INTRODUCTION

Recently, the "gravitostat", an entirely novel body weight homeostatic mechanism, was identified both in rodents and in humans. Its discovery offers the possibility of exploring new therapeutic avenues to reduce body fat, and thus body weight, in obese individuals. The existence of the gravitostat was first demonstrated by increasing weight loading in rodents via an implanted artificial...
weight (corresponding to 15% of their biological weight); this caused a reduction in biological weight, such that total body weight (biological + capsule) was approximately maintained. Importantly, studies in mice revealed that those of higher body weight (induced by offering a high-fat diet) were more sensitive to the gravitostat body weight gain-preventing effects than lean animals. This turned out to be of considerable clinical importance because obese human subjects had a reduced body weight and body fat in response to increased loading in a very recent randomised clinical trial.

The gravitostat sensor is assumed to reside in weight loading bones because mice lacking osteocytes do not reduce their biological weight, nor exhibit decreased fat mass in response to loading. The effector systems of the gravitostat reduce food intake, implying the engagement of brain pathways and potentially also endocrine signals of importance for feeding control. The anorexigenic homeostatic hormone leptin was rapidly ruled out as a mediating system because the body weight reducing effects of weight loading could also be detected in mice that lack leptin. Moreover, obese rodents and humans become leptin resistant, yet they remain responsive to the gravitostat. Although weight loading does not appear to engage leptin, it possibly targets pathways downstream to those shown to exert leptin's anorexigenic effects.

To better understand the reason behind the lower food intake in loaded animals, we established a gravitostat model in Sprague-Dawley rats, where we more precisely could measure meal size and meal frequency because it has been suggested that such data can provide useful information about the relative importance of the hypothalamus versus brainstem in the regulation of meal patterns. In addition, we aimed to explore the effects of loading on parameters linked to energy expenditure, namely caloric efficiency (CE) and locomotor activity, which arguably are more reliable in rats than in mice. Finally, in addition to verifying beneficial effects of loading on glucose homeostasis and leptin levels, we assessed plasma levels of the orexigenic hormone ghrelin, aiming to determine whether decreased levels could contribute to the weight loss effect.

2 | MATERIALS AND METHODS

2.1 | Animals

Because loading appears to impact similarly on body weight progression in male and female mice, only adult male Sprague-Dawley rats (10 weeks of age at arrival; Charles River, Sulzfeld, Germany) were used (n = 32). They were kept under standardised non-barrier conditions of a 12:12-hour light/dark photocycle (lights on 07.00 AM) at 20-22°C and approximately 50% relative humidity. Initially, the rats were group-housed (two or three rats per cage) and had ad lib. access to standard chow (Teklad diet #2016; Harlan Laboratories, Cambridge, UK; macronutrient composition: 22% protein, 66% carbohydrate, 12% fat by energy, 3.0 kcal g⁻¹) and water. After 1 week of acclimatisation, half of the rats switched diet and were given ad lib. access to a high-fat diet (HFD) (#D12492; Research Diets, New Brunswick, NJ, USA; macronutrient composition: 20% protein, 20% carbohydrate, 60% fat by energy, 5.24 kcal g⁻¹) throughout the study to induce diet-induced obesity (DIO group). The remaining 16 rats continued with ad lib. chow to keep normal weight after the acclimisation period (lean group). Procedures were authorised by the local ethical committee for laboratory animal care and use in Gothenburg, Sweden (permit 132-2016) and complied with European guidelines (Decree 86/609/EEC).

2.2 | Weight loading surgery

DIO and lean groups were further divided into load and control groups balanced between groups by their pre-surgical body weight: DIO load (n = 8), DIO control (n = 8), lean load (n = 8) and lean control (n = 8). Rats were i.p. implanted with capsules (diameter 1.8 cm, length 6.5 cm, weight 7.3 to 7.8 g; manufactured in-house) under isoflurane anaesthesia at 15 weeks of age. Prior to surgery, capsules were either loaded with wolfram granulate (Edstraco AB, Rönninger, Sweden) weighing 15% of the body weight (load) or left empty, weighing 1.3% of the body weight (control). Capsules were attached with suture to the peritoneum, after which the peritoneum was closed with suture and the skin was closed with clips. An analgesic (5 mg mL⁻¹ Rimadyl®, dose: 1 mL kg⁻¹ rat; Orion Pharma Animal Health, Solletuna, Sweden) was injected s.c. Rats were individually housed after surgery.

2.3 | Analysis of body weight, diurnal feeding, meal pattern and CE

Post-loading, body weight and food consumption were monitored regularly. The duration of the study (3 weeks) was informed from studies in mice showing that the loading effect on body weight stabilises during week 2 and persists until the end of the study at 7 weeks. Biological body weight was calculated by subtracting the capsule weight from the total body weight. Food intake was measured by weight (g) and converted into energy intake (kcal) to enable a better comparison between the different diets. Data showing the change in biological body weight during the first 13 days and food intake on day 10 and 16 have been reported previously in load and control obese rats. An automated feeding and drinking monitoring system (TSE LabMaster, Project 4261; TSE Systems, Bad Homburg, Germany) was used to analyse detailed diurnal feeding, drinking and meal pattern. Food hoppers and water bottles were suspended on calibrated sensors that register food and water consumption. HFD-fed DIO rats (both load and control groups) were transferred into the cages on day 10 after surgery for detailed 6-day measurements. Chow-fed lean rats (both load and control groups) were housed in the cages for the entire study period and the same 1-week period was used for diurnal feeding, drinking and meal pattern analysis. Data for meal analysis were collected as binary data every 10 seconds. Meal analysis was undertaken using LabMaster software (TSE Systems, Bad Homburg, Germany).
Systems), whereby all meals occurring during the study period were recorded chronologically to allow the evaluation of single feeding bouts. The start of a meal was defined by food removal (≥ 0.5 g) and the meal ended when no further food removal occurred before the end of the inter-meal interval of 10 min. Caloric intake, meal frequency and meal size were summarised over different periods: dark phase (12 hours), light phase (12 hours) and total day (24 hours), and then averaged per rat and group. A 10-minute inter-meal interval and a minimum meal size of 0.5 g are commonly used in defining meals in rats. CE was calculated as: CE = (body weight change/caloric intake) × 100.

### 2.4 | Locomotor activity

To monitor locomotor activity, 16 operant conditioning chambers (30.5 × 24.1 × 21.0 cm; Med-Associates Inc., St Albans, VT, USA) with four infrared beams located along the floor were used. Rats were allowed to explore the chambers for 1 hour during a habituation session on day 17 post-surgery. Locomotor activity monitoring occurred on day 18 post-surgery. This included 3-hour test sessions during the mid-light phase and the early dark phase. Food and water were withheld during the habituation and test sessions. Rats were always brought to the testing room 30 minutes prior to starting the measurements. In this paradigm, anxiety is unlikely to influence locomotor activity because, in addition to the 1-hour habituation session, the data were collected over 2 × 3-hour time periods, during which the rats should have adapted to their environment.

### 2.5 | Sacrifice, blood samples and body composition

All rats were sacrificed on day 24 post-surgery after an overnight fast. Rats were anaesthetised with isoflurane. A tail prick blood sample was taken for blood glucose concentration (Accu-Chek® Compact Plus; Roche Diagnostics Scandinavia AB, Bromma, Sweden). Blood samples, taken by heart puncture, were either processed to serum for analysis of circulating hormones (leptin and insulin) and metabolites (cholesterol and triglyceride) or processed to plasma using 4-(2- aminoethyl)-benzenesulfonyl-fluoride hydrochloride as a protease inhibitor for analysis of total ghrelin. Post-mortem, white adipose tissue (WAT, s.c. WAT from the hind legs, perirenal including retroperitoneal WAT) and the gastrocnemius muscle from the right leg was dissected and weighed as a measure of lean body mass.

### 2.6 | Quantification of circulating hormones, circulating metabolites and insulin sensitivity

Samples were assayed in duplicate for leptin, insulin and total ghrelin using enzyme-linked immunosorbent assay kits (rat leptin #EZRL-83K, rat/mouse insulin #EZRMI-13K and rat/mouse total ghrelin #EZRGRT-91K; EMD Millipore Corporation, Billerica, MA, USA). The sensitivity of the assay was 0.2 ng mL⁻¹ for leptin and insulin, and 0.156 ng mL⁻¹ for ghrelin. The intra-assay coefficients of variation (CV) were 6.80%, 2.06% and 5.61% for the leptin, insulin and total ghrelin assays, respectively. Samples from both cohorts were run simultaneously for each assay. The inter-assay CVs were 6.00%, 1.33% and 2.74% for the leptin, insulin and total ghrelin assays, respectively. Cholesterol and triglyceride were assayed in duplicate by colorimetric enzyme assays (cholesterol #TR13421, triglyceride #TR22421; Infinity™; Thermo Scientific, Middletown, VA, USA) using a multiconstituent calibrator (#1E65-05; Abbott Laboratories, Chicago, IL, USA). The intra-assay CVs were 12.18% and 7.83%, respectively. The homeostatic model assessment for insulin resistance (HOMA-IR) index was used for estimation of insulin sensitivity. It was calculated as: HOMA-IR = fasting serum insulin (mIU L⁻¹) × fasting blood glucose (mmol L⁻¹)/22.5.

### 2.7 | Statistical analysis

Analyses were conducted using the SPSS, version 22 (IBM Corp., Armonk, NY, USA). Data were first checked for equal variances and normal distribution. Importantly, to be able to treat DIO and lean rats as separate groups, either two-way repeated measures (RM) ANOVA (for continuous variables) or two-way ANOVAs were always run first. We handled DIO and lean groups separately only when at least one of these three conditions was met to obtain (i) a significant Diet × Loading interaction; (ii) a significant effect of Diet and/or Loading with a near-significant Diet × Loading interaction (set at P < 0.1); and (iii) only a significant effect of Diet and/or Loading, but always in conjunction with a scientific argument that would justify such data split. After splitting data according to Diet, continuous variables were analysed using one-way RM ANOVAs with Time as the within-subject factor and Loading (load vs control) as the between-subject factor, whereas discrete variables were analysed using one-way ANOVA (Loading). In general, one-way ANOVA tests were conducted when applicable at each data point after RM ANOVA analyses. P < 0.05 was considered statistically significant. Data are presented as the group mean ± SEM. Statistical details of the main analysis include the P value, its corresponding F ratio value and, in parentheses, the degrees of freedom of the numerator and denominator used to calculate the F ratio.

### 3 | RESULTS

#### 3.1 | Effect of weight loading on biological body weight and food intake

The body weights before weight loading surgery were 586.6 ± 6.5 g (n = 16) for the DIO group and 559.3 ± 9.1 g (n = 16) for the lean group (Loading: F₁,₃₀ = 5.928, P = 0.021). With these data, we were able to link the effectiveness of loading to different body weights
and adiposity levels determined at the start of the procedure. The first two-way RM ANOVA “filter” for the entire period (day 0 to day 20 post-surgery) revealed a Time × Diet interaction \((F_{9,20} = 7.459, P < 0.001)\), a Time × Loading interaction \((F_{9,20} = 5.363, P = 0.001)\), and general effects of the factors Diet \((F_{1,28} = 9.644, P = 0.004)\) and Loading \((F_{1,28} = 4.501, P = 0.043)\) on body weight progression. Collectively, these results suggest that following the implantation of the capsules, all rats initially lost weight regardless of the weight of the implanted capsules, including those in both control groups, as a response to the surgical trauma. However, the control groups recovered faster from surgery than the load groups and, after approximately 2 weeks, the control groups reached or were above their start weight, whereas load groups remained in a negative weight progression during the first week post-surgery, the factors Diet and Loading did not interact significantly, suggesting that, initially, both DIO and lean rats responded similarly to the surgical trauma (and to loading) by losing weight to the same extent. Initially, both DIO and lean rats responded similarly to the surgical trauma (and to loading) by losing weight to the same extent. However, from day 8 post-surgery, the body weights of both DIO and lean rats started to diverge significantly \((\text{Time} \times \text{Diet interaction: } F_{4,25} = 9.569, P < 0.001; \text{Time} \times \text{Loading interaction: } F_{4,25} = 4.364, P < 0.001; \text{Time} \times \text{Diet} \times \text{Loading interaction: } F_{4,25} = 3.314, P = 0.026)\). Therefore, data from DIO rats and lean rats were handled separately. Further tests confirmed that loading protected DIO rats from HFD-induced weight gain, starting from day 13 (day 13: \(P = 0.010\); day 15: \(P = 0.006\); day 20: \(P = 0.004\)) (Figure 1B), whereas it did not explicitly change body weight in lean-loaded rats compared to their non-loaded controls (Figure 1A). The eventual weight difference at day 20 after loading was greater in the DIO group (−13.0% vs DIO controls) compared to the lean group (−4.9% vs DIO controls) \((P = 0.041)\) (Table 1). Additionally, we also detected a Diet × Loading interaction \((F_{1,28} = 9.582, P = 0.004)\) and a general effect of the factor Loading \((F_{1,28} = 36.456, P < 0.001)\) on body weight change from pre-surgery and day 20 after loading surgery (Figure 1C).

Following capsule implantation, all rats showed a very low caloric intake, including the control rats, while recovering from surgery (Figure 1D,E). Two-way RM ANOVA analyses revealed a Time × Diet interaction \((F_{13,26} = 6.321, P < 0.001)\) and a Time × Loading interaction \((F_{13,26} = 2.939, P = 0.022)\), as well as general effects of the factor Loading \((F_{1,28} = 34.356, P < 0.001)\) and an almost significant Diet × Loading interaction \((F_{1,28} = 3.156, P = 0.087)\) on caloric food intake. Therefore, the results from DIO rats and lean rats were treated separately. Overall, loaded animals were the most affected regardless of the group they belonged to (Loading effect: DIO rats, \(F_{1,14} = 20.580, P < 0.001\); lean rats: \(F_{1,14} = 14.320, P = 0.002\) (Figure 1D,E). Nevertheless, further tests revealed that weight-loaded DIO rats had a reduced caloric food intake compared to control DIO rats starting on day 2 post-loading. Food intake

![Figure 1](attachment:image1.png)

**Figure 1** Effect of weight loading on biological body weight and caloric food intake. Effect of weight loading on biological body weight in (A) Lean rats on chow and (B) diet-induced obese (DIO) rats on a high-fat diet (HFD) over 20 days after loading surgery. (C) Body weight change between pre-surgery and day 20 after loading surgery. Effects of weight loading on caloric intake of (D) chow in lean rats and (E) HFD in DIO rats over 14 days after loading surgery. Data are presented as the mean ± SEM. *\(P < 0.05\), **\(P < 0.01\) and ***\(P < 0.001\) by one-way ANOVA tests (load vs control), \(n = 8\) per group.
remained significantly decreased over the whole study period in the DIO group, with the exception of day 4 (day 2: \( P = 0.020 \); day 3: \( P = 0.014 \); day 5: \( P = 0.035 \); day 6: \( P = 0.002 \); days 7-8: \( P < 0.001 \); day 9: \( P = 0.009 \); day 10: \( P = 0.001 \); day 11: \( P = 0.004 \); day 12: \( P = 0.042 \); day 13: \( P = 0.005 \) and day 14: \( P = 0.003 \)) (Figure 1E), in line with previous results. By contrast, the effect of loading mainly remained for 7 days in the lean group (day 2: \( P = 0.012 \); days 3-5: \( P = 0.001 \); day 6: \( P < 0.001 \); day 7: \( P = 0.016 \); day 8: \( P = 0.020 \)) (Figure 1D) and thereafter became more diffuse (day 10: \( P = 0.026 \); day 11: \( P = 0.021 \) and day 14: \( P = 0.024 \)).

### TABLE 1  Relative effect of weight loading on different parameters to compare diet-induced obese (DIO) and lean rats

| Parameter                        | ΔLoad versus Control in DIO (%) | ΔLoad versus Control in lean (%) | DIO versus lean |
|---------------------------------|---------------------------------|---------------------------------|-----------------|
| Body weight (day 20)            | -13.0                           | -4.9                            | \( P = 0.041 \)  |
| Epididymal WAT weight           | -36.5                           | 9.3                             | \( P < 0.001 \)  |
| Subcutaneous WAT weight         | -51.7                           | -37.3                           | NS              |
| Perirenal WAT weight            | -56.6                           | -35.4                           | NS              |
| Total WAT weight                | -49.2                           | -24.8                           | \( P = 0.009 \)  |
| Caloric intake (total day)      | -31.7                           | -8.8                            | \( P = 0.017 \)  |
| Caloric efficiency (mean)       | -66.7                           | -3.6                            | \( P = 0.047 \)  |
| Serum leptin                    | -52.4                           | -39.8                           | NS              |
| Serum insulin                   | -27.8                           | -19.5                           | NS              |
| Plasma total ghrelin            | 42.1                            | 28.8                            | NS              |
| HOMA-IR                         | -50.5                           | -8.1                            | NS              |

Note: Data for various parameters are presented as relative change (Δ) as a percentage (%) in weight loaded versus control rats; data from weight loaded rats were normalised to their respective control group. One-way ANOVA was used to determine significant differences in effect of weight loading in DIO versus lean rats. HOMA-IR, homeostatic model assessment for insulin resistance; NS, non-significant; WAT, white adipose tissue.

### FIGURE 2  Effect of weight loading on terminal body composition parameters.

Effect of weight loading on white adipose tissue (WAT) weights (epididymal WAT, s.c. WAT from hind legs, perirenal including retroperitoneal WAT) as a measure of body fat mass in (A) lean rats on chow and (B) diet-induced obese (DIO) rats on a high fat diet (HFD). (C) Total WAT weight is the weight of all measured WAT taken together. Effect of weight loading on the weight of the gastrocnemius muscle as a measure of body lean mass in (D) Lean rats fed chow and (E) DIO rats on a HFD. Data are presented as the mean ± SEM. *\( P < 0.05 \) and **\( P < 0.01 \) by one-way ANOVA tests (load vs control), \( n = 8 \) per group.
3.2 | Effect of weight loading on body composition

Three different WAT pads were measured to assess body fat mass. Two-way ANOVA revealed significant effects of the factor Diet on epididymal WAT ($F_{1,27} = 26.953, P < 0.001$), perirenal WAT ($F_{1,27} = 18.546, P < 0.001$) and the sum of the three WAT pads ($F_{1,27} = 11.506, P = 0.002$), but not the s.c. WAT, as well as significant effects of the factor Loading on epididymal ($F_{1,27} = 8.062, P = 0.008$), s.c. ($F_{1,28} = 22.247, P < 0.001$), perirenal ($F_{1,27} = 16.619, P < 0.001$) and the sum of the WAT pads ($F_{1,27} = 19.929, P < 0.001$). Likewise, we found Diet × Loading interactions for the epididymal ($F_{1,27} = 13.534, P = 0.001$), perirenal ($F_{1,27} = 5.740, P = 0.024$) and total WAT ($F_{1,27} = 5.935, P = 0.022$). DIO rats responded to loading with a decrease in epididymal ($P = 0.001$), s.c. ($P = 0.001$) and perirenal WAT ($P = 0.002$) (Figure 2B), whereas lean rats only had a reduction in s.c. WAT ($P = 0.020$) (Figure 2A). The sum of the three WAT pads was reduced by loading in the DIO ($P = 0.001$) but not the lean group (Figure 2C). The loading-dependent decrease in weight of the epididymal WAT (−36.5% for DIO rats vs 9.3% for lean rats; $P < 0.001$) and total WAT (−49.2% for DIO rats vs −24.8% for lean rats; $P = 0.009$) was greater in DIO than lean rats (Table 1). The weight of the gastrocnemius muscle was not affected by either Diet or Loading factors (Figure 2D-E).

3.3 | Effect of weight loading on diurnal feeding, meal pattern and CE

Food intake microstructure was analysed on days 10 to 16 post-loading. Two-way ANOVA analyses for the entire 24-hour period revealed significant (or nearly-significant) effects of the factor Diet on caloric intake ($F_{1,28} = 3.604, P = 0.068$) and meal size ($F_{1,28} = 4.917$).
Effects of weight loading on caloric intake (P = 0.035), but not the frequency of meals, as well as significant effects of the factor Loading on caloric intake (F_{1,28} = 16.352, P < 0.001), meal size (F_{1,28} = 12.754, P = 0.001) and meal frequency (F_{1,28} = 5.481, P = 0.027). Likewise, we found a Diet × Loading interaction on caloric intake (F_{1,28} = 6.729, P = 0.015). Although we did not detect Diet × Loading interactions for either meal size or meal frequency, we nevertheless split data according to Diet because, arguably, diet and/or loading-dependent differences in caloric intake should have been caused by changes in at least one of these two variables (meal size or frequency). In the DIO load group, there was a decrease in dark phase feeding (P = 0.032), light phase feeding (P = 0.006) and in 24-hour feeding (P = 0.003) (Figure 3B). Only dark phase food intake was significantly suppressed in the lean group by loading (P = 0.023) (Figure 3A). Additionally, the decrease in mean 24-hour caloric intake as a result of loading was greater in the DIO (−31.7% vs DIO controls) than in the lean group (−8.8% vs lean controls) (P = 0.017) (Table 1). Loading also impacted upon meal pattern. Rats in the DIO load group had a reduced meal size during the light phase (P = 0.010) (Figure 3D). In the lean group, loading decreased meal size during the dark phase (P = 0.018), the light phase (P = 0.001) and the total day (P = 0.006) (Figure 3C). Meal frequency (ie, meal number) in the lean group, unlike that of the DIO group, was increased by loading during the light phase (P = 0.001) (Figure 3E-F).

CE was analysed under the same time period (days 10, 13 and 15 and as a mean for each rat over the 3 days). Two-way ANOVA revealed general effects of the factors Diet (F_{1,26} = 47.331, P < 0.001) and Loading (F_{1,26} = 6.355, P = 0.018), as well as an almost significant Diet × Loading interaction (F_{1,26} = 3.162, P = 0.087) on CE. Following data split, we detected a significant effect of loading on mean CE (P = 0.035) in the DIO group (Figure 3H). By contrast, CE was unaffected by loading in the lean group (Figure 3G). The effect of loading on mean CE was greater in the DIO (−66.7% vs DIO controls) than in the lean group (−3.6% vs lean controls) (P = 0.047) (Table 1).

3.4 | Effect of weight loading on locomotor activity

Two-way ANOVA revealed a Diet × Loading interaction for the dark-phase locomotor activity (F_{1,23} = 4.825, P = 0.038). Loading reduced locomotor activity during the dark phase only in the lean group (P = 0.005) (Figure 4A) and not in the DIO group (Figure 4B). There were no effects of either Diet or Loading on light-phase locomotor activity (Figure 4A-B).

3.5 | Effect of weight loading on circulating hormones, circulating metabolites and insulin sensitivity

Two-way ANOVA revealed significant effects of the factor Diet on leptin (F_{1,26} = 10.131, P = 0.004), insulin (F_{1,28} = 27.223, P < 0.001), total ghrelin (F_{1,28} = 13.397, P = 0.001), HOMA-IR (F_{1,27} = 20.016, P < 0.001) and glucose (F_{1,28} = 9.542, P = 0.005), as well as significant effects of the factor Loading on leptin (F_{1,26} = 19.071, P < 0.001), insulin (F_{1,28} = 5.009, P = 0.033), total ghrelin (F_{1,28} = 10.321, P = 0.003) and HOMA-IR (F_{1,27} = 4.583, P = 0.041). Likewise, we found almost significant Diet × Loading interactions for leptin (F_{1,26} = 3.008, P = 0.095) and HOMA-IR (F_{1,27} = 3.940, P = 0.057). Loading decreased serum levels of leptin similarly in both DIO (P = 0.004) and lean (P = 0.034) rats (Figure 5A). In DIO animals, loading also decreased plasma insulin levels (P = 0.037) (Figure 5B) and HOMA-IR index scores (P = 0.042) (Figure 5D), whereas it increased plasma total ghrelin levels (P = 0.021) (Figure 5C). In lean rats, loading did not affect total ghrelin levels (Figure 5C), insulin levels or HOMA-IR index scores (Figure 5B, D). Nonetheless, direct comparisons of the effects of loading between both DIO and lean rats did not reach statistical significance for any of the blood parameters studied (Table 1). As expected, non-loaded DIO rats displayed a metabolic profile that was in accordance with their obese-like phenotype. Specifically, they exhibited higher leptin (P = 0.005), insulin (P < 0.001), total ghrelin (P = 0.018), glucose (P = 0.008) and...
HOMA-IR index scores ($P = 0.002$) compared to their non-loaded lean peers. Triglycerides and cholesterol were not affected by either Diet or Loading (Table 2).

**4 | DISCUSSION**

The recent discovery that challenging the gravitostat mechanism through artificial weight loading reduces body fat and body weight in obese human subjects\(^2\) provides an entirely new research field with respect to the pursuit of novel targets for the treatment of obesity in humans. To introduce a new therapy in humans, it is of utmost importance to first thoroughly investigate the mechanisms behind the anti-obesity effect, which requires work in experimental animals. Here, we demonstrate that the reduction of food intake caused by weight loading appears to reflect especially a decrease in meal size. This is important because meal size is especially regulated by pathways involved in satiation in the hindbrain,\(^{12}\) thereby implicating these pathways in the effects of loading. We also found that CE was suppressed by loading in DIO rats, implying increased energy expenditure. Taken together with our finding that loading does not impact on locomotor activity in DIO rats, we may infer that loaded rats use more calories to move a given distance. Changes in circulating levels of leptin, insulin, and ghrelin as well as insulin sensitivity that were consistent with weight loss and energy deficit.

**4.1 | The gravitostat decreases body fat and food intake more effectively in obese than in lean rats**

Comparing weight loss curves between DIO and lean rats, it appears that those animals with the greatest amount of body fat benefit most from the protective effect of loading on weight gain. Post mortem weighing of dissected tissues showed that the effects of loading were the result of a decrease in fat mass rather than a decrease in lean mass, again with the largest effect in the DIO group that had more fat to begin with. The plasma leptin levels, which reflect fat mass,\(^5\) mirrored the body fat data, confirming a loss of fat by loading. Insulin levels in DIO rats were more than double those of the lean group (probably linked to the greater need for insulin in obese animals that gain insulin resistance) and these could also be reduced by loading in the DIO group. In addition, loading of DIO rats
decreased the HOMA-IR index, indicating increased insulin sensitivity. Collectively, these data suggest that the gravitostat mechanism can be recruited by loading for weight loss and improved glucose metabolism in obese animals.

The duration of the present study in rats was 3 weeks. Our previous studies in mice have shown that the loading effect on body weight stabilises during week 2 and persisted until the end of that study at 7 weeks. Further studies are required to determine how long the loading effect persists in obese rats and mice. What is known is that removal of the artificial weight reverses the suppression of biological body weight, supporting the assumption that the loading effect is not a result of stress.

4.2 | Gravitostat-induced decrease in fat mass is mainly caused by decreased food intake

In all loading studies performed in rodents to date, including the present study, the loss in fat mass (and body weight) appears to reflect a reduced food intake rather than an increased energy expenditure. Indeed, it was previously shown that DIO mice pair-fed to the load group lose the same amount of body weight as seen by loading, suggesting that reduced food intake is the main factor causing the weight loss. Our finding that loading suppressed food intake, an effect that was greater in DIO rats, supports this conclusion. However, we demonstrate here that CE is decreased by loading in DIO rats, which could be indicative of enhanced energy expenditure, a logical effect of carrying an extra load. Thus, loading decreases fat mass mainly by decreasing food intake, although the increase in energy expenditure likely contributes to this effect.

4.3 | The gravitostat and locomotor activity

We did not detect any effect of loading on locomotor activity in the DIO group, in line with data from mice. Rats in the lean control group were more active during the dark phase than those in the DIO control group, which could contribute to the low fat mass of the lean group. Loading decreased locomotor activity in lean but not in DIO rats, and it might be speculated that this is a counter-regulatory effect that partly explains why fat mass is less decreased by loading in lean rats.

4.4 | The gravitostat in relation to caloric restriction

An extensively studied animal model of weight loss is caloric restriction in which animals have a limited supply of food over an extended time period. It typically ranges from 50% to 80% of ad lib. feeding. In addition to reduced body weight, rats on caloric restriction have reduced body fat mass and reduced insulin and leptin, as also seen here after loading. One interesting difference is that the caloric restriction decreases energy expenditure, an effect for which no evidence could be found after loading. Putting these observations together, it would appear that the reduction in food intake by loading is insufficient to cause energy-saving adaptations (eg, increased CE and/or reduced locomotor activity). However, it cannot be excluded that increased effort when carrying a weight could mask a decrease in energy expenditure caused by weight loss.

4.5 | The gravitostat in relation to ghrelin

Loading changed the levels of several circulating hormones. This included an increase in ghrelin in addition to decreases in circulating leptin and insulin. These effects are consistent not only with a reduced fat mass, but also a state of energy deficit and heightened hunger. Ghrelin is a stomach-derived hormone that is released during hunger and drives food intake and food motivated behaviour. Heightened ghrelin in the absence of feeding in loaded rats resonates with that observed in models of anorexia nervosa and could indicate resistance to ghrelin’s orexigenic effects. Arguing against ghrelin resistance in loading, however, is our observation that ghrelin receptor knockout mice respond normally to weight loading with a decreased body weight and reduced food intake.

4.6 | The gravitostat neural circuitry

The neural identity of the circuits mediating the gravitostat mechanism remains elusive. Ideally, these circuits would be identified (by activity and/or gene expression studies) during the time window when body weight homeostasis is adjusting to loading but, unfortunately, this partly corresponds to a period of recovery from surgical trauma, which could also impact on these pathways. Candidate
hypothalamic mediators have been explored in mice after 5 days of loading, including the orexigenic peptides agouti-related peptide (AgRP) and neuropeptide Y (NPY), comprising peptides that are co-expressed in neurons targeted by ghrelin. However, AgRP and NPY mRNAs were both increased by loading, which would align with a failed compensatory mechanism attempting to restore body weight that is secondary to weight loss. Loading studies in knockout mice demonstrated that the preventive effect in body weight gain was independent of signalling at various receptors including for glucagon-like peptide 1, ghrelin melanocortin 4 and oestrogen receptor alpha.

We have not specifically explored engagement of the stress axis in the weight loss effects of loading, although we did not detect changes in circulating levels of markers of acute or chronic stress (catecholamines and corticosteroids, respectively) or inflammation (ie, cytokines).

Pioneering work by Grill identified a role for hindbrain circuits in the regulation of meal size and more forebrain (including hypothalamic) circuits in the control of meal frequency. Thus, our finding that weight loading primarily reduced meal size, as observed in both DIO and lean groups, would be consistent with engagement of hindbrain pathways involved in satiation and meal termination, including those sensitive to cholecystokinin. Lean rats on chow diet ate fewer kilocalories overall than DIO rats fed a HFD. When the lean rats were loaded, they did not show much change in overall food intake. This could be because any reduction in meal size was compensated for by increased meal frequency, especially during the light phase. That said, our results suggest that, unlike lean rats, such a decrease in meal size did not cause an increase in meal frequency in DIO rats, highlighting their inability for compensation. This might therefore imply a hypothalamic dysfunction in DIO rats. On the other hand, because we did not analyse diurnal feeding prior to implantation of the capsules, we do not know to what extent surgery might have impacted upon meal patterning. However, our main objective was to explore the effects of loading (thus, post-surgery) in lean and DIO rats.

4.7 | The gravitostat and the leptin system as guards against obesity

Our data in the present study seem to point to a more effective gravitostat-body-weight-regulating mechanism in DIO rats compared to leaner chow-fed rats. This in accordance with our previous finding that the gravitostat is more effective in obese than in lean mice. In line with this, there were only small and inconsistent effects of loading in lean animals in some early studies. Very recently, we also found that loading is effective in suppressing body weight in obese humans. It could be speculated that obese animals (even severely obese ones) would be even more obese in the absence the gravitostat mechanism. As one example, Kim et al have shown that severely obese leptin deficient ob mice can be manipulated to become even more obese. It could also be speculated that the gravitostat mechanism must be suppressed or reset during pregnancy, such that the mother can sustain her body weight at the same time as supporting foetal growth. Other examples of physiological roles of the gravitostat are regulation of prepubertal growth and possibly the timing of puberty, as we recently reported. However, a remaining question concerns which pathophysiological events inactivate the gravitostat during adulthood, and thereby contribute to obesity.

Ablation of the fat-derived hormone leptin causes severe obesity that can be reversed by leptin replacement in animals and humans. This indicates that leptin can exert powerful biological effects and that it is needed to prevent grave obesity.

In conclusion, the leptin system and the gravitostat are the only two known homeostatic regulators of body fat. It will be of great interest to investigate how combined manipulations of these two powerful homeostatic systems may provide the long sought after cure of obesity.

ACKNOWLEDGEMENTS

We thank research engineer Staffan Berg for manufacturing the weight capsules, Dr Pol Solé-Navais for statistical advice and Jakob Bellman for assistance with blood lipid analysis. The research was supported by the EC FP7 project “Nudge-it” (607310), the Swedish Research Council for Medicine (Vetenskapsrådet, 2019-01051 and 2019-01164), Novo Nordisk Foundation (NNF057328, NNF17OC0027206 and NNF19OC0056694) and Hjärnfonden (FO2018-0262 and FO2019-0086), as well as by Avtal om Läkarutbildning och Forskning (ALFGBG-723681, ALF-138741 and ALFGBG-724341). In addition, it was supported by Jane and Dan Olsson (JADO) Foundation, the Torsten Söderberg Foundation, and The Knut and Alice Wallenberg’s Foundation. The funding bodies did not have a role in the study design, nor in the collection, analysis and interpretation of data or the writing of the report, as well as the decision to submit the article for publication.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Tina Bake: Conceptualisation; Formal analysis; Investigation; Writing – original draft; Writing-review & editing. Fiona Peris-Sampedro: Formal analysis; Investigation; Writing – original draft; Writing – review & editing. Zita Wáczek: Formal analysis; Investigation; Writing – review & editing. Claes Ohlsson: Writing – review & editing. Vilborg Pálsdóttir: Investigation; Writing – review & editing. Suzanne L Dickson: Conceptualisation; Funding acquisition; Writing – original draft; Writing-review & editing. John-Olov Jansson: Conceptualisation; Funding acquisition; Writing – original draft; Writing-review & editing. Zita Wáczek: Formal analysis; Investigation; Writing – review & editing.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/jne.12997.
REFERENCES

1. Jansson JO, Palsdottir V, Hagg DA, et al. Body weight homeostat study are not publicly available, but are available from the corresponding author upon reasonable request.

2. Ohlsson C, Gidestrand E, Bellman J, et al. Increased weight loading. Nature. 1999;402(6762):656-660.

3. Cummings DE, Frayo RS, Marmonier C, Aubert R, Chapelot D. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. Am J Physiol Endocrinol Metab. 2004;287(2):E297-304.

4. Jansson JO, Palsdottir V, Hagg DA, et al. Body weight homeostat study are not publicly available, but are available from the corresponding author upon reasonable request.

5. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homolog. Nature. 1994;372(6505):425-432.

6. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat Med. 1995;1(12):1311-1314.

7. Heymsfield SB, Greenberg AS, Fujioka K, et al. Recombinant leptin-induced resistance to leptin action. JAMA. 1995;274(21):1657-1658.

8. McCarter RJ, McGee JR. Transient reduction of metabolic rate by food restriction. Am J Physiol. 1989;257(2 Pt 1):E175-E179.

9. Johansson A, Fredriksson R, Winnergren S, Hulting AL, Schioth HB, Lindblom J. The relative impact of chronic food restriction and acute food deprivation on plasma hormone levels and hypothalamic neuropeptide expression. Peptides. 2008;29(9):1588-1595.

10. Schwartz A, Doucet E. Relative changes in resting energy expenditure during weight loss: a systematic review. Obes Rev. 2010;11(7):531-547.

11. Kohut B, Peris-Sampedro F, Waczk Z, et al. The gravitostat protects diet-induced obese rats against fat accumulation and weight gain. J Neuroendocrinol. 2021;00:e12997. https://doi.org/10.1111/jne.12997