The Journal of Experimental Biology 207, 2859-2866
Published by The Company of Biologists 2004
doi:10.1242/jeb.01120

Gender dimorphism of body mass perception and regulation in mice

Petra Wiedmer[1], Michael Boschmann[2] and Susanne Klaus[1,*]

[1]*German Institute of Human Nutrition (DIfE) Potsdam-Rehbruecke, Nuthetal, Germany* and [2]*Franz-Volhard Clinical Research Center Berlin, Medical Faculty of the Charité, Humboldt-University, Berlin, Germany*

*Author for correspondence (e-mail: Klaus@mail.dife.de)

Accepted 1 June 2004

Summary

According to the set-point theory of body mass, changes in body mass are perceived by the body, leading to activation of compensatory feedback mechanisms, which in turn restores the set-point body mass. However, this theory is still under debate. To test if mass *per se* might be sensed and regulated, we implanted loads corresponding to 10% (HI) or 2% (LO, control) of body mass into mice in addition to sham-operated mice (SO). We recorded body mass, food intake, energy expenditure and body composition over 14 weeks. Both male and female mice showed an initial stress-induced loss of body mass, which was more pronounced in males. Subsequently, male HI mice displayed a permanently decreased biological body mass (M_{BB}, body mass exclusive of the implant mass), equivalent to approximately half of the mass of the implant, and obtained by a decrease in fat mass compared to SO males. In contrast, female HI mice rapidly recovered and maintained their initial M_{BB} and body composition following a mass load. Initial lean body mass was maintained in all male and female groups, and energy intake was similar in all male and female groups. Body mass changes could not be explained by measurable changes in energy intake or expenditure. We conclude that changes in body mass are perceived and partially compensated in male but not in female mice, suggesting that mass-specific regulation of body mass might not play a major role in overall body mass regulation. Different compartments of the body are possibly regulated by different signals and stimuli. Our results suggest that lean body mass rather than body mass *per se* seems to be tightly regulated. Higher efficiency of energy utilization in females compared to males could explain the gender-specific changes in energy balance.

Key words: body mass set-point, energy expenditure, body composition, ponderostat, obesity, gravity, sex difference, mice.

Introduction

The set-point theory of body mass regulation assumes that maintenance of an individual body mass is the result of a regulated mechanism (Cabanac, 2001). It is suggested that changes in body mass can be perceived by the body, which generates an endogenous signal, the ponderostat, which is correlated with the present body mass. Changes in the extent of the ponderostat signal following deviations from set-point body mass activate feedback control mechanisms,which in turn act on food intake and energy expenditure to regenerate the individual set-point mass (Cabanac et al., 1971; Bradley, 1978; Keesey and Corbett, 1984; Harris, 1990; Cabanac, 2001). Failure to identify the ponderostat (Macdonald, 2003; Berthoud, 2002), and the fact that changes in body mass are mainly reflected by changes in fat mass (Harris, 1990), led to the proposition that body mass regulation depends on a lipostatic feedback system, with leptin as a lipostatic signal (Friedman and Halaas, 1998). However, the widely accepted hypothesis of lipostatic regulation of body mass has been disputed (Speakman et al., 2002; Lacy et al., 2003). There is evidence that gravity and mass *per se* also influence body mass regulation. Under conditions of hypergravity induced by centrifugation, body mass of rats was decreased through loss in fat mass and energy expenditure was increased; energy intake initially decreased and returned to normal or increased levels in the long-term (Warren et al., 1997). Furthermore, it has recently been reported that intraperitoneal implantation of a metabolically inert weight into male deer mice *Peromyscus maniculatus* resulted in a loss of body mass (Adams et al., 2001).

Most mammals show gender dimorphisms in body mass and composition, and there is ample evidence that energy homeostasis and substrate utilization are regulated differently in males and females (Cortright and Koves, 2000). Very recently it was shown that male and female rats display differential sensitivity to central insulin and leptin levels with regard to food intake regulation (Clegg et al., 2003). However, even extensive discussions of body mass regulation do not address the possibility of gender differences in the basal regulatory mechanisms (Jequier and Tappy, 1999).

We therefore tested the hypothesis that body mass *per se* is perceived and regulated, and that there might be gender differences in this regulation. In order to increase body mass artificially, we implanted inert weights corresponding to 10%

of initial body mass into the abdominal cavity of mice. If mice are able to detect the increase in total body mass (M_{TB}) and there exists an individual set point of body mass we would expect them to show a compensatory decrease in biological body mass (M_{BB}, body mass exclusive of the weight load) to restore the set-point M_{TB}. To control for surgical stress and the volume effect of the implant, we included a sham operated group (SO) and a group with an implant of the same volume, but lower mass, corresponding to only 2–3% of body mass (LO), in addition to the 10% implant group (HI).

Materials and methods

Animals

All experiments were performed according to the federal guidelines of Brandenburg Ministry of Agriculture and Environment. Male and female mice (FVB, in-house breed) were maintained under standard conditions at 25°C and a 12 h:12 h dark:light cycle. They had free access to water and standard rodent chow diet containing 19% protein, 4% fat and 50% carbohydrates (Altromin 1324 fortified, Lage, Germany). Mice were 7 months old at the time of weight load implantation. Body mass and energy intake were recorded regularly for at least 4 weeks prior to weight implantation. As basal values of physiological parameters the following measurements were used: energy intake, mean over 3 weeks; energy expenditure, mean over 2 weeks; body mass, mean over 1 week before weight implantation; body composition, immediately prior to implantation of the weight load. Because of technical limitations not all animals were subjected to the same measurements; animal numbers were 12–34 for each gender and parameter and exact numbers are reported in the figures and tables.

Implantation of weight loads

Animals were divided randomly into three treatment groups: a sham-operated group (SO), a group that received light weight loads (LO) and a group that received heavy weight loads (HI). LO and HI weight loads corresponded to 2–3% and 10% of initial body mass, respectively. Implants were composed of a rod-shaped core (1.4 cm length, 0.8 cm diameter) and a wax coating (Elvax Wax, Minimitter Co., Sunriver, OR, USA). The core of LO weight loads consisted of plastic tubing filled with cotton. HI weight load cores consisted of a metallic cylinder. For implantation of the weight loads, mice were anaesthetized with ketamin (1 µl g^{-1}; Ketamin Gräub, A. Albrecht, Aulendorf, Germany) and xylazinhydrochlorid (0.1 µl g^{-1}; Rompun: BayerVital, Leverkusen, Germany). Weight loads (see below) were disinfected and implanted into the abdominal cavity. The abdominal cavity was sutured using absorbable surgery thread (PGA Resorba, Resorba, Nürnberg, Germany), and skin was closed with clips (Becton Dickinson, Sparks, MD, USA) that were removed 1 week after the operation. SO animals were treated exactly like the implantation groups except that no weight was introduced. Weights were removed 14 weeks after implantation.

Measurements

Body mass and energy intake were measured 3 times per week throughout the experiment by weighing (BP 2100, Sartorius AG, Göttingen, Germany; detection limit 0.01 g). We define total body mass (M_{TB}) as the measured mass of the animals, i.e. including the implant, and biological body mass (M_{BB}) as the measured mass minus the implanted weight load.

Energy expenditure was measured by indirect calorimetry of mice housed in metabolic cages, receiving food and water *ad libitum*. Measurements were performed 2 weeks before implantation (background measurement) as well as 2 and 12 weeks after implantation. During indirect calorimetry, urine was collected daily for determination of nitrogen excretion using a Kjeldahl method (Proll et al., 1998). Gas analysis was performed using the analyzing system Advanced Optima (Hartmann & Braun GmbH & Co. KG, Frankfurt/Main, Germany) containing an oxygen (Magnos 16) and a carbon dioxide (Uras 14) analyzer. Mice were measured for two consecutive days. Mice from six cages were measured in parallel and every 6 min. Energy expenditure was calculated using the following equation (Frenz, 1999):

$$E_{TE} = 16.17\dot{V}_{O_2} + 5.03\dot{V}_{CO_2} + 5.98\dot{N}\,, \qquad (1)$$

where E_{TE} is total energy expenditure (kJ d^{-1}), $\dot{V}_{O_2}$ is rate of oxygen consumption (l d^{-1}), $\dot{V}_{CO_2}$ is rate of carbon dioxide production (l d^{-1}), $\dot{N}$ is excreted nitrogen in the urine (g d^{-1}).

Resting energy expenditure (E_{RE}) was defined as the mean of the 10 lowest daily values for energy expenditure (Klaus et al., 1998). Respiratory quotient (RQ; $\dot{V}_{CO_2}/\dot{V}_{O_2}$) and physical activity level (PAL; E_{TE}/E_{RE}) were calculated.

Body composition was measured at the time of surgery, prior to implantation, and when the weight load was removed after 14 weeks, using dual-energy X-ray absorptiometry (Lunar Piximus, Janesville, WI, USA) in anesthetized animals.

Calculations and statistical analysis

Most of the data are expressed relative to basal values measured 3 weeks (energy intake), 2 weeks (total energy expenditure, resting metabolic rate) and 1 week (body mass) prior to implantation, or at implantation of the weight load (fat mass, lean body mass), corresponding to 100%. Data are shown as means ± S.E.M. To achieve normal distribution, data for body mass values at 1 week after weight load and for energy intake were log-transformed. Baseline characteristics were compared using pooled or separate variances *t*-test for equality of means where applicable. Homogeneity of variances was tested by Levene's Test for equality of Variances.

Single measurement data were analyzed using analysis of variance (ANOVA), and Dunnett's-test was used as a *post-hoc* test for multiple comparisons against SO group. Development of body mass, energy intake, and energy expenditure were analyzed using repeated-measures ANOVA. Gender, mass implantation group and an interaction term between group and gender were included in the ANOVA models. To exclude any effects of post-surgical stress in these analyses, body mass and energy intake starting 2 weeks after surgery were included.

Significant differences within the sexes were tested using one-way ANOVA including mass implantation group as independent factor. Significance was assumed at $P<0.05$. Analysis was performed using SPSS 8.0 (SPSS Inc., 1998, Chicago, IL, USA).

Results

Baseline phenotype

Phenotypic characterization of male and female mice before implantation revealed a number of gender differences (Table 1). Males showed higher levels of body mass, energy intake, absolute lean body mass, absolute fat mass, total energy expenditure and resting metabolic rate compared to females. RQ was similar in males and females but PAL was higher in females than in males. Initial body mass and gross energy intake were similar in all treatment groups of male and female mice (Table 2). In order to render the results more comparable between males and females, we subsequently normalized most of the data relative to basal measurements.

Table 1. *Phenotypic characterization prior to weight implantation*

	Male	*N*	Female	*N*	*P*
Body mass (g)	35.3±0.4	34	26.0±0.3	33	<0.001
Energy intake (kJ d^{-1})	76.7±1.8	34	69.7±1.7	33	0.005
Lean body mass (g)	27.9±0.3	23	20.8±0.4	20	<0.001
Fat mass (g)	7.5±0.3	23	4.1±0.3	20	<0.001
E_{TE} (kJ d^{-1})	55.7±1.0	17	46.6±1.5	12	<0.001
E_{RE} (kJ d^{-1})	38.9±0.9	17	27.7±1.4	12	<0.001
RQ	0.98±0.01	17	0.97±0.02	12	0.587
PAL	1.44±0.03	17	1.76±0.07	12	0.001

Values for body mass and gross energy intake are means of 1 week or 3 weeks prior to weight load, respectively. Body composition parameters were measured immediately before surgery; energy expenditure parameters were obtained 2 weeks prior to weight load by measurement over a 48 h period.

E_{TE}, total energy expenditure; E_{RE}, resting energy expenditure; RQ, respiratory quotient; PAL, physical activity level.

Values are means ± S.E.M. Gender-specific differences were tested using Student's *t*-test.

Body mass development

Fig. 1 shows development of M_{TB}, M_{BB} and energy intake throughout the experiment. Acutely after surgery, all groups showed a reduction in M_{TB} and M_{BB} (Fig. 1A,B). Body mass reduction after 1 week of weight load was more pronounced in HI mice than SO mice (M_{TB}, $P<0.001$; M_{BB}, $P<0.001$). M_{TB} of male HI mice was 99.6+1.1%, and was the same as M_{TB} of SO males ($P=0.109$), whereas M_{TB} of female HI mice was 102.7+0.8% of the initial value and significantly higher than in SO females ($P<0.001$). Male mice thus showed complete initial compensation of M_{TB}, in contrast to female mice, and this effect was still apparent 2 weeks after weight load (see Fig. 1A).

Further change in M_{TB} was influenced by gender ($P<0.001$) and by the mass of the implant ($P=0.016$). HI mice of both genders showed significantly increased M_{TB} throughout the study period compared to SO mice (Fig. 1A).

Changes in M_{BB} were significantly affected by group ($P=0.004$) and sex ($P<0.001$). Up to week 4, M_{BB} was significantly reduced in HI males compared to SO, whereas from week 5 on, changes in M_{BB} were similar in all male mice. However, the M_{BB} of HI males was permanently slightly reduced, by about 3–5% compared to SO and LO mice. In females, M_{BB} was similar in all groups from week 2 on. Interestingly, from 8 weeks on, female LO mice showed the highest M_{BB} (Fig. 1B) resulting in M_{TB} levels comparable to those of HI females (Fig. 1A).

The gender differences are more obvious in Fig. 2, which shows the time needed to regain initial body mass. Females recovered initial body mass earlier than males ($P=0.003$). Male HI mice took longer to recover body mass compared to SO males ($P=0.011$). In females the weight of the implant had no effect on the time required for body mass recovery.

Food intake

During the first week, energy intake was significantly decreased in both genders (Fig. 1C). There were significant effects of group ($P=0.026$) and sex ($P<0.001$) on reduction of energy intake after 1 week. Males decreased their food intake to lower levels than females. Energy intake of male mice after 1 week was 80.1±3.2% in SO, 61.5±1.7% in LO, and 54.5±3.1% in HI relative to basal values. Energy intake of female mice after 1 week was 77.8±2.8% in SO, 67.4±3.8% in LO, and 66.1±3.6% in HI relative to basal values. From the

Table 2. *Body mass and energy intake prior to weight load separated by treatment groups*

	Sex	SO	*N*	LO	*N*	HI	*N*	*P*
Body mass (g)	M	35.9±0.7	12	34.9±0.6	12	35.1±0.8	10	0.546
	F	25.5±0.8	11	26.6±0.6	12	26.0±0.6	14	0.474
Energy intake (kJ d^{-1})	M	77.8±2.6	12	74.3±3.3	12	78.3±3.7	10	0.622
	F	73.0±2.7	11	69.3±4.0	12	62.5±4.5	14	0.173

SO, sham-operated mice; LO, mice containing implants corresponding to 2% of body mass; HI, mice containing implants corresponding to 10% of body mass; M, males; F, females.

Values for body mass and energy intake are means of 1 week or 3 weeks prior to weight load, respectively.

Values are means ± S.E.M. Differences were tested using one-way ANOVA (factor group).

second week on, energy intake of males and females was similar among the implantation groups. Increased energy intake following weight load was more pronounced in females of all groups than in males (P=0.042), presumably because of slightly higher values in HI females. However, comparison of the different female groups did not reveal significant differences; P-values for a given time point were between 0.1 and 0.2 for comparison between HI and SO (Dunnett's-test).

When cumulative energy intake from weeks 1 to 14 was calculated, no significant effect of gender could be detected (Fig. 3). The mean cumulative energy intake of LO and HI males seemed to be lower compared to SO males but was only significant for LO males (P<0.05), probably due to lower variation of values in the LO group. Although HI females displayed higher cumulative energy intake compared to SO and LO females there are no significant differences between female groups.

Body composition

Fig. 4 shows changes in body composition 14 weeks after

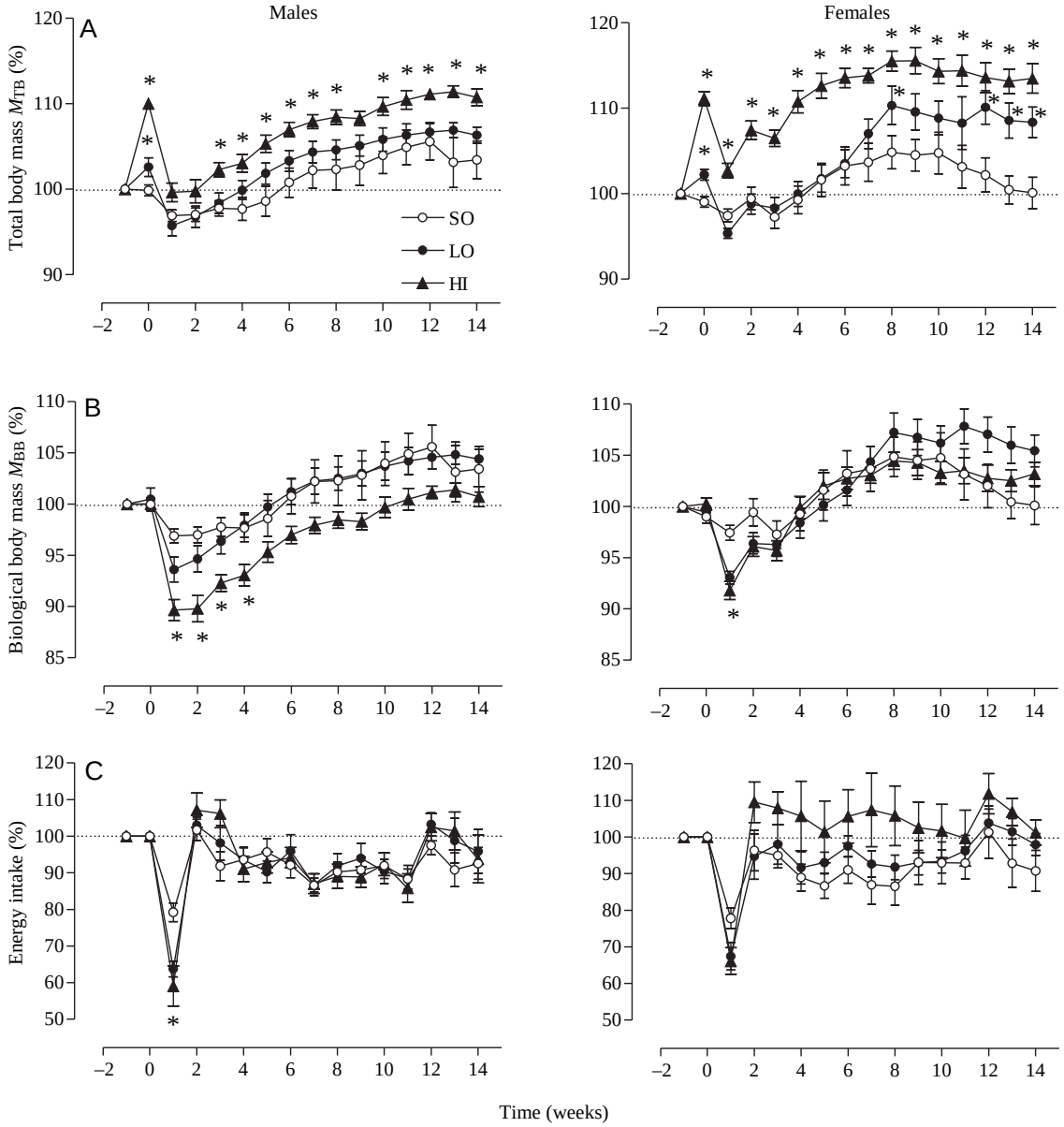

Fig. 1. Changes in total body mass (M_{TB}; A), biological body mass (M_{BB}; B), and energy intake after implantation of weight load (C) into male and female mice. SO, sham-operated mice (open circles); LO, mice containing implants corresponding to 2% of body mass (closed circles); HI, mice containing implants corresponding to 10% of body mass (closed triangles). Data are expressed relative (%) to values measured 3 weeks (for energy intake) and 1 week (for body mass) prior to implantation of the weight load (time 0). Body mass at time 0 was measured immediately after weight implantation; body mass at other time points is the mean of three measurements taken during 1 week. Values are means ± S.E.M., N (male/female): SO (12/11), LO (12/12), HI (10/14), *P<0.05 *vs.* SO (Dunett's-test).

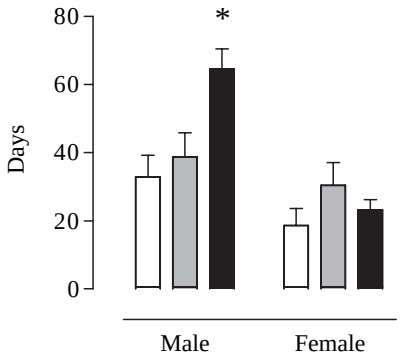

Fig. 2. Time required for initial biological body mass (M_{BB}) to be regained in male and female mice following acute mass loss after weight implantation. SO, sham-operated mice (white bars); LO, mice containing implants corresponding to 2% of body mass (grey bars); HI, mice containing implants corresponding to 10% of body mass (black bars). There was a significant gender effect (P=0.003). Values are means ± S.E.M., N (male/female): SO (12/11), LO (12/12), HI (10/14). *P=0.011 *vs.* SO (Dunett's-test).

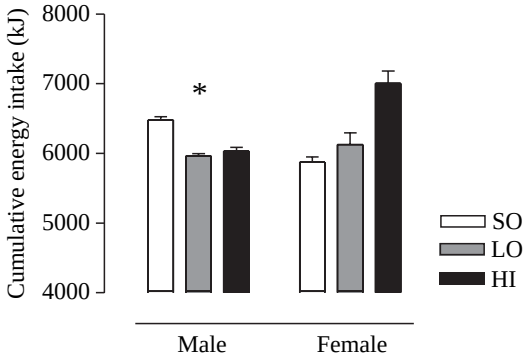

Fig. 3. Cumulative energy intake from week 1 to week 14 following weight load. SO, sham-operated mice (white bars); LO, mice containing implants corresponding to 2% of body mass (grey bars); HI, mice containing implants corresponding to 10% of body mass (black bars). Values are means ± S.E.M., N (male/female): SO (12/11), LO (12/12), HI (10/14). *P<0.05 *vs.* SO (Dunett's-test).

weight load in relation to initial values. Increase in fat mass at 14 weeks after weight implantation was lower in males than in females (Fig. 4; P=0.046). SO and LO males increased their fat mass compared to initial fat mass whereas HI males maintained their initial fat mass, the differences reaching borderline significance (P=0.052). In contrast, females increased fat mass in all treatment groups and there were no differences between the groups (P=0.456). Lean body mass was not affected by gender or the implanted weight and did not change compared to initial values (Fig. 4B).

Energy expenditure

E_{TE} was mainly determined by E_{RE} (data not shown). E_{RE} was influenced by gender but not by the implanted weight load (Table 3). Absolute E_{RE} was lower in females than in males (P=0.006). Following weight load females increased E_{RE} to a higher extent than males relative to initial levels (P=0.001). Due to the high variability in E_{RE}, no significant effect of the implanted weight could be detected in male and female mice. Physical activity level (PAL) was significantly affected by sex (P=0.041) with higher values in females that were consistent with baseline measurements (Table 1), but it was not influenced by the implanted weight (Table 3). Respiratory quotient (RQ), i.e. substrate oxidation, was not affected by weight load or by gender (data not shown).

Discussion

The present results show that male and female mice react differently to the augmentation of body weight by implantation of an artificial weight. In the medium and long term, HI males displayed slightly decreased M_{BB} compared to SO males; by contrast in females there was clearly no difference in M_{BB} between the treatment groups, except for a slightly increased M_{BB} in LO females in the second half of the study. Long-term changes in body mass could not be explained by measurable changes in energy intake and energy expenditure.

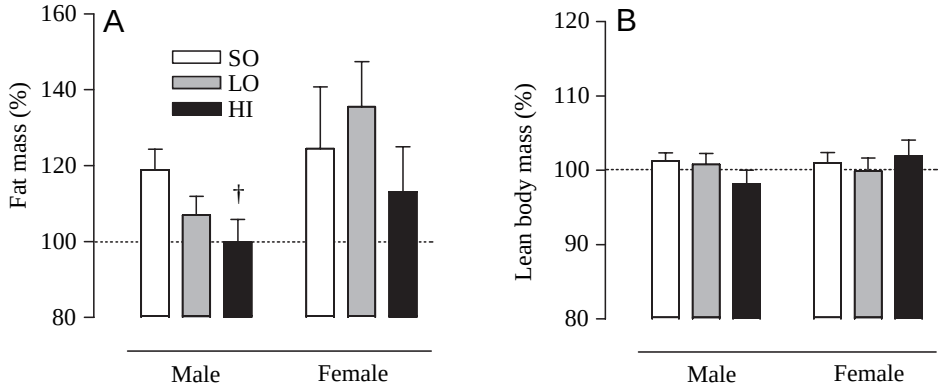

Fig. 4 Changes in body composition at 14 weeks after weight implantation in male and female mice relative to initial body composition. (A) Relative changes in fat mass. There was a significant gender effect on changes in fat mass (P=0.046). (B) Relative changes in lean body mass. SO, sham-operated mice (white bars); LO, mice containing implants corresponding to 2% of body mass (grey bars); HI, mice containing implants corresponding to 10% of body mass (black bars). Data (%) are expressed relative to values measured at weight implantation, and are means ± S.E.M., N (male/female): SO (7/5), LO (9/7), HI (7/7). †P=0.052 *vs.* SO (Dunett's-test).

Table 3. *Resting energy expenditure and physical activity level 2 weeks before and 2 and 12 weeks after weight load*

		Male			Female			
	Week	SO (6)	LO (6)	HI (5)	SO (4)	LO (4)	HI (4)	P_{gender}, P_{group}
E_{RE} (kJ d^{-1})	–2	37.9±1.1	39.2±1.0	39.8±2.5	29.4±3.9	23.3±2.1	26.8±1.4	
	+2	38.4±2.8	38.9±1.4	43.5±1.8	32.2±3.4	27.2±2.6	31.4±5.6	0.006, 0.787
	+12	38.0±1.6	36.7±2.2	34.7±2.7	34.6±3.3	27.5±3.0	34.8±2.4	
E_{RE} (%)	–2	100	100	100	100	100	100	
	+2	102.0±8.7	99.6±5.0	111.6±10.0	112.2±7.8	116.9±5.8	115.1±15.0	0.001, 0.889
	+12	100.5±4.6	94.0±7.0	88.2±7.9	120.6±7.1	120.7±15.8	129.7±3.5	
PAL	–2	1.44±0.03	1.46±0.07	1.41±0.03	1.69±0.16	1.90±0.12	1.69±0.08	
	+2	1.45±0.08	1.38±0.03	1.37±0.01	1.62±0.11	1.80±0.12	1.72±0.18	0.041, 0.640
	+12	1.47±0.03	1.44±0.06	1.53±0.08	1.55±0.02	1.62±0.10	1.56±0.02	

SO, sham-operated mice; LO, mice containing implants corresponding to 2% of body mass; HI, mice containing implants corresponding to 10% of body mass; M, males; F, females.

Values for resting energy expenditure E_{RE} (kJ d^{-1}) are means of the10 lowest values for total daily energy expenditure, measured using indirect calorimetry over a 48 h period. E_{RE} (%) was expressed relative to mean E_{RE} of basal measurement (–2 weeks).

+2, 2 weeks after weight load; +12, 12 weeks after weight load.

Values are means ± S.E.M.; number of measured animals is given in parentheses.

Differences were tested using two-way repeated-measures ANOVA (factors: gender, group)

Both genders showed an acute reduction in food intake accompanied by a reduction in body mass, which was obviously due to post-surgical stress. Male mice carrying the heavy implant decreased M_{BB} during weeks 1 and 2 following weight load to an extent that M_{TB} corresponded to basal values. This observation seems to comply with the set-point theory of body mass and a mass-dependent regulation of body mass. However, this effect was only transiently present as M_{TB} increased thereafter and became significantly higher compared to SO males. In addition, the transiently observed recovery of the basal body mass was not present in HI females, which acutely lost less M_{BB} and regained it rapidly to levels similar to SO and LO females. HI males displayed a permanently lower M_{BB} following weight load compared to SO males, the difference accounting for about half of the implanted mass (5%). Although not significant, the permanence of the reduction seems to suggest a real effect. Therefore, in the long-term there might be only a partial compensation of the increased body mass and only in male and not female mice.

The partial compensation observed in males occurred mainly at the expense of fat mass; lean body mass was not changed. This seems reasonable considering that a certain muscle mass is necessary to move an acutely heavier body mass. In addition, since fat contains much more energy per mass unit compared to lean mass, fat loss due to negative energy balance would lead to a lower overall body mass reduction than a loss in lean mass.

Energy intake was not affected by the additional weight in the long-term. After the initial reduction, male mice of all groups returned to their initial levels of food intake by the second week after implantation. One could suggest that the lack of an increase in energy intake in HI males that compensated for the acute weight loss led to the slightly decreased M_{BB} compared to SO males. However, HI females also showed normal energy intake but displayed comparable M_{BB} to SO and LO females. This suggests that males are either more sensitive in their perception of an increased body mass or that they showed a higher stress response to the additional weight compared to females. There are some studies to support the first suggestion. Firstly, our observations in male HI mice confirm the results of Adams et al. (2001), who described a compensatory decrease in M_{BB} following intraperitoneal implantation of 1–3 g weights into male deer mice *Peromyscus maniculatus*. This reduction was paralleled by a reduction in food intake, which was significant only for the heaviest implant group (3 g). The authors suggested the existence of a ‘mechanical set point’, referring to the loading of the musculoskeletal system that leads *via* unknown mechanisms to perception of the animal’s body mass. However, they did not show a time course of body mass change or energy intake and investigated male mice only for a period of 5 weeks. The overall reduction in food intake they described could thus have resulted from the initial, stress induced reduction after surgery rather than being the result of a regulated process to approach the set-point mass. In addition, the authors described that the reduction of M_{BB} in the 3 g implanted group corresponded to about 1.5 g. This represents a M_{BB} reduction of half the implanted mass, similar to our study. Therefore, the conclusions drawn from our study could also be applied to the study by Adams et al. (2001).

Secondly, several studies have examined the effect of hypergravity induced by centrifugation, thereby multiplying the mass load on the body. Overall they show that hypergravity leads to an acute decrease in body mass in rodents, associated with acutely decreased energy intake and increased maintenance feed requirements (Wade et al., 1997, 2002; Warren et al., 1997). The decrease in body mass resembles that of male HI mice in our study. In fact, most of these studies

were done in male rodents only. One study that included male and female rats showed that female rats raised in hypergravity (2.5 ***g***) showed a much less pronounced body mass reduction than male rats (Wubbels and de Jong, 2000), suggesting a stricter maintenance of M_{BB} in females similar to our results.

Thirdly, after i.p. implantation of a telemeter with a weight corresponding to about 12% of initial body mass into male Swiss Webster mice, M_{BB} was acutely reduced by the mass of the implant but was increasing above basal levels by the end of a 14 day period (Perry et al., 2000). However, a control group was not included in this study.

It seems that at least in male rats and mice an acutely increased body mass is partially compensated by a reduction in M_{BB}, although to a lower extent than would be expected from the mass of the implant if the maintenance of a mass-specific set-point is assumed. This suggests that a mass-specific set-point regulation of body mass is not accurate or might be impaired by still unknown competing mechanisms. The fact that males in general needed longer to recover their initial M_{BB} than females could point to a higher stress susceptibility of male mice.

We expected an increase in energy expenditure in HI mice, considering the increased energy demand needed for carrying the additional weight. However, E_{RE} was only slightly and not significantly increased in HI animals 2 weeks after weight implantation. The failure to detect significant group differences in energy expenditure is probably due to the small sample size, but also to the high intra-individual variability of energy expenditure. It should be emphasized that only very small effects on energy expenditure could be expected, since the maximum weight load was only 10% of body mass, i.e. around 2–3 g. This is not much considering that mice normally display daily mass fluctuations in the range of 1–2 g according to our own measurements (not shown). Interestingly, physical activity was apparently also not affected by implantation, as evident from the PAL values. However, in mice implanted with a telemetry transmitter, their willingness to practice in running wheels decreased after i.p. implantation (Perry et al., 2000), suggesting a decrease in physical activity. In contrast, subcutaneous implantation in rats of telemetry transmitters corresponding to 15% of the animals' initial mass did not impair activity levels during a 5 h measurement period (Moran et al., 1998) supporting the observation that energy expenditure was not influenced by the additional weight. These different observations suggest that the site of implantation (intraperitoneal *versus* subcutaneous) might have an impact on physical activity and overall energy expenditure. Intraperitoneal implantation seems rather more likely to impair physical performance and energy expenditure than subcutaneous implantation. In addition, studies in humans carrying additional weights suggested that an increase in energy expenditure occurs only if a certain mass threshold is obtained, usually higher than 10% of initial body mass (Maloiy et al., 1986; Jones et al., 1987). Possibly, artificial augmentation of body mass by 10% in mice in the present study lies below this threshold and thus fails to increase energy expenditure due to increased physical work required to move the body.

Data on changes of energy expenditure and physical activity following weight implantation remain contradictory, depending on the species, implant mass, and site of implantation, and need further investigation. In addition, direct measurements, e.g. by use of cages equipped with infrared beams, could be more useful for evaluation of physical activity than a calculation of PAL.

The gender-specific differences in body mass point to different strategies in males and females to cope with a situation affecting energy demands and body mass. There are a several rodent and human studies to support this suggestion. In addition to gender-specific responses in energy balance under hypergravity conditions as mentioned above, different catecholamine responses to space flight, i.e. in microgravity, have been reported in male and female astronauts (Stein and Wade, 2001), pointing to gender-specific hormonal effects on body mass regulation. Gender specific responses were also reported in rats subjected to a change in energy expenditure by forced and voluntary exercise. Male rats decreased body mass under the influence of forced exercise whereas female rats increased energy intake and thereby maintained their body mass (Nance et al., 1977; Cortright et al., 1997). Interestingly, similar effects were observed in humans subjected to different levels of weekly fitness training. Despite an increased daily energy expenditure, men did not increase energy intake to compensate for the energy loss (Stubbs et al., 2002a), while women at least partially increased their energy intake (Stubbs et al., 2002b). In the present study, HI females showed a more efficient gain in body mass (both M_{TB} and M_{BB}) compared to HI males, although energy intake was not significantly increased in HI females compared to SO and LO females. This implies a higher efficiency of energy utilization and conservation of females compared to males. This seems reasonable considering the main evolutionary responsibility of females for reproduction (Cortright and Koves, 2000; Hoyenga and Hoyenga, 1982) and the necessity to maintain adequate energy stores throughout gestation and suckling period. It was suggested that this higher efficiency is a result of higher selection pressures on females during evolutional development (Cortright and Koves, 2000).

Another interesting, gender-specific phenomenon is our observation that LO females showed increased M_{BB} in the second half of the study period compared to SO and HI females and also displayed the highest mean fat increase after 14 weeks. Obviously, in females but not in males the implant volume has an impact on energy balance. It is conceivable that the implant leads to an abdomen distension similar to that experienced during gestation, causing metabolic responses to maintain and increase energy stores rather than to maintain a certain body mass set-point, which in our model would mean to decrease energy stores. During gestation this regulation would ensure an adequate energy supply for the offspring. It is therefore possible that there are two different systems competing with each other: mass-specific set-point regulation of body mass overlapping with a volume-specific response that increased energy resources supposedly by mimicking

gestation. The latter should be female-specific and hence would not appear in males.

Conclusions

We found gender-specific responses following an acute, artificial body mass increase. Perception of body mass *per se* and a compensatory decrease in M_{BB} following artificial body mass augmentation appeared only partially and in males rather than in females. This compensation happened mainly at the expense of fat mass; lean body mass was maintained at basal levels in both genders. Long-term energy intake was not affected by the weight implantation. Also measurements of energy expenditure revealed no significant influence on body mass, possibly because the sensitivity of the measurement was insufficient to detect the range of actual changes. In females the volume of the implanted weight seemed to have a more important impact on body mass development than the actual weight. It therefore seems that body mass *per se* might not be a major player in the set-point regulation of body mass. In females especially, the distension of the peritoneum induced by the volume of the weight could have caused the observed gender-specific body mass development. The real influence of this suggestion could be further investigated by implanting weights into other sites of the body different from the peritoneum. Overall, our results do not support the set-point theory of body mass control with weight *per se* being regulated. It seems that different compartments of body mass are perceived and regulated differently. Notably, the strict conservation of lean body mass compared to the evident changes in body fat mass suggests that the ponderostat is rather linked to lean body mass, thus arguing against a predominant lipostatic regulation of body mass.

We thank Carola Plaue for excellent technical assistance and Dr Cornelia Koebnick for statistical advise. We are grateful to Dr Sylvia Ortmann and Andreas Subke for the design and manufacture of the metal implant cores, respectively.

References

Adams, C. S., Korytko, A. I. and Blank, J. L. (2001). A novel mechanism of body mass regulation. *J. Exp. Biol*. **204**, 1729-1734.

Berthoud, H.-R. (2002). Multiple neuronal systems controlling food intake and body weight. *Neurosci. Biobehav. Rev*. **26**, 393-428.

Bradley, P. (1978). The ponderostat and a physiological model of obesity. *Am. J. Clin. Nutr*. **31**, 1975-1977.

Cabanac, M. (2001). Regulation and the ponderostat. *Int. J. Obes.* **25**, **Suppl.** 5, S7-S12.

Cabanac, M., Dulaux, R. and Spector, N. H. (1971). Sensory feedback in regulation of body weight: is there a ponderostat? *Nature* **229**, 125-127.

Clegg, D. J., Riedy, C. A., Smith, K. A., Benoit, S. C. and Woods, S. C. (2003). Differential sensitivity to central leptin and insulin in male and female rats. *Diabetes* **52**, 682-687.

Cortright, R. N. and Koves, T. R. (2000). Sex differences in substrate metabolism and energy homeostasis. *Can. J. Appl. Physiol.* **25**, 288-311.

Cortright, R. N., Chandler, M. P., Lemon, P. W. R. and DiCarlo, S. E. (1997). Daily exercise reduces fat, protein and body mass in male but not female rats. *Physiol. Behav*. **62**, 105-111.

Frenz, U. (1999). Whole body calorimetry. In *Handbook of Thermal Analysis and Calorimetry*, Vol. 4 (ed. P. B. Kemp), pp. 511-555. Amsterdam, Lausanne, New York, Oxford, Shannon, Singapore, Tokyo: Elsevier Science.

Friedman, J. M. and Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature* **395**, 763-770.

Harris, R. B. S. (1990). Role of set-point theory in regulation of body weight. *FASEB J*. **4**, 3310-3318.

Hoyenga, K. B. and Hoyenga, K. T. (1982). Gender and energy balance: sex differences in adaptations for feast and famine. *Physiol. Behav*. **28**, 545-563.

Jéquier E. and Tappy L. (1999). Regulation of body weight in humans. *Physiol. Rev*. **79**, 451-480.

Jones, C. D., Jarjou, M. S., Whitehead, R. G. and Jequier, E. (1987). Fatness and the energy cost of carrying loads in African women. *Lancet* **2**, 1331-1332.

Keesey, R. E. and Corbett, S. W. (1984). Metabolic defense of the body weight set-point. In *Eating and its disorders* (ed. A. J. Stunkard and E. Stellar), pp. 87-96. New York: Raven Press.

Klaus, S., Münzberg, H., Trüloff, C. and Heldmaier, G. (1998). Physiology of transgenic mice with brown fat ablation: obesity is due to lowered body temperature. *Am. J. Physiol*. **274**, R287-R293.

Lacy, E. L. and Bartness T. J. (2003). Autologous fat transplants influence compensatory white adipose tissue mass increases after lipectomy. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **286**, R61-R70.

Macdonald, I. A. (2003). The body weight set-point – the case against. In *Progress in Obesity Research*, Vol. 9 (ed. G. Medeiros-Neto, A. Halpern and C. Bouchard), pp. 724-728. Montrouge, France: John Libbey Eurotext Ltd.

Maloiy, G. M., Heglund, N. C., Prager, L. M., Cavagna, G. A. and Taylor, C. R. (1986). Energetic cost of carrying loads: have African women discovered an economic way? *Nature* **319**, 668-669.

Moran, M. M., Roy, **R. R., Wade, C. E., Corbin, B. J. and Grindeland, R. E.** (1998). Size constraints of telemeters in rats. *J. Appl. Physiol.* **85**, 1564-1571.

Nance, D. M., Bromley, B., Barnard, R. J. and Gorski, R. A. (1977). Sexually dimorphic effects of forced exercise on food intake and body weight in the rat. *Physiol. Behav*. **19**, 155-158.

Perry, A. **M., Huettemann, D. A., Brockway, B. P., Zwiers, L. M., Gelsema, A. J. M., Schwartz, R. S. and Kramer, K.** (2000). A new method for measurement of blood pressure, heart rate, and activity in the mouse radiotelemetry. *J. Appl. Physiol*. **88**, 1537-1544.

Proll, J., Petzke, K. J., Ezeagu, I. E. and Metges, C. C. (1998). Low nutritional quality of unconventional tropical crop seeds in rats. *J. Nutr*. **128**, 2014-2022.

Speakman, J. R., Stubbs, R. J. and Mercer, J. G. (2002). Does body mass play a role in the regulation of food intake? *Proc. Nutr. Soc*. **61**, 473-487.

Stein, T. P. and Wade, C. E. (2001). The catecholamine response to spaceflight: role of diet and gender. *Am. J. Physiol. Endocrinol. Metab*. **281**, E500-E506.

Stubbs, R. J., Sepp, A., Hughes, D. A., Johnstone, A. M., Horgan, G. W., King, N. and Blundell, J. (2002a). The effect of graded levels of exercise on energy intake and balance in free-living men, consuming their normal diet. *Eur. J. Clin. Nutr.* **56**, 129-140.

Stubbs, R. J., Sepp, A., Hughes, D. A., Johnstone, A. M., King, N., Horgan, G. and Blundell, J. E. (2002b). The effect of graded levels of exercise on energy intake and balance in free-living women. *Int. J. Obes. Relat. Metab. Disord*. **26**, 866-869.

Wade, C. E., Harper, J. S., Daunton, N. G., Corcoran, M. L. and Morey-Holton, E. (1997). Body mass change during altered gravity: spaceflight, centrifugation, and return to 1G. *J. Gravit. Physiol*. **4**, 43-48.

Wade, C. E., Moran, M. M. and Oyama, J. (2002). Resting energy expenditure of rats acclimated to hypergravity. *Aviat. Space Eviron. Med.* **73**, 859-864.

Warren, L. E., Horwitz, B. A. and Fuller, C. A. (1997). Gravity and body mass regulation. *J. Gravit. Physiol.* **4**, P89-P92.

Wubbels, R. J. and de Jong, H. A. A. (2000). Vestibular-induced behaviour of rats born and raised in hypergravity. *Brain. Res. Bull*. **52**, 349-356.