# Increased Body Weight Reduces

# Voluntary Movement to Maintain Energy Expenditure

# of Rats Exposed to Increases in Gravity

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Running Head: Body Weight Modulation of Movement

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With the increase in obesity related diseases there is heightened interest in mechanisms regulating body weight. To assess the influence of increases in body weight on energy expenditure and intake in rats we employed variable levels of gravity. Our approach afforded the means to measure interactions of energy expenditure and intake in response to increases in body weight (body mass x gravity level). We found a dose relationship between rapid elevation of body weight and reduction of voluntary movement, such that the energy requirements for activity are unchanged, and total energy expenditure and intake maintained. Reduction of movement appears to be a response to increased body weight, rather than a contributing factor, suggesting a new regulatory pathway.

Maintenance of body weight in humans and animals is tightly regulated in normal adults. The balance of total energy expenditure and intake is essential for the regulation of body weight. This balance is attained by a combination of physiological and behavioral adjustments. To understand the interactions of energy expenditure and intake on body weight, previous investigators have manipulated these components independently. In response to a forced increase in body weight, induced by raising energy intake, there is an increase in total energy expenditure attempting to rectify body weight (1, 2). The increase in energy expenditure is predominately due to an increase in spontaneous physical activity. With reductions in energy intake opposite responses occur. Investigators have also decreased activity levels causing a lower total energy expenditure (3). This

maneuver results in a decrease in energy intake to sustain body weight. An increase in activity elicits the opposite response (4). Thus, from studies of individual components, a highly integrated and redundant regulatory system has been proposed that maintains body weight within narrow limits over a prolonged period of time.

Obesity affects a large percentage of the population, and the associated diseases (diabetes, hypertension, and cardiovascular disease) are major public health concerns (5, 6). In most cases these diseases are associated with inactivity as well (7, 8). Voluntary movement is reduced in animal models of obesity (9, 10, 11), in contrast to the reduction noted when body weight is reduced by restriction of energy intake (12, 13). In obesity models correction of the disorder contributing to the abnormal elevation in body weight leads to an increase in activity. These conflicting responses suggest independent pathways for the integration of spontaneous movement with changes in energy intake or body weight.

The use of centrifugation, altering the gravity load placed on an animal, has been suggested as a possible tool to investigate the control of body weight (14, 15). Body weight is the product of the body mass of the animal and the gravity field to which it is exposed. In the normal environment an animal is exposed to a 1.0 G gravity load, thus body mass equals body weight. An increase in the gravity field to 2.0 G doubles the body weight of the animal. Rats have been shown to effectively acclimate to gravity levels greater than 2.0 G (14, 16, 17). In response to alterations in gravity rats rapidly adjust body mass establishing a new steady state, and demonstrate that this new body mass is

tightly regulated. The use of centrifugation to increase gravity therefore allows body weight to be directly manipulated, and the responses and interactions of total energy expenditure, activity and energy intake to be studied.

To investigate the interaction of total energy expenditure, energy intake and activity in response to increases in body weight we studied young growing Sprauge-Dawley derived male rats acclimated to varying gravity levels up to 2.0 G, thus increasing body weight by as much as a factor of two (18). We hypothesized that an increase in body weight induced by increasing gravity, would reduce body mass, an increase energy expenditure and intake and no change in the level of activity. Further the magnitude of these changes would be dose related to the level of gravity to which the animals were exposed.

In response to increases in gravity to 1.25, 1.5 and 2.0 G there was a reduction in body mass compared to control animals at 1.0 G (Fig. 1A). As a percentage of baseline, body mass prior to experimental manipulation, there was decrease with an overall effect of gravity level [$F_{(3, 44)} = 56.61$; $P < 0.0001$] and time [$F_{(13, 572)} = 607.13$; $P < 0.0001$], along with a significant group time interaction [$F_{(39, 572)} = 6.37$; $P < 0.0001$] (19). The reduction in body mass was associated with a failure to initially gain mass at a rate equivalent to that of controls, and a decrease in fat mass (16, 17). The decrease in body mass was initially due to inhibition of feeding (Fig. 1B). For food intake there was an overall effect of gravity level [$F_{(3, 44)} = 4.62$; $P = 0.0068$] and time [$F_{(13, 572)} = 43.03$; $P < 0.0001$], along with a significant group time interaction [$F_{(39, 572)} = 17.17$; $P < 0.0001$]. Food intake recovered within the 96 hours of the onset of the increase in

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gravity level. There were also transient reductions in water intake and urine output, but no change in total fluid balance (20). Following this initial acclimation period animals establish a new body weight that is tightly maintained. The mean rate of gain in body mass over days 8 -14 at 1.25 G was greater than the 1.0 G [F (3, 44) = 3.59; P= 0.0210], but was similar to that of the other groups (Fig. 1C). Upon dissection on day 14 there was a significant reduction in epididymal fat pad mass indicative of a reduction in total body fat [F (3, 44) = 8.65; P< 0.0001] (Fig. 1D) (21). Both increases and decreases in gravity levels, produce prompt alteration of energy intake to establish a new body mass (14, 16, 17). During centrifugation in response to food restriction there was a loss of mass, which upon re-alimentation was rapidly corrected to the previous body mass by increased intake. We found with imposed increases in gravity, once the animal is acclimated, body mass is tightly regulated at a reduced level. Measurements of the effect of increased body weight were therefore assessed over days 8 to 14. Though body mass was reduced compared to 1.0 G controls, with increasing gravity there was a dose related increase in body weight (BW=BM x G-level) [F (3, 44) = 363.70; P< 0.0001] (Fig 1E).

We found increasing body weight by exposure to elevated gravity levels to not dramatically alter total energy expenditure or intake. Total energy intake was not significantly different between gravity levels (Fig. 2A). Average total energy expenditure was measured by the doubly labeled water method over seven days, days 8-14 (22). A small, but significant, increase in energy expenditure was noted for animals at 2.0 G compared to 1.0 G controls [F (3, 43) = 3.47; P= 0.024] (Fig. 2B). There was no significant difference in mean body temperature (18) between treatments over days 8-14

(37. 5 ± 0.03 $^0$C) (9). Energy balance was not significantly altered until animals were exposed to a gravity level of 2.0 G [F (3, 43) = 3.19; P= 0.033] (Fig. 2C).

Energy expenditure due to activity is the most labile component of total energy expenditure (5). Vacillation of energy expenditure due to activity may be reflective of its importance in the maintenance of energy balance and thus body weight. With the onset of exposure to increased gravity there was a pronounced reduction in movement (Fig 3A) (18). For movement expressed as a percent of baseline levels there was a reduction with an overall effect of gravity level [F (3, 42) = 29.11; P< 0.0001] and time [F (13, 546) = 14.61; P< 0.0001], along with a significant group time interaction [F (39, 546) = 4.34; P< 0.0001]. Within 96 hours the amount of movement stabilized with the change over time similar between groups. Over days 8-14 there was a reduction in voluntary movement related to increasing gravity levels compared to baseline values [F (3, 43) = 12.35; P< 0.0001] (Fig. 3B). The reduction in movement has been shown to persist for over 50 days in rats exposed to 2.0 G (23). The proportional reduction in voluntary movement from baseline levels was inversely related to the percent increase in body weight from baseline (Fig. 3C). We equated the energy cost of activity to be proportional to the relative amount of movement times relative body weight (energy cost of activity = movement x BW). We assumed no change in work efficiency of the rats to the increase in body weight. At varying gravity levels the reduction in movement coupled with the increase in body weight led to no significant change in the energy cost of activity. These adjustments defend energy balance and suggest this is the variable being regulated rather than body weight.

The reduction in voluntary movement in response to an increase in body weight in the present study is in contrast to the increase in activity when body weight is increased by involuntary caloric supplementation, or to the decrease of activity with reductions in body weight due to caloric restriction (1, 2, 12, 13). Further these alterations induce changes in energy expenditure to maintain body mass. In the present study there was no dramatic difference in total energy expenditure or intake in an attempt to rectify body weight.

In obese individuals there is a decrease in the level of spontaneous movement that is postulated to be a contributing factor to the increase in body weight due to reduced activity related energy expenditure (24). The present study would suggest that the reduction in movement is a result of the increase in body weight. This is supported by studies in which absolute energy expenditure associated with activity is similar among groups with varying body weights (25). Further, during exercise in obese subjects when adjusted for body weight there is no difference in energy requirements at a given workload (26). Thus, a decrease in movement maybe the result of the increase in body weights rather than a contributing factor in the etiology of obesity.

Obesity in most instances has been associated with a decrease in movement (9, 10, 12). This would correspond to our finding of a reduction in movement with increased body weight. The obese individual would reduce movement such that energy cost of activity would be constant. As noted these observations are in contrast to the increase in body mass by forced overfeeding where movement is increased. These disparate findings

suggest that two independent regulatory pathways may mediate the extent of movement. One-pathway responds to acute alterations in energy intake and stores, and the other pathway responds to changes in body weight. Thus, the association of inactivity with obesity may occur due to an alteration in the response to feeding behavior, or represent the change in body weight modulating the level of movement.

We recognize that the ability to reduce voluntary movement to maintain energy balance is limited. There is a minimal level of movement necessary to sustain life. When this level is attained, in response to increases in gravity level, and thus body weight, there would have to be an increase in energy expenditure. We may have approached the level of minimal movement, as there is a trend for an increase in energy requirements at the highest gravity level of 2.0 G. It is interesting to note previous studies demonstrating increases in energy intake and expenditure in response to increased gravity levels are at body weights greater than those in the present study (14, 27).

With the increase of body weight by centrifugation other factors may contribute to the reduction in voluntary movement. These include the Coriolis effect of rotation, and the reduction in body fat energy reserves (15). These factors appear to be minimal as the control animals at 1.0 G showed similar changes in voluntary movement in the face of increased body weight due to growth. Over days 8 to 14 of the experiment the control animals at 1.0 G increased body weight from baseline values to $127 \pm 4$ % (Fig. 1) and reduced movement to $80 \pm 4$ % (Fig 2). These animals were not rotated and are assumed to be increasing body fat during maturation. Their response is similar to the changes induced in animals exposed to centrifugation suggesting that factors, other than increased body weight, are of minor influence.

Our study is the first to investigate the effects of direct alterations in body weight (body mass x gravity level), on interactions of energy expenditure and intake. In contrast to previous studies manipulating caloric intake or activity (1, 2, 12, 13), increases in body weight yielded minimal change in energy expenditure or intake. Energy balance was maintained by a reduction in spontaneous movement proportional to the increase in body weight, such that the energy cost of activity was not altered. These findings are similar to the reduction in activity noted in obese animals. Our study does not support the concept of a "body weight set point" (28, 29). Increases in body weight to almost double that of control animals at 1.0 G did not result in pronounced changes in energy expenditure or intake. It appears there is an "energy balance set point", of which the primary defense mechanism in response to an alteration in body weight is modulation of voluntary movement such that the energy cost of activity is preserved. Further, there appears to be an independent pathway for the interaction of body weight and movement that may offer a means for intervention in the management of body weight disorders.

Reduction in the level of exercise is postulated to be a primary contributor to the recent increase in incidence obesity (5, 6). We suggest, from the present findings, that the decrease in exercise level may be a function of the increase in body weight, rather than a contributing factor to the occurrence of obesity.

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18. 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. 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 three day acclimation period. Food and water were provided *ad libitum*. Throughout the study the rats were maintained on a12:12-hour light dark cycle (06:00 on:18:00 off). Room temperature was maintained at $23 \pm 2°C$. The acclimation period was followed by surgery to implant a telemeter (Data Sciences Inc., St. Paul, MN) into each rats' abdomen for measurement of body temperature and movement (M.M. Moran et al. *J. Appl. Physiol.* **85**, 1564 (1998)). Movement was measured by changes in the strength of the telemetry signal as the animal moved in the cage. For example, for any distance the animal moved a digital pulse was generated and counted by the data acquisition system. The daily total counts were used as an arbitrary measure of the movement of the animal. Values were expressed as a percentage of the baseline movement levels to account for differences in the sensitivities of individual telemeters with specific receivers. Following a seven-day surgical recovery period, there was a seven-day baseline data collection period. During the baseline and 14-day test period, 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 of the urine collection. Animals were fed a powdered diet (Purina Rat Chow no. 5012). 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. Daily data collection (food intake, urine output and body mass) and animal health checks occurred at 08:00 and lasted for 45 minutes. To change body weight varying levels of centrifugation were used. The centrifuge can accommodate two different G-loads at a time, thus the study was conducted in two runs. In the first run rats were exposed to centrifugation at 2.0 G (12 ft. 21.1 RPM), 1.5 G (8ft, 21.1 RPM), or were stationary 1 G controls (n=8/group). In the second run rats were centrifuged at 1.5 G, (12 ft. 16.06 RPM), 1.25 G (8 ft, 16.06 RPM), or were stationary 1 G controls (n=8 rats/group). Responses at 1.0 and 1.5 G in the two experiments were compared and no significant differences were noted. Therefore, the data from the two runs at each gravity level were pooled.

19. *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. Each parameter was compared as a percentage of the baseline levels.

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21. *Dissection.* A dissection was performed on each rat at day 14 of the test period. The rats were anesthetized with isoflurane and killed by decapitation. Bilateral epididymal fat pads were collected, and weighed. Previous data collected in our laboratory has

shown a high correlation between epididymal fat pad weight and total body fat in rats ($r^2$ =0.797, n=22). In addition, this technique has been proven a successful indicator of percent body fat in other rodents (E. J.Elisen, J. M. Leatherwood *Growth* **45**, 100 (1981)).

22. Measurements of metabolism were taken over days 8 – 14 of the experiment. This period was selected as previous investigators have shown rats to stabilize body mass gain after 5 of increased gravity (J. Oyama, W. T. Platt *Am. J. Physiol.* **212**, 164 (1967); C. E. Wade, et al. *J. Grav. Physiol.* **4**, 43 (1997)). Energy intake was determined from the amount of food used daily. The animals were fed a certified diet (Purina Rat Chow no. 5012) with a known caloric content of 3.3 kcal/g. We used the doubly labeled water (DLW) expenditure ($^2H_2{}^{18}O$) method for measuring energy expenditure. The labeled water was given via intra-peritoneal injection in the morning upon initiation of the experimental period. Daily urine samples were collected from each rat and analyzed. 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 (corrected for the weight of the oil), the oil was removed, and the samples were centrifuged. The daily samples were frozen at –20 °C for later analysis. All isotope analyses were done with a Europa ANCA (Europa Inc., Cleveland, Ohio) isotope ratio mass spectrometer. Standard curves are run after every 20-30 samples. All samples were run in duplicate and each sample tube was sampled

twice. The isotopic enrichment of the urine was calculated from the standard curves. For this study, we assumed that the isotope distribution space was equal to the total body water. The rate of carbon dioxide production was calculated from the equation of Schoeller, et al. (*Am J Physiol.* **250**, R823, (1986)). The Weir equation was used to convert the rate of carbon dioxide production into energy expenditure values.

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**Fig. 1.** Effect of increased gravity levels on body mass, food intake and body weight. (A) Body mass of rats as a percent of baseline over 14 days of increased gravity. Baseline body mass for each group was: 1.0 G, 242 ± 2.2 g; 1.25 G, 236 ± 2.0 g; 1.5 G, 243 ± 1.4 g; and 2.0 G, 247 ± 2.2 g. The number of animals in each group were 16, 8, 16, and 8, respectively. (B) Food intake as a percent of baseline over 14 days of varying level of gravity. Baseline food intake per 100 grams of body mass (BM) for each group was: 1.0 G, 24.2 ± 0.22 g/ 100 g BM /day; 1.25 G, 23.6 ± 0.2 g/ 100 g BM /day; 1.5 G, 24.3 ± 0.1 g/ 100 g BM /day; and 2.0 G, 24.7 ± 0.2 g/ 100 g BM /day. The baseline body masses and energy intakes were not statistically different between groups (*10*). Shown in (C), (D) and (E) are average daily body mass gains over days 8 to 14, epididymal fat pad masses expressed per 100 g of body mass, and mean body weights at variable gravity levels over days 8 t0 14. Body weight is the product of body mass times the level of gravity. Groups labeled with the same letter are not statistically different from one another. Numbers of animals per group were 8-16.

**Fig. 2.** Effect of increased gravity on energy intake, expenditure and balance. Values are the cumulative average of seven days of measurement. The time period was over days 8 to 14 following the initial period of acclimation (*7, 8*). (**A**) Energy intake was measured from the quantity of food consumed. (**B**) Energy expenditure was measured by the doubly labeled water expenditure method (*13*). (**C**) Energy balance is the difference between energy intake and expenditure. All rats were in a positive energy balance and gaining weight. Groups labeled with the same letter are not statistically different from one another.

**Fig. 3.** Effect of increased gravity on spontaneous movement and activity. (**A**) Spontaneous movement of rats as a percent of baseline over 14 days of increased gravity. Baseline movement for each group was: 1.0 G, 893 ± 43 counts/day; 1.25 G, 936 ± 86 counts/day; 1.5 G, 878 ± 86 counts/day; and 2.0 G, 792 ± 72 counts/day. (**B**) Activity level of rats at varying gravity levels. Percent activity was the product of movement as a percentage of baseline times body weight as a percentage of baseline level at 1.0 G divided by 100. Groups labeled with the same letter are not statistically different from one another. (**C**) Linear regression of the increase in body weight compared to baseline in relation to the reduction in movement compared to baseline (r= 0.69, n= 47, F= 40.82, p<0.0001). The solid line represents the relationship, while the dash line indicated activity being maintained. Activity is the product of the bodyweight times movement.
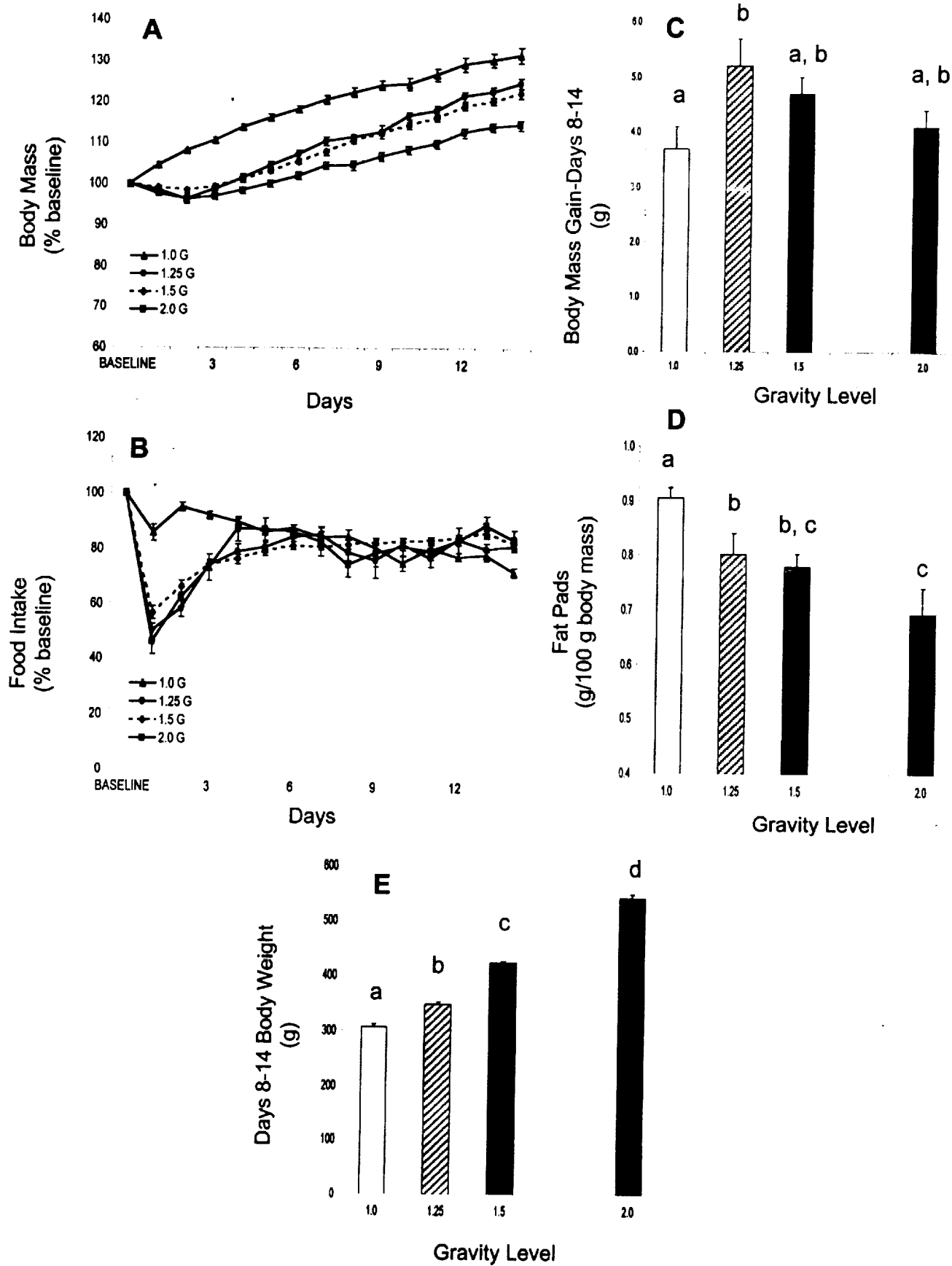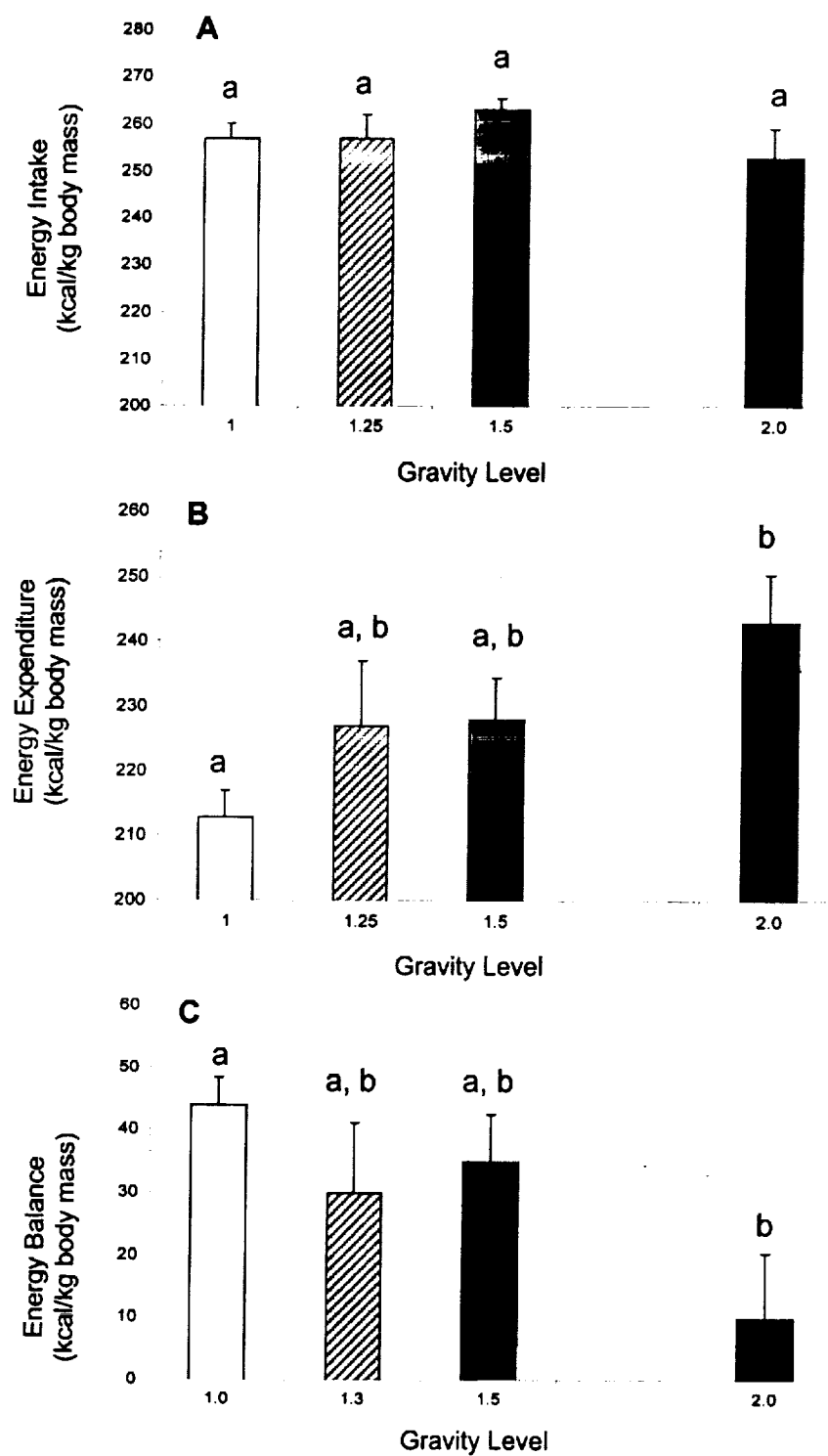
## Figure 1

**Figure 2**

**Figure 3**