The Journal of Experimental Biology 204, 1729–1734 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB3083

A NOVEL MECHANISM OF BODY MASS REGULATION

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Accepted 5 February; published on WWW 23 April 2001

Summary

While significant attention has been devoted to the identification of hormonal factors that control body mass, little attention has been paid to the role of mechanical loading on animal mass. Here, we provide evidence that intraperitoneal implantation of metabolically inert mass results in a compensatory reduction in tissue mass. Deer mice (*Peromyscus maniculatus*) were surgically implanted with weights of 1, 2 or 3 g. There was a resulting loss of tissue mass (total body mass minus implant mass) that was proportional to the mass of the implant. This reduction in tissue mass followed a reduction in food intake in animals with 3 g implants. Evaluation of body composition failed to identify any single component that contributed to the loss of tissue mass. Removal of implants led to a transient restoration of body mass to levels similar to the total body mass of those control animals in which the implant had not been removed. However, within 12 days of implant removal, body mass again declined to the level seen before implant removal. These results suggest the existence of a set point that is sensitive to changes in the perception of mass and that is transduced *via* neural pathways.

Key words: body mass, mechanical regulation, food intake, deer mouse, *Peromyscus maniculatus*, diet restriction.

Introduction

The regulation of body mass is incompletely understood, in large part because it is affected by numerous regulatory components. One theory suggests that body mass is regulated by a neural set point. The set point adjusts body mass by integrating information from multiple efferent pathways that reflect changes in energy balance (Schwartz and Seeley, 1997; Weigle and Kuijper, 1996). The neural basis for a set point and the putative regulatory pathway(s) that effect changes in it have not been completely described. There is considerable evidence that endocrine factors, such as leptin, adjust body mass by modifying some function of the set-point mechanism (Pelleymounter et al., 1995; Halaas et al., 1995; Campfield et al., 1995). Certainly, changes in metabolic rate due to any number of physiological states (e.g. pregnancy, pubertal growth spurt, ageing) are known to produce dramatic and sustained changes in body mass, and many of the endocrine changes associated with these states are recognized to effect changes in body size (Bowman and Miller, 1999; Youngman, 1993; Wolden-Hanson et al., 2000). It is known that variation in loading of the musculoskeletal system alters bone growth and development, which subsequently affect body size and therefore mass (Gordon et al., 1993; Carter et al., 1991). Little or no attention has been paid to how changes in mechanical loading of the musculoskeletal system might affect the body mass set point.

We undertook the present study following an observation that suggested that mechanical loading might play an important role in the regulation of body mass in adults. In studies of rodent body temperature, using intraperitoneally implanted temperature sensors weighing approximately 10 % of total body mass, we noted that there was a compensatory and equivalent loss of tissue mass. On the basis of this observation, we developed the hypothesis that sensory perception of total body mass is an important regulatory signal in determining the biological body mass set point. Here, we describe a study in which we artificially increased deer mouse (*Peromyscus maniculatus*) total body mass (body mass + implant mass) and evaluated the compensatory adjustments in metabolically active tissue mass. The findings show that tissue mass (body mass minus implant mass) declines in a dose-dependent manner in animals implanted with inert weights. We suggest that a previously undescribed pathway related to mechanical loading of the musculoskeletal system may be involved in the neural regulation of body mass.

Materials and methods

Animals and breeding conditions

The adult deer mice (*Peromyscus maniculatus* (King, 1968)) used in these experiments were selected from the F_2 and F_3 generations of an outbred F_1 breeding colony maintained at Kent State University, Kent, OH, USA. Parental breeders were

captured in Wind Cave National Park, Hot Springs, SD, USA (latitude 43°30′W; longitude 103°34′N). In the laboratory, breeding pairs and their offspring were maintained under a long photoperiod (16 h:8 h L:D) from birth and provided with food (Purina Lab Chow; Ralston, St Louis, MO, USA) and water *ad libitum*. The animals used in these experiments were at least 90 days old and were sexually mature. They were housed singly. Activity during the study periods was not recorded.

Food intake

Food intake was assessed as described previously (Blank et al., 1994). Briefly, minced food was placed beneath a 2 mm^2 wire mesh in a porcelain cup, and the decline in the mass of food in the container was used to determine the daily food intake for each animal. Food intake data were discarded where there was obvious evidence of food spillage. This method of presentation allows the animals free access to food and has been shown previously to have no effect on body mass (Blank and Desjardins, 1983).

Implants

Small plastic capsules (Minimitter Corp. Sunriver, OR, USA), approximately 13 mm in length and 9 mm in diameter, were used as implants in all experiments. The implants were either empty (mean mass 0.4 g) or contained small stainless-steel pellets, resulting in final implant masses of approximately 1, 2 or 3 g. Each capsule was coated with Elvax wax (Minimitter Corp. Sunriver, OR, USA), weighed to determine exact implant mass and placed within the peritoneum according to previously described methods (Blank and Desjardins, 1986). Briefly, the animals were anesthetized, the abdomen and peritoneum were opened, the implant was inserted and the incision was closed with sutures and staples. Animals were allowed to recover and returned to their cages. A 3 day course of antibiotics was given.

Experiment 1: effects of implants on tissue mass

Body mass and food intake of adult males (N=50) were measured (to 0.1 g) every 3 days for 18 days to identify an initial baseline mass. Animals were body-mass-matched to ensure no differences between groups (Fig. 1A). Food intake was also recorded for the preoperative period (Fig. 1B). Thereafter, animals were either sham-operated (N=10) or implanted with one of four capsule weights: control (0.4 g), 1 g, 2 g or 3 g (N=10 per group). Two control groups (sham-operated and empty capsule or implant control) were chosen since, on the basis of changes in available peritoneal space, the presence of the capsule in the peritoneal cavity might regulate mass. Total body mass (=tissue mass + implant mass) and food intake were then recorded for 5 weeks, after which the implants were removed from half the animals within each group. Animals were once again anesthetized, the peritoneal cavity was opened, the implants were removed, the incisions were sutured and stapled, and the animals were allowed to recover. Total body mass and food intake were then determined for an additional 45 day period.

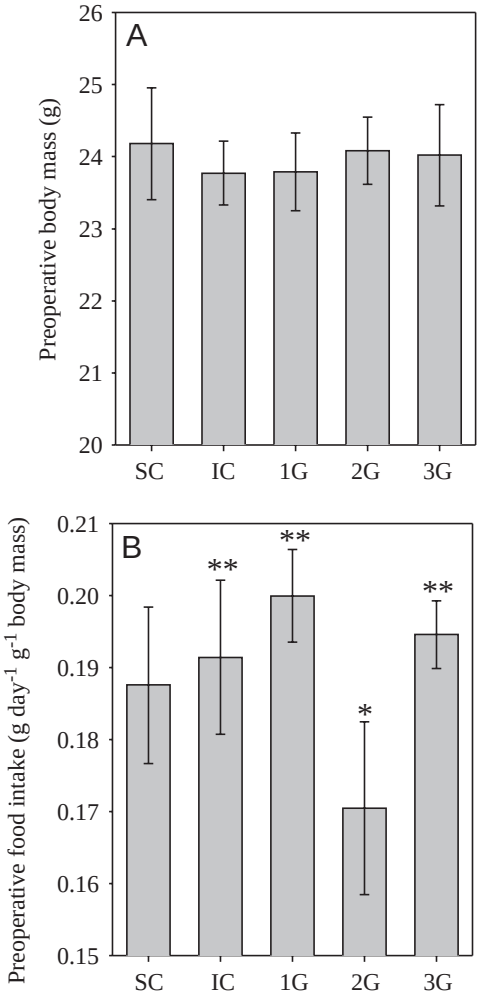

Fig. 1. Preoperative body mass (A) and food intake (B) of male deer mice used in weight implantation experiments. Body mass and food intake was recorded for 18 days prior to implantation at 3 day intervals. Values are means ± S.E.M. (N=10 per group). SC, sham-operated control; IC, implant control; 1G, 1 g implanted group; 2G, 2 g implanted group; 3G, 3 g implanted group. *Significantly different from sham controls (P<0.05); **significantly different from 2 g implanted group (P<0.05).

Experiment 2: effects of implants on body composition

A second group of adult male mice (N=50) was used to determine the effects of the implants on body composition. These mice were allocated to five experimental groups (N=10 per group) as in experiment 1 (sham-operated, implant control, 1 g, 2 g and 3 g), and total body mass and food intake were measured for 5 weeks. The mice were then killed by cervical dislocation, the implants were removed and the animals were weighed before removing their hair with manual shears and a depilatory agent (Carter and Wallace, New York, USA). The digestive tract was removed, manually emptied of food residues and replaced inside the carcass, and the animals were reweighed to give a final wet carcass mass. Body composition, including water, fat, protein and ash content, was determined (Cortright et al., 1996).

To assess water content, carcasses were frozen at −110 °C and ground in a Waring commercial blender containing 180 ml of deionized water for 10–15 min until thoroughly homogenized. The resulting homogenate was placed in a preweighed freeze-dry vessel, sealed, frozen in a cryogenic bath and freeze-dried to constant mass. Total water content was calculated as the difference between wet and dry carcass mass. Lyophilized tissue was subsequently subdivided into three samples for determination of fat, protein and ash content. Fat analysis was performed in triplicate on approximately 0.5 g of the powdered sample. Briefly, tissue was combined with 0.5 ml of ethyl alcohol (100 %) and 10.0 ml of diethyl ether, shaken manually for 30 min and then centrifuged for 10 min at 500 ***g***. The organic phase was decanted. This process was repeated on the residue, and the organic phases were pooled. Samples were dried overnight in an oven at 67 °C. Fat content was calculated as the difference between the original mass of the sample and the final mass of the residue. A Perkin-Elmer (model 2400 CHN) elemental analyzer was used to determine the total protein content of each sample (Cortright et al., 1996). Dried tissue (2–10 mg) was combusted and reduced, the elemental carbon, hydrogen and nitrogen were separated by gas chromatography and the absolute amount of each was detected by thermal conductivity. The nitrogen content was then multiplied by 6.25 to calculate the total protein content. Ash content was determined using standard methods (Cortright et al., 1996). Briefly, samples were combusted at 800 °C for at least 90 min or until constant mass had been achieved. The true ash content of each dried sample was calculated.

Statistical analyses

Capsule mass was subtracted from total body mass for each individual to determine tissue mass. Tissue mass was subtracted from each animal's preoperative mean mass. Changes in body mass, total body mass and tissue mass from the preoperative mean across groups were evaluated using a two-way analysis of variance (ANOVA) with time from implantation as a covariate. Statistical tests were carried out on all the data; however, the figures, where noted, present the means and standard errors of the mean (S.E.M.) from all days combined. Individual group differences were assessed by a *post-hoc* Student–Newman–Keuls test. In all statistical analyses, $P<0.05$ was considered to be significant.

Results

Experiment 1: effects of inert implants on tissue mass

Implantation of inert weights caused a significant and sustained loss of tissue mass (total body mass minus implant mass); the magnitude of this effect was dependent on implant mass (Fig. 2). Five weeks after implantation, the total body mass (tissue mass + implant mass) of the animals showed an increase for both the 2 and 3 g implanted groups (Fig. 2A). This increase was significantly greater than that of the implant controls and the 1 g implanted group, and the change in total body mass of the 3 g implanted group was also significantly different from that of the 2 g implanted group (Fig. 2B).

Tissue mass was reduced in the implant groups in comparison with controls (Fig. 2C). Both the implant control and the 1 g implanted group showed a significantly lower tissue mass then the sham controls. The tissue mass of the 2 and 3 g implanted groups was significantly lower than those of all other groups.

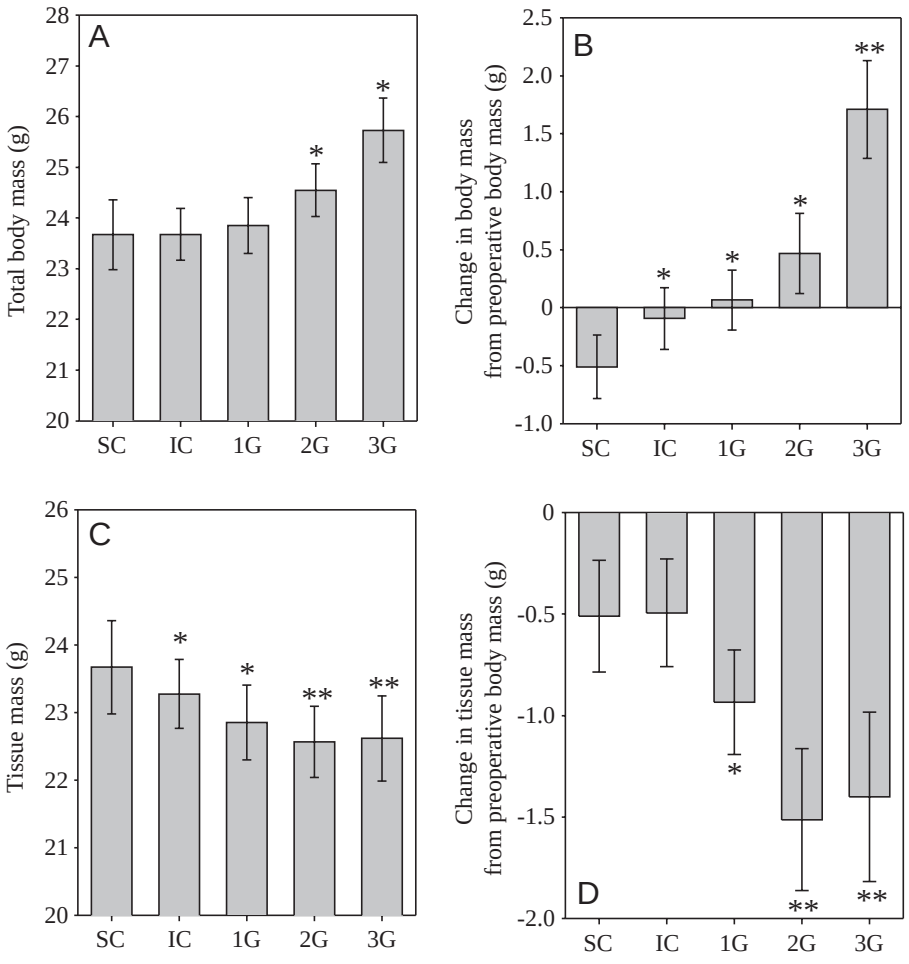

Fig. 2. Mean body mass 5 weeks after implantation of inert weights into deer mice. Values represent means ± S.E.M. (N=10 for each group). (A) Total body mass for each group. (B) Body mass minus initial body mass: data are expressed as the change in body mass from the preoperative mean. (C) Tissue mass (body mass minus implant mass). (D) Tissue mass minus initial preoperative body mass. SC, sham control; IC, implant control; 1G, 1 g implanted group; 2G, 2 g implanted group; 3G, 3 g implanted group. *Significantly different from sham controls (P<0.05); **significantly different from sham control, implant control and 1 g implanted group (P<0.05).

Presentation of these data as the change from the respective preoperative mean (Fig. 2D) indicates that the sham and implant controls did not differ from each other but did differ significantly from all three implant groups. Mice in the 1 g implanted group showed a significant loss of tissue mass, while mice in the 2 and 3 g groups had significantly lower tissue masses than the sham and implant controls as well as the 1 g implanted group.

To determine whether tissue mass loss was related to food intake, we determined the amount of food consumed by the mice over the same period. We found a clear trend for a reduction in food uptake with increased implant mass, although only in the 3 g implant group, which exhibited the greatest loss of tissue mass, was this reduction significant (Fig. 3).

Five weeks after implantation, animals in each of the 1, 2 and 3 g groups were allocated to two subgroups, and the implants were removed from the animals in one subgroup. Fig. 4 compares the changes in total body mass and tissue mass of mice with implants with those of mice from which the implants had been removed. For the 1 g implanted group, removal of the implant caused an initial statistically insignificant small increase in body mass (Fig. 4A). However, by day 6 after removal, the body mass of this subgroup was indistinguishable from the tissue mass of the subgroup that retained the implant, and this effect was noted over the following 42 days. From day 12 onwards, a two-way ANOVA indicated that there was a statistically significant difference between the body mass of the group without the implant and the total body mass of the subgroup that retained the implant. Removal of the 2 g implant resulted in an immediate increase in body mass (Fig. 4B) that was statistically significant on day 6. This increase was seen over the first 9 days of the experimental period. From day 12 onwards, the body mass of this subgroup was indistinguishable from the tissue mass of the retained implant subgroup and was significantly different from the total body mass of the retained implant subgroup. The 3 g implanted subgroup also showed a transient increase in body mass following implant removal (Fig. 4C). This increase was statistically significant on day 3, while values on days 6 and 9 were not significantly different from either the total body mass or the tissue mass of the retained implant group. From day 12 onwards, the body mass was indistinguishable from the tissue mass of the retained implant subgroup and was significantly different from the total body mass of the retained implant subgroup.

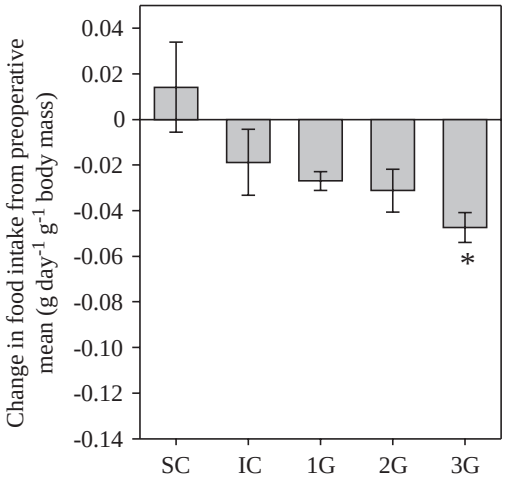

Fig. 3. Change in food intake 5 weeks following implantation. Food intake was recorded every 3 days over the 5 week experimental period. Values represent means ± S.E.M. (N=10 for each group). SC, sham control; IC, implant control; 1G, 1 g implanted group; 2G, 2 g implanted group; 3G, 3 g implanted group. *Significantly different from sham controls (P<0.05).

Experiment 2: effects of inert implants on body composition

To determine whether the reductions in metabolic tissue mass described above affected body composition, body water, fat, protein and ash contents were determined. Again, mice were body-mass-matched prior to the experiment, and these mice showed the same responses to implantation of weights as described above. After 5 weeks of implantation, there were no significant changes in body composition (Table 1).

Discussion

In this study, we present data suggesting the existence of a biological set point for body mass that is initially established by the animal's perception of its own body mass. We refer to this set point as a 'mechanical set point' because of the assumption that the sole change in the physiology of the animal caused by

Table 1. *Body composition of* Peromyscus maniculatus *calculated as a percentage of carcass mass*

Group	Water	Fat	Protein	Ash
Sham-operated control	59.58±1.78	18.82±2.64	20.26±1.01	2.29±0.40
Implant control	63.52±1.52	12.40±2.02	19.16±0.74	2.26±0.30
1 g implant	65.22±2.41	12.59±2.24	18.10±0.99	1.87±0.33
2 g implant	64.73±1.84	12.11±2.02	19.07±0.57	1.52±0.21
3 g implant	64.96±1.48	12.29±1.87	18.26±0.62	1.92±0.29
P	0.174	0.148	0.354	0.394

Values are given as the mean ± S.E.M. (N=10) percentage of total carcass mass (equal to total measurable body mass minus mass of inert implant).

P values are taken from a one-way ANOVA.

our manipulation is an increase in the loading of the musculoskeletal system. Thus, when we added artificial mass to these animals, we saw a compensatory loss of body tissue mass. Although the loss of tissue mass was proportional to the mass of the implant, differences were apparent. It is noteworthy that none of the animals in any of the three experimental groups ever lost more than 1.5 g or approximately 7 % of its original body mass. We tentatively suggest that this point may be a threshold beyond which other pathways are activated that can serve to modify body mass further.

It is clear, at least in the 3 g implant group, that food intake was reduced following implantation. The other implanted groups also showed a trend towards a reduction in food intake. While it is reasonable to assume that mass loss occurred as a result of decreased food consumption in relation to the change in tissue mass, the magnitude of the reduction in food intake is minor. Furthermore, deer mice are known to modify energy balance *via* pathways other than food intake (e.g. reduced metabolic rate), and it remains possible that these pathways are responsible for the observed tissue mass loss. Body composition analysis showed that the tissue composition of implanted and control animals was very similar, indicating that neither protein nor fat is preferentially removed when the set point is activated.

Three days following the removal of the implant, the body masses of the animals had increased to the level of the total body mass of the retained-implant group (Fig. 4). In other words, animals in this group regained a mass proportional to the mass of the implant that had been removed. More surprising, however, was the observation that, within 12 days of implant removal, the body mass of animals in all three groups had returned to pre-implant-removal tissue mass levels. From that time point on, there was no significant difference between the body mass of the implant-removed group and the tissue mass (i.e. total body mass minus implant mass) of animals that retained their implant. Therefore, the animals rapidly lost the mass they had gained following removal of the implant. In addition, the highest body mass recorded during this 12 day period was at day 3 for all three implanted groups, after which there was a gradual decrease in body mass until day 12. The animals sustained this body mass for the remainder of the experimental period, approximately 5 weeks.

The results discussed above suggest that, following initial implantation, the normal tissue turnover of the animals is disturbed in favor of catabolism until a new steady state is achieved. Because implant removal involves a loss of non-

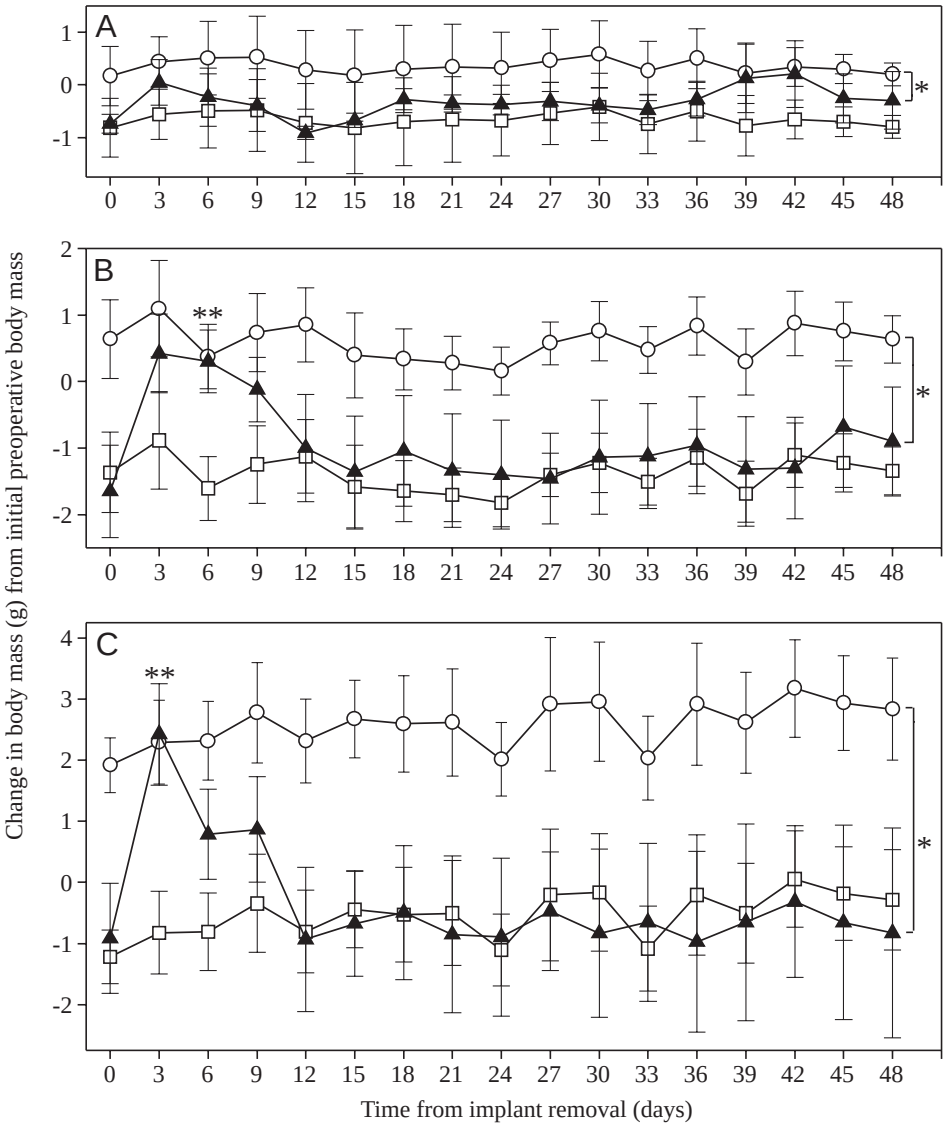

Fig. 4. Change in body mass relative to preoperative body mass following implant removal. Five weeks after implantation, the implants were removed surgically from half the animals in each group. The mean body mass of these animals (N=5) is represented by the filled triangles. The remainder of the animals (N=5) retained their implants: the total body mass (open circles) and the tissue mass (open squares) of these animals are plotted. (A) 1 g implanted group. (B) 2 g implanted group. (C) 3 g implanted group. *Significant difference between the body mass of animals with implants removed and the total body mass of animals that retained implants (significant differences shown are from a two-way ANOVA performed on the data for days 12–48; P<0.05). **Significant difference between the body mass of animals with implants removed and the tissue mass of animals that retained implants (significant differences shown are from a one-way ANOVA performed only on data from that day; P<0.05).

metabolically active mass, it is unlikely that there is an associated change in levels of afferent humoral signals such as leptin. When the newly established set point is challenged by implant removal, the primary stimulus should be that of a change in the neural perception of body mass. We suggest that this stimulus is transduced into an anabolic response, which results in a mass gain. It is entirely possible that this anabolic response is humoral in nature. In fact, it is probable that that is the case. However, this anabolic response should also increase afferent humoral signals that result in a second catabolic response that returns body mass to the reset level. An additional implication of this work is that the initial implantation eventually separates information transduced by the perception of body mass from that mediated by humoral signals, although levels of hormones related to body mass were not measured in our study. With the removal of the implant, the disparity between the signals provided by these two stimuli results in the changes in body mass.

We suggest that the change in body mass is initially mediated by mechanical loading, possibly as a result of some form of sensory input. How an increase in body mass mediates the change in set point was not determined. The possibility that the presence of the implant within the peritoneal cavity obstructed food passage and consequently altered food intake is unlikely, given the similar responses of the sham and empty (0.4 g) implant control groups. Furthermore, tissue composition analysis showed no modification in water content among implant groups, despite a dose-dependent reduction in tissue mass; qualitative abnormalities in gut morphology or length at autopsy were not detected (results not shown).

While these findings offer no direct evidence for a sensory pathway, mechanisms exist that could serve to effect changes in body mass. For example, numerous mechanoreceptors are located within muscles and tendons, many of which have unknown functions. The muscle spindle and Golgi tendon organ, which measure muscle fiber length and tension, respectively (Schmidt, 1985; Carpenter, 1984), have both been demonstrated to have afferent pathways to multiple loci within the cerebral cortex (Oscarsson and Rosen, 1963; Rosen, 1969; McIntyre et al., 1985). Either of these receptors could provide the necessary information for activation of a mechanical set point. It is important to state that we do not present any evidence regarding the efferent arm of this phenomenon. It is entirely possible that previously elucidated pathways regulate the mass loss and gain demonstrated in these animals on the efferent side.

Taken together, these data suggest that sensory perception of body mass is a critical regulator of metabolic tissue mass and is capable of resetting a biological set point of body mass in this small rodent. We surmise that a mechanical signal is used to control and maintain perceived body mass. It seems clear that, when perceived body mass is increased, excess mass is reduced by an alteration in metabolism that translates, in part, to a reduction in food intake. It is possible that, by resetting the mechanical set point, a metabolic set point is also reset and that, together, they serve to regulate tissue mass.

We thank Superintendent Martin Ott and Park Biologist Larry Hayes of the US Department of Interior, Wind Cave National Park, Hot Springs, SD, USA, for assistance in collecting parental stocks of deer mice and Dr Irving Shapiro for his contribution to the preparation of this manuscript. All animal work was approved and reviewed by the Animal Care and Use Committee. This research was supported by the Department of Biological Sciences, Kent State University, Kent, OH 4424, USA.

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