A Body Weight Sensor Regulates Prepubertal Growth via the Somatotropic Axis in Male Rats

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Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; GH, growth hormone; GHRH, growth hormone–releasing hormone; IGF, insulin-like growth factor; PCR, polymerase chain reaction.

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Abstract

In healthy conditions, prepubertal growth follows an individual specific growth channel. Growth hormone (GH) is undoubtedly the major regulator of growth. However, the homeostatic regulation to maintain the individual specific growth channel during growth is unclear. We recently hypothesized a body weight sensing homeostatic regulation of body weight during adulthood, the gravitostat. We now investigated if sensing of body weight also contributes to the strict homeostatic regulation to maintain the individual specific growth channel during prepubertal growth. To evaluate the effect of increased artificial loading on prepubertal growth, we implanted heavy (20% of body weight) or light (2% of the body weight) capsules into the abdomen of 26-day-old male rats. The body growth, as determined by change in biological body weight and growth of the long bones and the axial skeleton, was reduced in rats bearing a heavy load compared with light load. Removal of the increased load resulted in a catch-up growth and a normalization of body weight. Loading decreased hypothalamic growth hormone releasing hormone mRNA, liver insulin-like growth factor (IGF)-1 mRNA, and serum IGF-1, suggesting that the reduced body growth was caused by a negative feedback regulation on the somatotropic axis and this notion was supported by the fact that increased loading did not reduce body growth in GH-treated rats. Based on these data, we propose the gravitostat hypothesis for the regulation of prepubertal growth. This states that there is
a homeostatic regulation to maintain the individual specific growth channel via body weight sensing, regulating the somatotropic axis and explaining catch-up growth.

Key Words: Prepubertal growth; growth hormone; homeostasis

In healthy conditions, prepubertal growth hormone (GH)–dependent growth occurring after infancy but before puberty (childhood growth in humans) is tightly regulated (1, 2), strictly following an individual specific tract. This is depicted in growth charts as a standard deviation–based body growth channel (mainly in Europe) or percentile (mainly in the United States) (1, 3). This means that a child has the same relative size in relation to its peers at different ages. If there is a growth retardation due to illness, malabsorption etc., the child will return to its normal growth channel when the factor causing the growth inhibition is gone (1, 4). Thus, a growth canal is the part of a growth chart within which the size of a healthy child will normally stay (3). The growth channel is specific for that child, but there is no established clinical definition of how wide a growth channel is. The higher than normal growth rate that an individual needs to reach its predicted growth channel after a period of stunted growth is referred to as catch-up growth (1). Catch-up growth was first described in animals in the early 1900s (5), and the term “catch-up” was introduced into a pediatric context by Prader and coworkers 50 years later (6). Although catch-up growth has been recognized for a long time, the mechanisms causing this phenomenon are to a large part unknown. Thus, it is completely unknown how the body of a growth-retarded individual knows that there is growth retardation in relation to its normal growth channel (1). There is even less knowledge about the mechanism for how prepubertal growth is tightly regulated in the healthy individual to keep it within its predefined growth channel (1). GH, acting both via induction of liver-derived circulating insulin-like growth factor 1 (IGF-I) and via direct effects on target tissues, is the major regulator of prepubertal childhood growth, but the homeostatic regulation to maintain the individual specific growth channel during prepubertal GH-dependent growth is unknown (7-9).

We recently hypothesized a leptin-independent body weight sensing homeostatic regulation of body weight during adulthood, the gravitostat (2, 10). This system includes registration of body weight via sensors in the lower extremities, presumably depending on the osteocytes being the sensors of strain within the long bones (7, 10-14). Very recently, we obtained evidence that body weight sensing per se might be the high precision sensor of the actual body size. Based on the data in the present study we put forward the gravitostat hypothesis for homeostatic regulation to maintain the individual specific growth channel during prepubertal growth.

Materials and Methods

Animals

All animal procedures were approved by the Ethics Committee on Animal Care and Use in Gothenburg University. Male Sprague–Dawley rats (Charles River Laboratories, Sulzfeld, Germany) were housed in standard conditions on a 12/12 hour light/dark cycle at 20 to 22°C and 50% humidity. The animals had ad libitum access to normal chow (Harlan, NJ, USA) and water.

Loading

At age 26 days, young rats were divided in 2 groups matched for mean body weight and received a capsule that either weighed 20% (high load, capsule filled with tungsten powder) or 2% (low load, empty capsule) of the starting body weight implanted intraperitoneally under isoflurane anesthesia. After the implantation, the body weight was measured several times per week until the end of each experiment. The biological body weight was calculated as the total body weight minus the weight of the capsule. During the experiment, growth was calculated as percent increase relative to starting weight.

In the “removal of load” experiments, the capsules were exchanged 1 week after the first implantation. The rats were exposed to sustained high load (heavy capsule days 0-7 followed by heavy capsule days 7-14), sustained low load (light capsule days 0-7 followed by light capsule days 7-14), or removed high load (heavy capsule days 0-7 followed by light capsule days 7-14).

Food intake was recorded by weighing the food distributed to each rat housed individually and thereafter weighing the amount that was left after 24 hours. Given that the rats were growing, food intake was normalized to body weight.
Locomotor Activity
Locomotor activity was recorded in Basic Behavior Test boxes (med associates, St Albans, VT, USA). The rats were habituated to the boxes for 30, 60, 120, and 180 minutes on separate days during light phase, followed by 180 minutes in dark phase and the number of beam brakes was recorded.

X-Ray Analysis and Fluorochrome Labeling to Determine Bone Growth
To determine bone growth of the long bones (tibia, radius, and humerus) and the appendicular skeleton (crown–rump length), rats were analyzed by repeated X-ray analyses (Faxitron Bioptics, Tucson, AZ, USA, isotropic pixel size of 48 µm). The bone lengths were determined using the ruler-tool software provided with the scanner. All measurements of the lengths of the long bones were calculated as the average of the left and right bones. Percentage fat mass was analyzed using dual-energy X-ray absorptiometry (Faxitron Bioptics).

In addition, dynamic histomorphometric analyses were performed to determine the growth rate of the proximal tibia. The rats were injected intraperitoneally with the fluorochrome alizarin (Sigma Aldrich, 30 mg/kg) 4 days prior to sacrifice. After decalcification and sectioning, the sections were observed with fluorescent microscopy. Ten ruler measurement from the alizarin band to the end of the proximal growth plate was performed for each tibia, using blinded samples.

Growth Hormone Treatment
On the day of loading, rats received mini-osmotic pumps (Alzet 1002, Cupertino, CA, USA) implanted subcutaneously in the neck under isoflurane anesthesia. The mini-osmotic pumps were filled with either saline or GH (1 µg/g body weight; human GH, 1.8 IU/mg; Pfizer, New York, NY, USA). This dose of GH has previously been reported to approximately restore normal mean plasma GH levels in hypophysectomized rats (17). Rats were divided in a total of 4 groups; low load and high load treated with saline and low load and high load treated with GH.

Gene Expression
Hypothalamus and liver were dissected, snap frozen in liquid nitrogen, and kept in –80°C until analyses. mRNA from all tissues was extracted using RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). The mRNA concentration of the samples was measured by a NanoDrop spectrophotometer (Wilmington, DE, USA), and cDNA was synthesized from 1 µg of mRNA with iScript cDNA Synthesis Kit (Bio-Rad, Solna, Sweden).

Real-time polymerase chain reaction (PCR) was performed using the Step-One-Plus Real Time PCR System (Applied Biosystems, Forster City, CA, USA). The hypothalamus samples were analyzed by assays for growth hormone–releasing hormone (GHRH; Rn00580832_m1), somatostatin (Rn00561967_m1), ghrelin receptor (GHS-R1A; Rn00821417_m1). The pituitary samples were analyzed by an assay for adrenocorticotropic hormone (ACTH; Rn00595020_m1). RPLP0 (Rn03302271_gH) was used as a reference gene for all brain tissues. Liver IGF-1 (Rn00710306_m1) was analyzed with beta-actin as reference gene (Rn00667869_m1). The relative mRNA levels were obtained by using the comparative threshold cycle (Ct) method and calculated with the ΔΔCt equation.

Serum Analyses
At the end of the experiment, blood samples were collected and the serum was separated and kept in –80°C until analysis. Serum was analyzed in duplicates by enzyme-linked immunosorbent assays for IGF-1 (18) and corticosterone (19).

Statistics
Differences between rats implanted with low load and high load were analyzed using the 2-tailed Student t test. When more than 2 groups were compared, analysis of variance (ANOVA) followed by Turkey’s post hoc test was used. P < .05 was considered statistically significant. All data are presented as mean ± standard error of the mean (SEM). In analyses pooling data from several experiments, data are expressed as a percentage of the low load group and the pooling was done after first normalizing to the mean of the low load group in each experiment.

Results
Homeostatic Regulation to Maintain the Individual Specific Growth Channel via Sensing of Body Weight
Increased loading reduces body growth in young rats
To evaluate the effect of increased artificial loading on pre-pubertal growth, we implanted heavy (20% of body weight; high load) or light (2% of the body weight; low load) capsules into the abdomen of rapidly growing 26-day-old male Sprague–Dawley rats.

Increased loading reduced growth of biological body weight (Fig. 1A). Differences in growth of biological body weight...
weight between the high load and the low load groups were observed already at day 1 after surgery and became more pronounced throughout the experiment, being 12% at day 7 and almost 18% at day 14 (end of the experiment). At the start of the experiment (day 0, after implantation of the capsule) the total body weight (biological body weight + capsule weight) was 18% higher in the high load group compared to the low load group (Fig. 1B). As the body growth during the study was reduced in the high load group compared with the low load group, this difference in total body weight was almost completely abolished on day 14 at the end of the experiment (Fig. 1B). Calculations of the efficiency of the homeostatic regulation of the total body weight disclosed that almost all of the artificially increased loading was counteracted by reduced
growth of the biological body weight when evaluated at day 14 after initiation of the increased loading (Fig. 1C). Repeated X-ray analyses revealed that the growth of the long bones (tibia, femur, and humerus; Fig. 1D) and of the axial skeleton (crown–rump length; Fig. 1E) between day 0 and 14 of increased loading was reduced in the high load group compared with the low load group. Dynamic histomorphometric analyses of the proximal region of the tibia using fluorochrome labelling confirmed that the longitudinal tibia bone growth rate was reduced in the high load group compared with the low load group (Fig. 1F). Body composition was analyzed 12 days after surgery, with no significant difference in fat mass percentage between animals treated with high load and low load, (Fig. 1G). To verify that the rats exposed to high load did not gain less weight due to some unspecific effects such as stress, we performed several analyses. Serum corticosterone levels were measured at day 4 and day 7 after surgery. There was no significant difference in serum corticosterone between rats with low load and high load (Fig. 2A). Moreover, there was no difference between rats with high load and low load in pituitary ACTH mRNA (Fig. 2B), another part of the corticotropic axis. Food intake was recorded daily for 1 week and normalized to body weight, with no significant difference between control and load group (Fig. 2C). We also analyzed voluntary locomotion as determined by laser beams broken when placed in operant boxes. There was no significant difference between the groups as seen in Fig. 2D.

Catch-up growth after removal of increased loading

We next evaluated if removal of the increased load would result in catch-up growth. For this purpose, there was a start of load on day 0 and a change of load on day 7 (Fig. 3A). The rats were exposed to sustained high load (heavy capsule days 0-7 followed by heavy capsule days 7-14), sustained low load (light capsule days 0-7 followed by light capsule days 7-14) or removed high load (heavy capsule days 0-7 followed by light capsule days 7-14; Fig. 3). Similar to our previous results (see Fig. 1A), the growth of the biological body weight at day 7 of the 2 high load groups (sustained high load and removed high load) was significantly lower than the growth of the biological body weight of the sustained low load group (Fig. 3A and 3B). As expected, at day 7 similar growths of the biological weight were observed for the sustained high load and the removed high load groups that so far had been exposed to similar high load (Fig. 3A and 3B). Removal of the high load in the removed high load group at day 7 increased the biological body weight growth between day 7 and day 14 when compared with sustained high load and sustained low load (Fig. 3). Biological body weight of the removed high load group at day 14 (days 0-14) was no longer significantly lower than the sustained low load group (Fig. 3A). Moreover, the biological body weight growth between day 7 and day 14 for the removed high load group was significantly higher than for both the sustained low load group and the sustained high load group (Fig. 3B). Thus, removal of the increased load in the removed high load group resulted in a catch-up growth (Fig. 3A and 3B).

Homeostatic regulation to maintain the individual specific growth channel via sensing of body weight is mediated via the somatotropic axis.

As the somatotropic axis is the major regulator of prepubertal growth (7, 20, 21), we evaluated if it is involved in the homeostatic regulation to maintain the individual specific growth channel via sensing of body weight. Increased loading decreased hypothalamic mRNA levels of GHRH and ghrelin receptor, but did not affect somatostatin mRNA levels (Fig. 2A). Serum corticosterone was analyzed 4 (high load, n = 10; low load, n = 10) and 7 (high load, n = 9; low load, n = 10) days after loading, with no significant difference between the groups (A). Pituitary adrenocorticotrophic hormone (ACTH) was analyzed using real-time PCR (high load, n = 9; low load, n = 7) 4 days after loading, with no significant difference between the groups (B). Food consumption was measured the first week after loading (high load, n = 10; low load, n = 10) and it did not differ between the groups (C). Voluntary locomotor activity was analyzed 8 days after loading (high load, n = 9; low load, n = 10) and did not differ between the groups.

**Figure 2.** Increased loading does not cause adverse effects on growing rats. Two markers of stress was analyzed. Serum corticosterone was analyzed 4 (high load, n = 10; low load, n = 10) and 7 (high load, n = 9; low load, n = 10) days after loading, with no significant difference between the groups (A). Pituitary adrenocorticotrophic hormone (ACTH) was analyzed using real-time PCR (high load, n = 9; low load, n = 7) 4 days after loading, with no significant difference between the groups (B). Food consumption was measured the first week after loading (high load, n = 10; low load, n = 10) and it did not differ between the groups (C). Voluntary locomotor activity was analyzed 8 days after loading (high load, n = 9; low load, n = 10) and did not differ between the groups.
Furthermore, liver IGF-1 mRNA levels (Fig. 4B) and serum IGF-1 (Fig. 4C) were reduced in the high load group compared with the low load group. These data indicated that the reduced body growth was caused by a negative feedback regulation on the somatotropic axis. To functionally evaluate the involvement of modulation of the feedback regulation of the somatotropic axis for the effect of increased loading on body growth, we compared the effect of increased loading (high load vs low load) in saline and GH-treated rats. As expected, increased loading robustly reduced body growth as determined by change in body weight and tibia length (Fig. 4D and 4E) in the saline-treated rats. In contrast, no effect of increased loading on growth was observed in the GH-treated rats (Fig. 4D and 4E). Further statistical evaluation using the interaction term from 2-way ANOVA showed that the effect of loading on body weight was larger in the saline-treated rats than GH-treated rats at day 5 ($P < .05$). Thus, administration of exogenous GH reversed the suppression of growth caused by increased loading (Fig. 4D and 4E). Collectively, these findings indicate that the homeostatic regulation to maintain the individual specific growth channel via sensing of body weight is mediated via a feedback regulation on the somatotropic axis.

**Discussion**

In healthy conditions, prepubertal growth is a tightly regulated process, causing the body growth to follow an individual specific growth channel. Consequently, an individual has a similar body size in relation to its peers at different ages. However, the mechanism behind the tight homeostatic regulation to maintain the individual specific growth channel during prepubertal GH-dependent growth is unknown. We herein demonstrate that the homeostatic regulation to maintain the individual specific growth channel is dependent on body weight sensing, which regulates the somatotropic axis and thereby body growth. In a pathophysiological context, body weight sensing may explain catch-up growth after illness and other growth retarding conditions.

**Homeostatic Regulation to Maintain the Individual Specific Growth Channel via Sensing of Body Weight**

As the prepubertal GH-dependent growth occurring after infancy but before puberty (childhood growth in humans) is tightly regulated within a predefined growth channel, we hypothesized that this regulation must include a sensory input bringing information about the actual body size. We recently revealed a body weight sensing homeostatic regulation of body weight during adulthood in both rodents and humans, the gravitostat, which includes sensing of the body weight in the weight bearing long bones (10-13, 15). We propose that the body weight–induced strain within the weight bearing long bones, sensed by the osteocytes, constitutes the sensory input for the feedback regulation of not only body weight during adulthood (gravitostat; 10), but also prepubertal growth within a predefined growth channel.

During adulthood, the bone dimensions are relatively constant and the strain within the weight bearing bones is essentially directly proportional to the body weight. In contrast, during prepubertal growth, the bone dimensions...
Figure 4. Homeostatic regulation to maintain the individual specific growth channel via sensing of body weight in rats is mediated via the somatotropic axis. Effects of loading on (A) GHRH mRNA, somatostatin mRNA, and GHS-R1A mRNA in the hypothalamus (low load; n = 20; high load n = 20) 4 days after initiation of load. (B) liver IGF-1 mRNA (low load, n = 20; high load, n = 17) and (C) serum IGF-1 (low load, n = 17; high load, n = 16) on day 14 after capsule implantation. Effect of increased loading on growth in (D) biological body weight and (E) tibia length (days 0-14) in rats treated with saline (low load, n = 10; high load n = 9) or GH (1 µg/mL; low load, n = 9; high load n = 10). Data are expressed as means ± SEM. *P < .05, **P < .01, ***P < .001, high load vs low load. (F) The gravitostat hypothesis for homeostatic regulation to maintain the individual specific growth channel during prepubertal GH-dependent growth. (a) Balanced normal prepubertal growth. When the body weight is following the predefined growth channel, the body weight sensor indicates a normal body weight (lower panel; proposed by us to be represented by normal strain in
are increasing by age, making bones more resistant to body weight. Thus, to retain a constant bone strain by age, the body weight required at homeostasis must also be increased by age. We propose that during prepubertal growth, the set point for bone strain may be constant but the set point for body weight is increasing by age in proportion to the increase in cross-sectional bone dimensions. This would allow for a homeostatic regulation to maintain the individual specific growth channel during prepubertal growth. Our hypothesis is supported by the well-known fact that it is the magnitude of the resulting bone strain and not the magnitude of the artificial loading on the long bones that determines the local bone anabolic response (7, 14). However, further experimental studies are warranted to determine if our hypothesis of the involvement of bone strain in the weight bearing bones for the observed body weight sensing is valid. A homeostatic regulation to maintain the individual specific growth channel via body weight sensing is supported by the findings in the present study demonstrating that implantation of artificial high load to young growing rats reversibly decreased their body growth compared to rats with a low load.

In the present study we have used weight loading to investigate our gravitostat hypothesis that there is a feedback regulation of body growth. Some information applicable to the gravitostat hypothesis may also be gained from earlier studies on altered gravity. Most studies (including an extensive meta-analyses of 18 independent studies) demonstrate that hypergravity induced by centrifugation reduces the body weight gain (22, 23), supporting the gravitostat hypothesis. The findings from low gravity during spaceflight are less conclusive, possibly as it is difficult to perform accurate body weight measurement during space flight (22, 24).

Homeostatic Regulation to Maintain the Individual Specific Growth Channel via Sensing of Body Weight Is Mediated via the Somatotropic Axis

GH is the major regulator of growth. Although, several factors involved in the molecular and cellular feedback regulation of GH secretion have been extensively characterized (3, 7, 20, 25), it is unknown if the overall outcome of GH activity during growth, the body size, exerts a negative feedback regulation on the somatotropic axis. As GH transgenic rodents with constant high GH levels, devoid of normal negative feedback regulation of GH secretion, are unique in displaying a substantially increased body size, one may speculate that the homeostatic sensing of body weight is dependent on a normal body size-driven negative feedback regulation on the somatotropic axis. We evaluated, therefore, if the somatotropic axis is involved in the homeostatic regulation to maintain the individual specific growth channel during prepubertal growth. We found that increased loading decreased hypothalamic GHRH and GHS-R1A mRNAs and hepatic IGF-1 mRNA and serum IGF-1 levels. These data support the notion that the reduced body growth in loaded rats was caused by a negative feedback regulation on the somatotropic axis, reducing the overall growth in a systemic manner.

To functionally evaluate the involvement of modulation of the feedback regulation of the somatotropic axis for the inhibitory effect of increased loading on body growth, we compared the effect of increased loading in rats given saline or a constant GH treatment in a replacement dose (17). As increased loading decreased the growth of the salinetreated but not the GH-treated rats, we propose that the homeostatic regulation of body growth via sensing of body weight is mediated via modulation of the somatotropic axis (Fig. 3F). Hypothalamic GHRH and GHS-R1A (ghrelin receptor1A) activation stimulates secretion of GH from the pituitary that in turn stimulates growth either via hepatic IGF-1 secretion into the blood or directly on growing tissues (20, 26-32). We propose that increased loading during prepubertal growth is detected by the gravitostat sensor in the lower extremities which in turn sends a signal that reduces growth via inhibition of the somatotropic axis (Fig. 4F).

The Novel Gravitostat Hypothesis in Relation to Previous Models for Catch-up Growth

We propose that the experimental model used in the present study, implantation and removal of artificial weight capsules, can be regarded as a model for growth inhibition followed by catch-up growth. Catch-up growth is the higher than normal growth rate that an individual achieves to reach predicted size for age after a period of stunted

Figure 4: continued

the bone), resulting in a moderate negative feedback regulation on the somatotropic axis. (b) Reduced growth. After induction of artificially increased loading, the body weight sensor indicates elevated body weight (proposed by us to be represented by increased bone strain), resulting in increased negative feedback regulation on the somatotropic axis and thereby reduced body growth. Reduced growth can also be caused by illness via other mechanisms. (c) Catch-up growth. After removal of the increased loading, the body weight sensor indicates reduced body weight (proposed by us to be represented by reduced bone strain), resulting in reduced negative feedback regulation on the somatotropic axis and thereby increased body growth. (d) Normal balanced growth again. After the catch-up growth, the individual reaches its constitutional predefined growth channel and again grows at a normal rate associated with a neutral indication of the body weight sensor.
growth (1, 6), and the body weight changes after capsule removal seen in this study are in line with this definition (Fig. 4F). Based on our results we hereby propose the gravitostat hypothesis of homeostatic regulation to maintain the individual specific growth channel during prepubertal growth. According to this hypothesis, there is a homeostatic regulation to maintain the individual specific growth channel via body weight sensing, regulating the somatotropic axis and explaining catch-up growth. There are 2 main previous hypotheses for catch-up growth: the neuroendocrine hypothesis, first proposed by Tanner (4, 16) and the growth plate hypothesis suggested by Baron et al. (25).

The novel gravitostat hypothesis compared with the neuroendocrine hypothesis

Tanner and later other authors (4, 16) have proposed that catch-up growth is regulated by neuroendocrine mechanisms. According to the neuroendocrine hypothesis: There is a mechanism that is able to recognize the degree of mismatch between the size the organism ought to have (the target size) and the size it actually has, and consequently to adjust growth rate according to the degree of mismatch. They proposed that the target size is represented by the steadily increasing concentration (or organization) of something somewhere, most likely in the brain while the actual body size is sensed by a circulating inhibitory factor, produced by growing tissues in proportion to their size. The gravitostat hypothesis extends and modifies the neuroendocrine hypothesis. Tanner (16) and others suggested that there is a circulating inhibitory factor, produced by growing tissues in proportion to their size. After inhibition of growth the levels of this blood-borne inhibitory factor would decrease and thereby allow for catch-up growth (16). The present finding, that the biological body size is decreased by artificially increased loading, demonstrates that it is the total body weight per se that initiates a signal exerting a negative feedback regulation on body size. We propose that the afferent factor regulating prepubertal body growth is an endocrine or nervous signal originating from the body weight sensor in the weight bearing lower extremities in a similar manner as previously described by us for body weight regulation in adulthood (10, 12). Tanner suggested already in the 1960s that central neuroendocrine mechanisms are involved in catch-up growth (16), but this hypothesis has been supported by few data during the more than 50 years that have passed. Here we demonstrate that increased loading reduces hypothalamic GHRH expression associated with reduced IGF-I mRNA in the liver and reduced serum IGF-I, supporting that the effect of the regulatory factor is exerted at the level of the CNS, presumably the hypothalamus (Fig. 4F).

The novel gravitostat hypothesis compared with the growth plate hypothesis

Besides the neuroendocrine hypothesis described above, another hypothesis has been put forward to explain the mechanisms behind catch up growth. The growth plate hypothesis put forward by Baron and coworkers (25, 34) suggests that the cells of growth plate have a certain capacity for proliferation, and that this decreases over time; so called cell senescence. Temporarily stopped cell proliferation in the growth plate is afterwards compensated by local catch-up growth, because the temporarily stopped cells have larger unused proliferation capacity. Our proposed gravitostat hypothesis of a homeostatic regulation to maintain the individual specific growth channel and of catch-up growth does not exclude a contribution of local growth plate–regulated catch-up growth.

To determine if unspecific stress might contribute to the reduced body weight growth observed, we made a number of control measurements. However, we observed no indication of stress in the loaded animals compared to control animals. Animals with high load did not have an increase in the levels of serum corticosterone or pituitary ACTH mRNA compared with controls. Moreover, they continued to eat normally during the experimental period. Further, voluntary locomotion was not altered in rats carrying a heavy load. Taken together, these experiments argue against that the rats lost weight due to unspecific stress.

In a previous study from our laboratory (10), we showed that subcutaneously placed capsules in the lower back of the animals also promoted weight loss in adult rodents, further strengthening that weight loss promoted by artificial loading is a specific effect. Further studies are warranted to determine if the method and location of added weights may influence the effects on body weights in growing rats.

In this study, we use human GH which is known to also bind to the prolactin receptor as well as the GH receptor in rats (35). Therefore, it cannot be completely ruled out that the lack of weight loss in high load rats given GH is caused by another mechanism than via the somatotropic axis. However, murine prolactin receptor stimulation does not affect body growth (35) and we therefore assume that the effect seen in this study is exerted via the GH receptor. Further studies are warranted to determine if the effect of loading also can be observed in growing female rats, and for how long the effect of the gravitostat can be seen in growing rats.
In conclusion, we, hereby, propose the gravitostat hypothesis of homeostatic regulation to maintain the individual specific growth channel. According to this hypothesis, there is a homeostatic regulation to maintain the individual specific growth channel via body weight sensing in the long bones of the lower limbs, regulating the somatotropic axis and explaining catch-up growth (see schematic Fig. 4F).

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Author contributions: J.-O.J. and C.O. designed the study, interpreted data and wrote the manuscript. A.D.-G. performed animal experiments, interpreted data, and explained catch-up growth (see schematic Fig. 4F).

Additional Information

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Data availability: Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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