group means ± SE.
[d] >1.0 G.
[e] <2.0 G.
Significance $P \leq 0.05$.

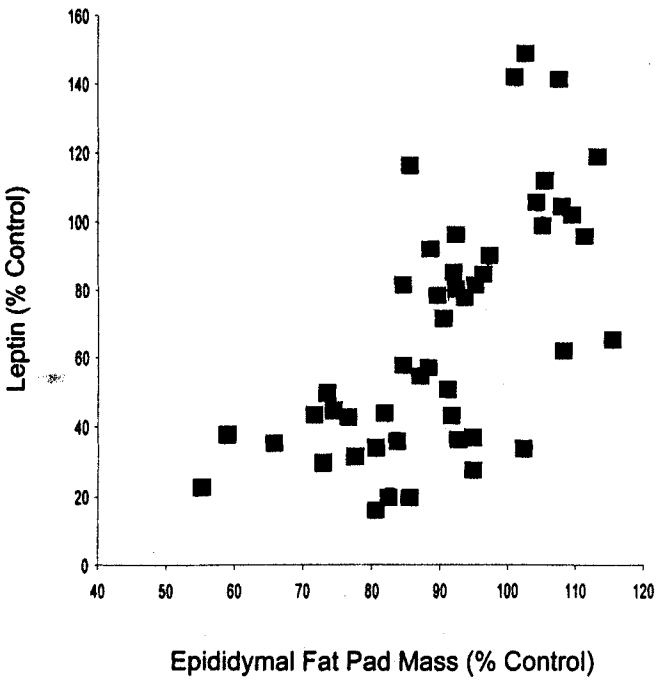

Figure 2. Regression analysis comparing plasma leptin concentration and fat pad mass. Values are covariants.

sizes the importance of catecholamines in achieving a new steady state during exposure to altered environments.

Corticosterone may inhibit food intake (23–25). However, no differences were found in corticosterone levels between the 1.0, 1.25, 1.5, and 2.0 G groups. Thus, it is unlikely that corticosterone played a role in preventing hyperphagia.

Circulating insulin levels are depressed in rats exposed to hypergravity, thus our results were expected (26). Although leptin and insulin act in a similar manner to inhibit food intake (27), a reduction in circulating insulin levels in hypergravity-exposed rats would not cause hyperphagia. Rats exposed to hypergravity exhibit an increase in insulin sensitivity and glucose uptake in skeletal muscles (26, 28). Thus, glucose is readily available to tissues, and subsequently, hypergravity rats remain euphagic.

The gravity-dependent responses observed in this study were particularly interesting, as the data demonstrated the plasticity of the energy balance system. Incremental increases in weight cause proportional alterations in the magnitude of the hormonal signal. Moreover, the similarities between the 1.25 and 1.5 G groups suggest that stimulation of 1.5 G or greater is required to clearly delineate a hormonal response. Note that the data were collected from young rats that were growing. It remains unclear whether similar hormonal changes would be found in older rats.

In conclusion, rats exposed to gravity levels ranging from 1.25 to 2.0 G lose fat and plasma leptin, but remain euphagic. It appears that euphagia was a result of other afferent signal pathways, such as the binding of catecholamines to β3-AR and possibly enhanced insulin sensitivity. Furthermore, increases in gravity level lead to a concomitant change in the magnitude of the hormonal signal. Thus, when body weight is increased by hypergravity exposure, the peripheral system that regulates energy intake remains intact.

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