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## Growth hormone receptor deficiency results in blunted ghrelin feeding response, obesity, and hypolipidemia in mice

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**Egecioglu, Emil, Mikael Bjursell, Anna Ljungberg, Suzanne L. Dickson, John J. Kopchick, Göran Bergström, Lennart Svensson, Jan Oscarsson, Jan Törnell, and Mohammad Bohlooly-Y.** Growth hormone receptor deficiency results in blunted ghrelin feeding response, obesity, and hypolipidemia in mice. *Am J Physiol Endocrinol Metab* 290: E317–E325, 2006. First published September 20, 2005; doi:10.1152/ajpendo.00181.2005.—We have previously shown that growth hormone (GH) overexpression in the brain increased food intake, accompanied with increased hypothalamic agouti-related protein (AgRP) expression. Ghrelin, which stimulates both appetite and GH secretion, was injected intracerebroventricularly to GHR<sup>-/-</sup> and littermate control (+/+) mice to determine whether ghrelin's acute effects on appetite are dependent on GHR signaling. GHR<sup>-/-</sup> mice were also analyzed with respect to serum levels of lipoproteins, apolipoprotein (apo)B, leptin, glucose, and insulin as well as body composition. Central injection of ghrelin into the third dorsal ventricle increased food consumption in +/+ mice, whereas no change was observed in GHR<sup>-/-</sup> mice. After ghrelin injection, AgRP mRNA expression in the hypothalamus was higher in +/+ littermates than in GHR<sup>-/-</sup> mice, indicating a possible importance of AgRP in the GHR-mediated effect of ghrelin. Compared with controls, GHR<sup>-/-</sup> mice had increased food intake, leptin levels, and total and intra-abdominal fat mass per body weight and decreased lean mass. Moreover, serum levels of triglycerides, LDL and HDL cholesterol, and apoB, as well as glucose and insulin levels were lower in the GHR<sup>-/-</sup> mice. In summary, ghrelin's acute central action to increase food intake requires functionally intact GHR signaling. Long-term GHR deficiency in mice is associated with high plasma leptin levels, obesity, and increased food intake but a marked decrease in all lipoprotein fractions.

agouti-related protein; appetite regulation; intracerebroventricular injection

GROWTH HORMONE (GH) IS ACTIVE in the central nervous system, influencing feeding behavior and the sense of well-being in humans (9, 48). In rodents, GH increases food intake (3, 10) and alters the pattern of feeding (46). In both human and rat brain, GH and the GH receptor (GHR) are present in regions known to participate in the regulation of feeding behavior, energy balance, and motivation, including the hypothalamus, hippocampus, and amygdala (19, 23, 28, 29, 35, 36), raising the possibility that GH may exert its effects on feeding in these central nervous system (CNS) areas.

It is possible that the GH effects in CNS on feeding will include an interaction with the hypothalamic circuits regulating

appetite and energy balance including also those involved in the action of ghrelin, an endogenous ligand for the GH secretagogue receptor (GHSR) (27). In addition to stimulating GH secretion, ghrelin and ghrelin mimetics (the GH secretagogues) increase food intake and body weight gain (via increasing fat accumulation) upon intracerebroventricular (ICV) injection and peripheral administration (30, 49), suggesting that it has a role in the regulation of feeding behavior and energy balance.

Expression of bovine (b)GH under transcriptional control of the glia fibrillar acidic protein (GFAP) promoter in the brain of transgenic mice results in a hyperphagic and severely obese mouse phenotype (10). In this GFAP-bGH model, GH is overexpressed in the brain. Total GH levels were not measured, and a direct peripheral effect of increased GH levels cannot be excluded. However, the results of this study suggest a role for GH signaling in the brain in controlling energy balance. Furthermore, ICV injection of bGH acutely increases food intake in C57BL/6 mice (10). Together, these findings suggest that increased levels of GH in the CNS induce alterations in the hypothalamic systems controlling satiety and orexigenic behavior similar to ICV injections of ghrelin.

GH also regulates lipid and glucose metabolism as well as adiposity in humans and rodents (7, 18, 31, 39, 42, 45). The GHR-binding protein gene-disrupted (GHR<sup>-/-</sup>) mice used in this study are growth retarded with decreased body weight and length, delayed sexual maturity, elevated serum GH levels, and extended life span (12, 14, 52). Furthermore, it has been reported that GHR<sup>-/-</sup> mice have decreased blood glucose levels, increased insulin sensitivity (21), and unchanged serum leptin and ghrelin levels (7, 38). It has also been reported in some, but not all, studies that GHR<sup>-/-</sup> mice become obese (7, 21, 45) and have increased food consumption early, but not later, in life (15). Hypothalamic arcuate neuropeptide Y (NPY) mRNA expression is, on the other hand, downregulated in GHR<sup>-/-</sup> mice (43). Moreover, gene expression analysis in liver of GHR<sup>-/-</sup> mice, mice with truncated GHR intracellular domains, and other GH-deficient mouse models, such as Ames dwarfs and Little mice, show alterations in genes involved in glucose, amino acid, and lipid metabolism (1, 2, 45).

In this paper, we examined the extent to which the acute effect of ghrelin on food intake is dependent on a functionally intact GHR. Furthermore, since GH has been shown to have profound effects on lipoprotein metabolism and body composition in GH-deficient humans and other models of GH defi-

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ciency, we sought to determine these parameters in  $GHR^{-/-}$  mice.

#### MATERIAL AND METHODS

**Animals.**  $GHR$  gene-disrupted ( $GHR^{-/-}$ ) mice were generated as described previously (52). Male and female heterozygous mice were intercrossed to generate  $GHR^{-/-}$  mice and wild-type (+/+) littermates (Sv129Ola-Balb/c). Male offspring were used in this study. Genotyping was performed by PCR as described earlier (52). The mice were group housed in a temperature- and illumination-controlled environment (12:12-h light-dark cycle with a 1-h dawn/sunset function), relative humidity between 45 and 55%, with unrestricted access to autoclaved tap water and standard pellet chow (R-34; Lactamin, Vadstena, Sweden) at Experimental Biomedicine, Göteborg University, Sweden. Maintenance of the mice was according to national and institutional guidelines. The experiments were carried out in accordance with the ethical certificate approved by the local ethics committee for animal experimentation in Gothenburg, Sweden.

**ICV cannulation.** The mice were anesthetized with an initial 4% isoflurane followed by a maintenance dose of 2% isoflurane and placed in a stereotaxic frame (Stoelting, Wood Dale, IL) to implant a permanent 31-gauge stainless steel guide cannula (Eicom, Kyoto, Japan) into the third ventricle (0.94 mm posterior to the bregma, 1.0 mm below the surface of the skull). Because no difference in the bregma-lambda distance was observed between  $GHR^{-/-}$  and +/+ mice, the same coordinates were used for both  $GHR^{-/-}$  and +/+ mice. The guide cannulas were held in position by dental cement (Heraeus Kulzer, Hannau, Germany) and attached to two stainless steel screws driven into the skull. A stainless steel obturator (Eicom) was inserted into guide to maintain cannula patency. The animals were allowed 4 days of postoperative recovery. ICV injections (1  $\mu$ l) were carried out during a short period of anesthesia with 2% isoflurane. Substances were injected by stainless steel injector inserted into and projecting 1.5 mm below the tip of the guide cannula. A 5- $\mu$ l Hamilton syringe (VWR international, Stockholm, Sweden) was connected to a plastic tube and used for injection.

**Food intake measurement and sample collection.** The mice were fasted for 16 h before ICV injection, which occurred at the beginning of the dark phase (19:00). In a first, randomized cross-over experiment,  $GHR^{-/-}$  mice and +/+ littermates ( $n = 7-8$ ; age, 3 mo) were ICV injected with either ghrelin (0.4  $\mu$ g rat *n*-octanoylated ghrelin; Bachem, Weli am Rhein, Germany) or an equal volume of Ringer solution (vehicle) over 45 s. The opposite treatment was given 4 days later at the same hour of the day. Fresh solutions of ghrelin were prepared before each experiment. Cumulative food consumption over 3 h was measured as described previously (8).

In a second experiment,  $GHR^{-/-}$  mice (4–5 mo) and +/+ littermates were divided into four groups ( $n = 6-9$  per group) and ICV injected with either ghrelin (0.4  $\mu$ g) or an equal volume of vehicle at 0900. Animals had unrestricted access to chow and water before the

injection. Food consumption was measured as explained above. A single injection of the same treatment (0.4  $\mu$ g ghrelin, or vehicle) was given to the mice 7 days later. The mice were anesthetized 30 min after injection with 4% isoflurane and killed by heart puncture. Both before and after injection, the mice had free access to food and water. Blood serum was collected after centrifugation (2,500 rpm, 10 min) and stored at  $-80^{\circ}\text{C}$  until assayed. Hypothalamus, the remaining brain, and various peripheral organs were dissected and stored at  $-80^{\circ}\text{C}$ .

**RNA extraction, DNase treatment, and cDNA synthesis.** Total RNA preparation from dissected organs was performed using TRIzol Reagent kit (Invitrogen, Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. The RNA pellet was dissolved in RNase-free  $\text{H}_2\text{O}$ , and the concentration was measured using a spectrophotometer. Aliquots from all samples were loaded on a nuclease-free TAE agarose gel (1%) to confirm RNA quality. To eliminate DNA contamination in the samples, all cDNA synthesis was initiated with DNase treatment using a DNA-free kit (Ambion, Austin, TX). Both reverse transcriptase (RT) and  $-RT$  controls were used. cDNA was synthesized using Superscript II RNase H<sup>-</sup> Reverse Transcriptase and random hexamer primers (Life Technologies, Frederick, MD), according to manufacturer's protocol. After the synthesis, cDNA samples were stored at  $-20^{\circ}\text{C}$  until analyzed.

**Real-time quantitative PCR analysis.** Quantification of mRNA levels was performed using Taqman real-time PCR with both FAM/TAMRA-labeled fluorescence probe with TaqMan Universal PCR Master Mix (Roche Molecular Systems, Branchburg, NJ) and SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK). All samples were run in triplicates, and each triplicate was normalized using mouse acidic ribosomal phosphoprotein PO (M36B4) as an endogenous control. Primers were optimized and linear amplification was confirmed. Mixing was done with a Tecan Genesis RSP 200 Robot (Tecan, San Jose, CA) and analyzed using ABI 7900 HT (Applied Biosystems). A triplicate of 7  $\mu$ l of master mix and 3  $\mu$ l of water was run along in each plate as a nontemplate control. Analysis of the data was done in SDS 2.1 (ABI Prism, Applied Biosystems). Sequences for the primers and probes used in the Taqman PCR are presented in Table 1.

**Dual-energy X-ray absorptiometry.** The total soft tissue lean body mass, body fat content, and bone mineral density (BMD) of mice fed standard chow diet was measured by dual-energy X-ray absorptiometry (DEXA). DEXA analysis was performed on isoflurane (Baxter, Kista, Sweden)-anesthetized male  $GHR^{-/-}$  mice ( $n = 7$ ) and +/+ littermates ( $n = 7$ ) at age 3.5 mo by densitometry using a PIXImus imager (Lunar GE Medical systems, Madison, WI).

**Organ weights.** The animals were killed, and adipose tissue, liver, and brain were dissected, weighed, and kept at  $-80^{\circ}\text{C}$  until assayed. Blood was collected and serum separated for later analysis, meanwhile stored at  $-80^{\circ}\text{C}$ .

Table 1. Sequences for Taqman PCR primers and probes

| Gene              | FP 5'-3'   | RP 5'-3'                   | Probe 5'-3'                         |
|-------------------|--|----------------------------|-------------------------------------|
| NPY precursor     | CTCCAAGCCGGACAATCC   | GAGCGGAGTAGTATCTGGCCAT     | CCTCTGCTGGCGGCTCCTCG                |
| AgRP precursor    | TTGGCGGAGGTGCTAGATC  | GACTCGTGCAGCCTTACACAG      |                                     |
| Pro-MCH precursor | GAATGGAGTTCAGAATACTGAGTCCA                                   | AGCATACACCTGAGCATGTCAAAAT  | TCCTTCCTATGGGAAATTTAGCTGAGTTTCTTCAT |
| CART              | AGTGCCGAGTGGCGAAA  | GAGGAAAGAAATTGCAAGAAGTTCTC | ACAGTCACACAGCTTCCCGATCCTGG          |
| POMC              | CGCAGAGCCGTGGGAGGAAGA  | TCCCTCTTGAACCTAGGGGAAA     | AGGCTCCTACTCCATGGAGCACTTCCGCTG      |
| Orexin precursor  | GCCGTCTCTACGAACTGTTGC  | CGTCTTCCCAGAGTCAGGATA      | CGGAGCTGGCAACCACGCTG                |
| GHSR              | Assay on demand from Bio-Applied Systems (ID Mm 00616415_ml) |                            |                                     |

FP, forward primer; RP, reverse primer. Neuropeptide Y (NPY), melanin-concentrating hormone (MCH), cocaine- or amphetamine-regulated transcript (CART), proopiomelanocortin (POMC), growth hormone secretagogue receptor (GHSR), and orexin were analyzed with FAM-labeled probes; agouti-related protein (AgRP) was analyzed with SYBR Green dye.

**Serum analysis of hormones, lipoprotein, and metabolite profiles.** Serum triglycerides and total cholesterol concentrations were measured using commercial reagent kits (Roche Diagnostics, Mannheim, Germany). The intra-assay coefficient of variation (CV) of triglyceride measurements was 1.5% (mean conc. 1.21 mM, limit of detection 0.05 mM). The intra-assay CV of cholesterol measurements was 0.8% (mean conc. 6.0 mM, limit of detection 0.05 mM). Glucose analysis was performed using a photometric assay kit HK 125 (ABX Diagnostics-Parch Euromedecine, Montpellier, France). The intra-assay CV of glucose measurements was 1.3% (mean conc. 5.2 mM, limit of detection 0.2 mM). The assays were performed using a Cobas Mira analyzer (Hoffman-La Roche, Basle, Switzerland). Serum apolipoprotein (apo)B was measured by an electroimmunoassay as previously described (18). Insulin and leptin was measured using mouse-specific radioimmunoassay kits (Linco Research, St. Charles, MO; RI-13 K and ML-82 K, respectively). The intra-assay CV of insulin determinations was 1.4% (mean conc. 120 pM); the intra-assay CV of leptin measurements was 4.0% (mean conc. 2.2 ng/ml). Corticosterone in serum was measured using an RIA kit (Amersham Life Science, Amersham International; RPA 548). The intra-assay CV of corticosterone measurements was 5.2% (mean conc. 200 ng/ml). For total ghrelin concentration, an RIA kit was used (Linco Research, GHRT-89HK). The intra-assay CV was 3.3% for the ghrelin measurements (mean conc. 1,500 pg/ml). The cholesterol distribution profiles were measured using a size exclusion high-performance liquid chromatography system, SMART, with column Superose 6 PC 3.2/30 (Amersham Pharmacia Biotech, Uppsala, Sweden) as described previously (33). The lipoproteins in a 10- $\mu$ l sample were separated over 60 min, and the area under the curve represents the cholesterol content. The peaks in the profiles are designated VLDL, LDL, and HDL for simplicity, even though it is clear that the separation is determined primarily by the size of the lipoproteins.

**Statistical analysis.** The serum profiles were analyzed by Student's *t*-test. Wilcoxon's paired rank sum test was used to analyze the difference in food intake in the paired study of fasted  $GHR^{-/-}$  mice and their  $+/+$  littermates. Results from mice fed ad libitum and administered vehicle or ghrelin were analyzed by one-way ANOVA followed by a Bonferroni post hoc test. Values were transformed to logarithms when appropriate. *P* values of  $<0.05$  were considered significant.

## RESULTS

**Effects of ghrelin on food intake and hypothalamic gene expression.** To determine whether a functionally intact GHR is required for ghrelin's CNS effects on food intake,  $GHR^{-/-}$  mice and littermate controls were injected with ghrelin. Because circulating endogenous ghrelin is influenced by states of energy balance, responses were examined in both fasted and fed mice.  $GHR^{-/-}$  mice and littermate controls that had been fasted for 16 h were ICV injected with ghrelin or vehicle at the beginning of the dark phase. Analysis of the difference in food intake after ghrelin and vehicle treatment in the same animals showed that the increase in food intake in response to ghrelin in the littermate control group differed significantly from that in the  $GHR^{-/-}$  group ( $P = 0.015$ ) (Fig. 1A). Thus the response to ghrelin was blunted in  $GHR^{-/-}$  mice. Moreover, the  $GHR^{-/-}$  mice had a significantly higher food consumption per gram body weight than control animals (80%,  $P < 0.01$ ; Fig. 1B).

In the next experiment, ad libitum-fed littermates and  $GHR^{-/-}$  mice were ICV injected with ghrelin or vehicle in the beginning of the light phase (Fig. 2A). Ghrelin treatment of the littermate controls increased the food intake 5.8-fold compared with the vehicle-treated control mice ( $P < 0.05$ ). In contrast,

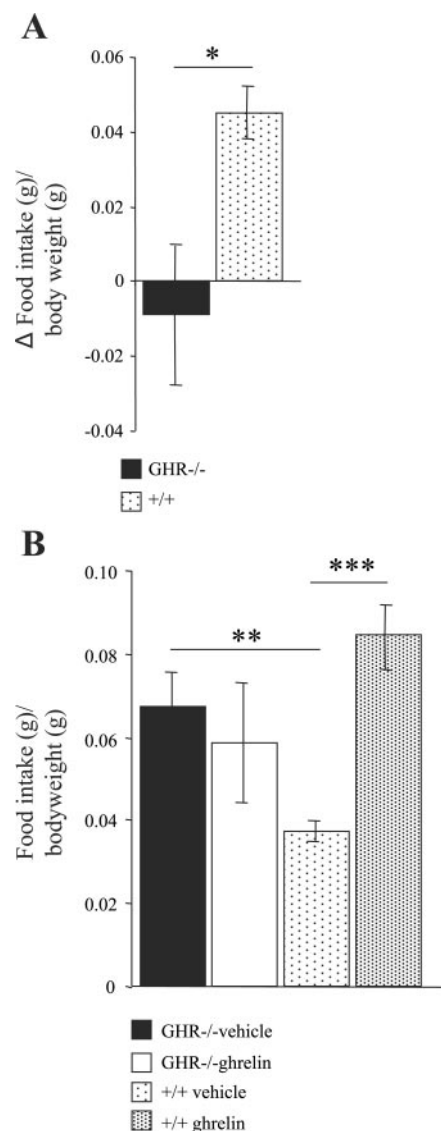


Fig. 1. Effect of acute intracerebroventricular (ICV) ghrelin injection on food intake in fasted growth hormone receptor-binding protein-deficient ( $GHR^{-/-}$ ) mice and their wild-type ( $+/+$ ) littermate controls. A:  $\Delta$ food intake/body wt compares the change in food intake between ghrelin treatment (0.4  $\mu$ g) and vehicle treatment, expressed per body weight in  $GHR^{-/-}$  ( $n = 8$ ) and  $+/+$  littermate controls ( $n = 7$ ). The study was paired as single animals received either vehicle or ghrelin on 2 separate occasions. Animals were fasted 16 h before ICV injections, and food intake was measured over a 3-h period postinjection. B: food intake in  $GHR^{-/-}$  ( $n = 8$ ) and  $+/+$  littermate control mice ( $n = 7$ ) compared with vehicle treatment. Values are expressed as means  $\pm$  SE. For A, Wilcoxon's rank sum test ( $*P < 0.05$ ), and for B, Student's *t*-test ( $***P < 0.001$ ,  $**P < 0.01$ ) were used.

ghrelin treatment of fed  $GHR^{-/-}$  mice did not result in a significant change in food intake. Thus the  $GHR^{-/-}$  mice are much less responsive to the feeding effect of ghrelin whether or not they are fasted. In this experiment, serum levels of ghrelin and other hormones were determined to investigate whether the altered responsiveness of  $GHR^{-/-}$  mice to ghrelin treatment was accompanied by other differences between the genotypes. ICV injection of ghrelin resulted in a similar increase in serum concentration of ghrelin in  $GHR^{-/-}$  and littermate mice (4.9- and 6.4-fold, respectively; Fig. 2B). However, the injection of ghrelin had no acute effect on serum leptin or corticosterone

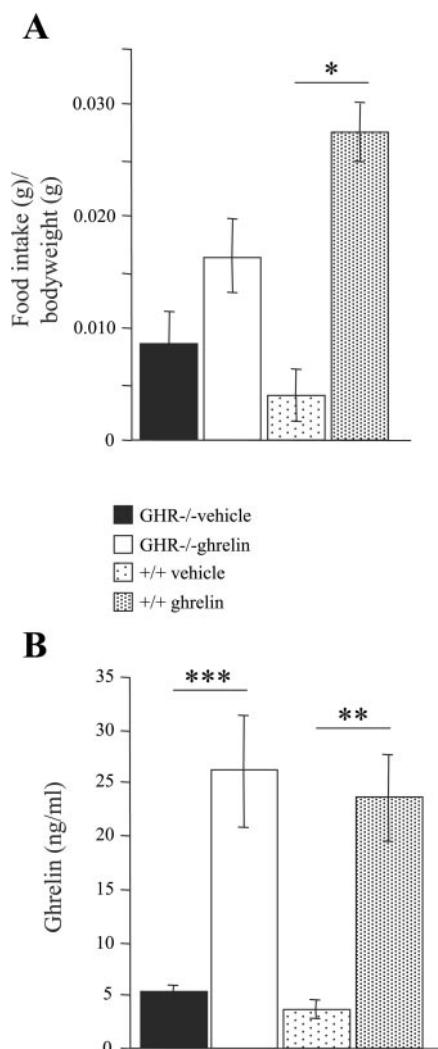


Fig. 2. Effect of acute ICV ghrelin injection on food intake in fed GHR<sup>-/-</sup> mice and +/+ littermate controls. *A*: ICV injection of ghrelin (0.4  $\mu$ g) to GHR<sup>-/-</sup> ( $n = 7$ ) and +/+ littermate control mice ( $n = 6$ ). Food intake was measured over a 3-h period postinjection. *B*: total ghrelin levels in GHR<sup>-/-</sup> ( $n = 7$ ) and +/+ littermate controls ( $n = 6$ ). Values are given as means  $\pm$  SE. Statistical analysis was done by 1-way ANOVA followed by Bonferroni post hoc test (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

levels in either GHR<sup>-/-</sup> mice or littermate controls (data not shown).

In this experiment, hypothalamic mRNA expression was analyzed by real-time PCR for several peptides that participate in the hypothalamic circuits controlling food intake and energy balance. There was no significant difference in AgRP mRNA expression between GHR<sup>-/-</sup> and littermate control mice given vehicle. However, when given ghrelin, littermate control mice had significantly higher AgRP mRNA expression than GHR<sup>-/-</sup> mice ( $P < 0.05$ ; Fig. 3A). Moreover, NPY mRNA tended to be higher (1-way ANOVA,  $P = 0.06$ ) in ghrelin-treated littermate controls than in ghrelin-treated GHR<sup>-/-</sup> mice (Fig. 3B). We did not detect any differences between the treatment groups on expression of proopiomelanocortin (POMC), melanin-concentrating hormone (MCH) mRNA, GHSR mRNA, orexin mRNA, or cocaine- or amphetamine-regulated transcript (CART) mRNA (Table 2).

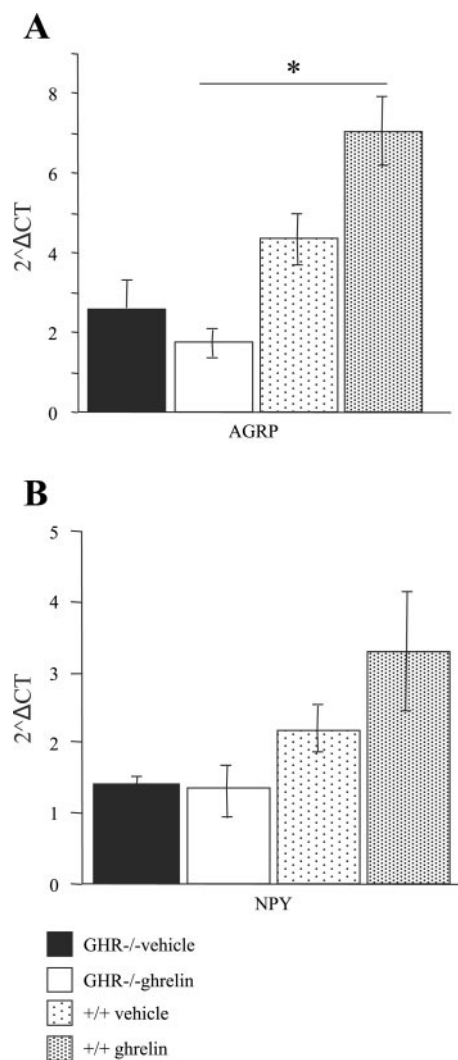


Fig. 3. Expression levels of agouti-related protein (AgRP) and neuropeptide Y (NPY) mRNA in hypothalamus. AgRP and NPY mRNA were quantified by Taqman real-time PCR. Values presented as 2<sup>-ΔCT</sup>, where ΔCT is the mean of triplicates normalized using mouse acidic ribosomal phosphoprotein PO (M36B4). Statistical analysis was done by 1-way ANOVA followed by Bonferroni post hoc test. Values are given as means  $\pm$  SE;  $n = 5$  in +/+ groups and  $n = 7$  in GHR<sup>-/-</sup> (\* $P < 0.05$ ).

Table 2. Expression levels of hypothalamic genes in freely fed GHR<sup>-/-</sup> and their +/+ littermates

| Gene   | GHR <sup>-/-</sup>  |                     | +/+                 |                     |
|--------|---------------------|---------------------|---------------------|---------------------|
|        | Vehicle             | Ghrelin             | Vehicle             | Ghrelin             |
| CART   | 0.0029 $\pm$ 0.0003 | 0.0029 $\pm$ 0.0003 | 0.0035 $\pm$ 0.0008 | 0.0031 $\pm$ 0.0006 |
| MCH    | 2.1 $\pm$ 0.19      | 1.72 $\pm$ 0.21     | 1.64 $\pm$ 0.44     | 1.49 $\pm$ 0.24     |
| Orexin | 2.44 $\pm$ 0.19     | 3.11 $\pm$ 1.18     | 2.35 $\pm$ 0.51     | 2.62 $\pm$ 0.94     |
| POMC   | 0.020 $\pm$ 0.003   | 0.022 $\pm$ 0.006   | 0.026 $\pm$ 0.006   | 0.039 $\pm$ 0.011   |
| GHSR   | 0.65 $\pm$ 0.07     | 0.47 $\pm$ 0.06     | 0.71 $\pm$ 0.09     | 0.83 $\pm$ 0.21     |

Values are means  $\pm$  SE and presented as 2<sup>-ΔCT</sup>, where ΔCT is the mean of triplicates normalized using mouse acidic ribosomal phosphoprotein PO (M36B4). GHR<sup>-/-</sup>, growth hormone receptor-binding protein-deficient; +/+, wild-type littermates. mRNA expression levels were quantified by real-time PCR. Statistical analysis was made with 1-way ANOVA followed by Bonferroni post hoc test.

Table 3. Effect of GHR deletion on body and organ weights

|  | Absolute Values |                    | Values Related to Body Weight,<br>%body wt |                    |
|--|-----------------|--------------------|--|--------------------|
|  | +/+             | GHR <sup>-/-</sup> | +/+  | GHR <sup>-/-</sup> |
| Body weight, g                               | 27.93 ± 0.76    | 14.29 ± 0.53†      |  |                    |
| Body length, cm                              | 10.31 ± 0.00    | 7.59 ± 0.053†      |  |                    |
| Brain weight, g                              | 0.44 ± 0.00     | 0.37 ± 0.00†       | 1.6 ± 0.05                                 | 2.6 ± 0.09†        |
| Liver weight, g                              | 1.05 ± 0.04     | 0.40 ± 0.01†       | 3.7 ± 0.07                                 | 2.8 ± 0.10†        |
| Retroperitoneal adipose tissue weight, g     | 0.17 ± 0.03     | 0.19 ± 0.04        | 0.6 ± 0.10                                 | 1.3 ± 0.25*        |
| Reproductive adipose tissue weight, g        | 0.74 ± 0.14     | 0.48 ± 0.06        | 2.6 ± 0.45                                 | 3.3 ± 0.32         |
| Interscapular brown adipose tissue weight, g | 0.14 ± 0.01     | 0.12 ± 0.00        | 0.5 ± 0.04                                 | 0.9 ± 0.05†        |

Values are given as means ± SE. Three and a half-month-old GHR<sup>-/-</sup> ( $n = 7$ ) and +/+ mice ( $n = 7$ ) were compared. Student's *t*-test was used for statistical analysis \* $P < 0.05$ , † $P < 0.001$ .

**Body weight and body composition.** Because food intake was altered in GHR<sup>-/-</sup> mice, GHR<sup>-/-</sup> mice and wild-type littermate controls of 3.5 mo of age were investigated with respect to body composition. As previously shown (47, 52), body weight and body length was markedly lower in GHR<sup>-/-</sup> mice compared with their littermate controls (Table 3). To determine whether deletion of GHR affected body composition, DEXA analysis was performed. The ratio between non-bone lean mass and crown-rump length was decreased by 43% ( $P < 0.001$ ) and the percent body fat was increased 2.4-fold ( $P < 0.001$ ) in GHR<sup>-/-</sup> compared with control mice (Fig. 4, A and B). The ratio between total bone mineral content to crown-rump length as well as total bone area to crown-rump length was decreased by 45 and 23% respectively in GHR<sup>-/-</sup> compared with +/+ mice.

To compare the relative organ mass, individual organ weights were related to the total body weight (Table 3). Weights of brain, retroperitoneal adipose tissue, and interscapular brown adipose tissue were disproportionately larger (brain  $164 \pm 3.4\%$ ,  $P < 0.001$ ; retroperitoneal adipose tissue  $227.5 \pm 18.5\%$ ,  $P < 0.05$ ; brown adipose tissue  $176 \pm 3.6\%$ ,  $P < 0.001$ ), whereas liver weights were disproportionately smaller ( $76 \pm 3.6\%$ ,  $P < 0.001$ ) in the GHR<sup>-/-</sup> mice compared with littermate controls.

**Serum hormones and lipoproteins.** In accordance with the increased body fat mass in the GHR<sup>-/-</sup> mice, serum leptin levels were increased 4.8-fold ( $P < 0.001$ ) in GHR<sup>-/-</sup> mice compared with littermate controls (Fig. 5A). Lower serum levels of nonfasting glucose and insulin (glucose  $-23\%$ ,  $P < 0.01$ ; insulin  $-75\%$ ,  $P < 0.01$ ) as well as elevated corticosterone levels ( $115\%$ ,  $P < 0.01$ ) were observed in GHR<sup>-/-</sup> mice compared with their controls (Fig. 5, B–D).

To investigate whether the alterations in serum insulin and glucose levels in addition to the changed body composition were accompanied by changes in serum lipoprotein levels, total serum levels of cholesterol, triglycerides, and apoB as well as serum lipoprotein profiles were determined in 3.5-mo-old male GHR<sup>-/-</sup> mice and their littermates. Serum cholesterol and triglyceride levels were lower in GHR<sup>-/-</sup> mice compared with littermate controls (cholesterol  $-40\%$ ,  $P < 0.001$ ; triglyceride  $-47\%$ ,  $P < 0.001$ ; Fig. 6, A and B). In addition, serum apoB levels were markedly lower in the GHR<sup>-/-</sup> compared with control mice ( $-71\%$ ,  $P < 0.001$ ; Fig. 6C). Size exclusion chromatography of serum lipoproteins was performed to investigate which lipoprotein fractions were influenced by GHR deficiency (Fig. 6D). The profiles indicate that GHR<sup>-/-</sup> mice

have decreased serum levels of all major lipoprotein fractions, although no significant change in VLDL levels was observed (HDL  $-40\%$ ,  $P < 0.001$ ; LDL  $-61\%$ ,  $P < 0.01$ ; VLDL  $-37\%$ ,  $P = 0.25$ ; data not shown).

## DISCUSSION

In this study, we sought to determine whether ghrelin's acute effects on food intake are dependent on functionally intact GHR signaling and, furthermore, the effects of GHR deletion in mice on body composition and serum lipoproteins. Apart from short status and decreased body weight, GHR<sup>-/-</sup> mice had decreased lean mass and increased total fat mass accompanied by increased serum leptin levels and increased food intake. Moreover, we found that central ghrelin injection increased food intake in +/+ littermates but not in GHR<sup>-/-</sup> mice, indicating that GHR<sup>-/-</sup> mice are less responsive to ghrelin than +/+ mice.

It is not clear why the GHR<sup>-/-</sup> mice showed a blunted feeding response following ICV ghrelin injection. One possibility is the lack of GH signaling onto ghrelin-responsive neurons in the hypothalamus, such as the NPY/AgRP neurons in the arcuate nucleus. Certainly, the majority of these NPY/AgRP neurons express GHR and NPY mRNA expression in these neurons has been shown to be positively regulated by GH (11, 24).

We did not find any differences in expression of AgRP mRNA in GHR<sup>-/-</sup> mice compared with the +/+ mice given vehicle, whereas +/+ mice given ghrelin had significantly higher AgRP mRNA expression than GHR<sup>-/-</sup> mice given ghrelin. This finding supports the hypothesis that the ghrelin-induced feeding response involving GHR signaling is exerted at the level of the AgRP/NPY neurons. However, we were not able to detect any significant effect of ghrelin on NPY mRNA. This could be explained by the findings by others that AgRP is more strongly regulated than NPY in response to fasting and central ghrelin injections (20, 25).

In a previous study (10), we demonstrated that specific overexpression of bGH in the brain generated a severely obese phenotype with elevated expression levels of hypothalamic AgRP and NPY. The obesity of this mouse model was due to hyperphagia and not changes in energy expenditure or lower levels of peripheral GH (10). Similarly, rats injected ICV with ghrelin also developed hyperphagia-induced obesity associated with induction of AgRP and NPY in the hypothalamus (26, 37, 49). Together with the present results, these findings indicate

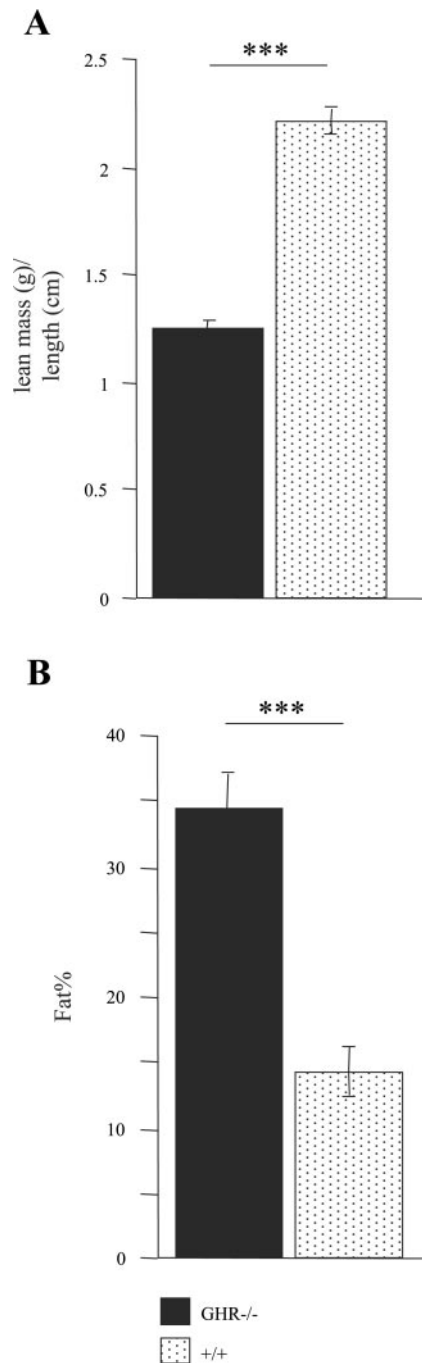


Fig. 4. GHR<sup>-/-</sup> mice are obese and have lower nonbone lean mass. DEXA measurements of lean mass per crown-rump length (A) and of %fat (B) in 3.5-mo-old GHR<sup>-/-</sup> ( $n = 7$ ) and +/+ littermate control mice ( $n = 7$ ). Values are given as means  $\pm$  SE. Student's  $t$ -test (\*\*\*)  $P < 0.001$ .

that ghrelin increases food intake, at least partly, via upregulation of AgRP and NPY and that this effect of ghrelin may be mediated, at least in part, by GH receptor signaling.

In contrast to our findings, GH-deficient rats showed increased feeding following ICV injection of ghrelin (37). Notably, the saline-injected GH-deficient rats in that study did not eat anything during the 2-h measurement period, and the effect of ghrelin in these rats was not compared with the effect in wild-type rats (37). Repeated peripheral injections of ghrelin to

GH-deficient dwarf rats, on the other hand, do not significantly increase cumulative food intake, although the authors noted a trend to overeat (49). To conclude, there are divergent results about the importance of GH signaling for the effect of ghrelin on food consumption that could be dependent on differences in species, models of deficient GH signaling, or other differences in the experimental situation.

The increased food consumption seen in vehicle-treated GHR<sup>-/-</sup> mice could have resulted from the hypoglycemia and/or the decreased circulating insulin levels. Our finding that POMC, NPY, and orexin mRNA expression was unchanged in the fed basal state is, on the other hand, not consistent with the general theory behind the appetite-promoting effects of hypo-

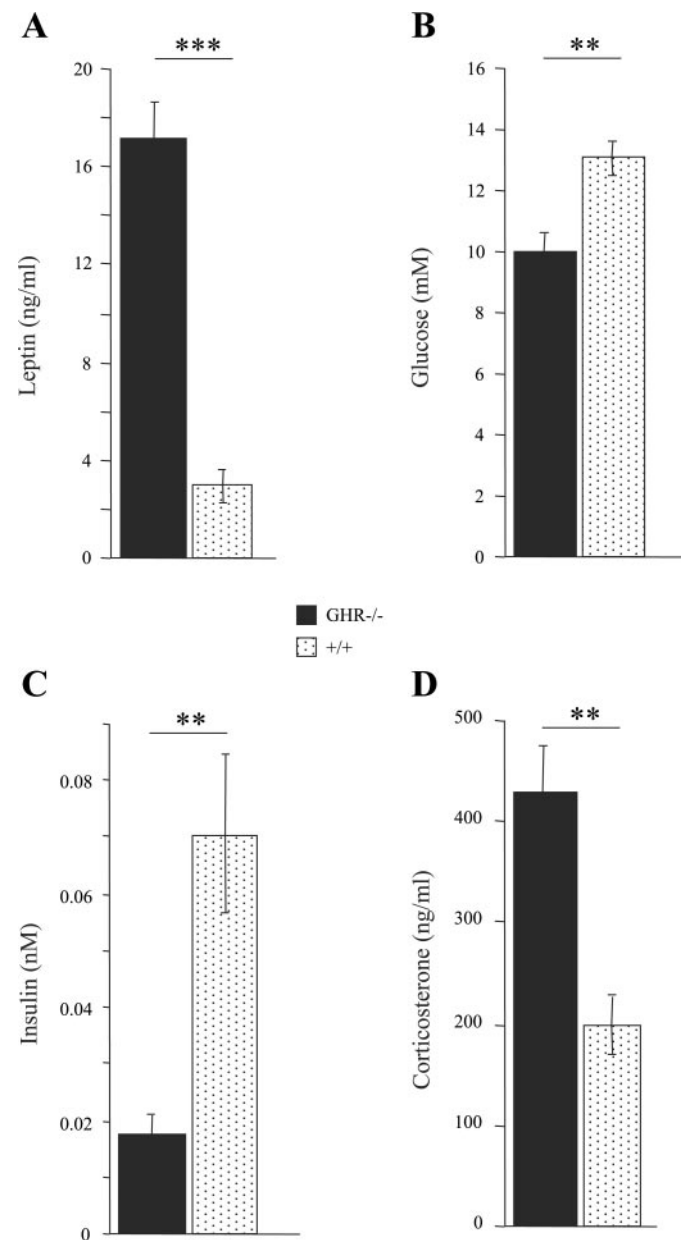


Fig. 5. Serum chemistry analysis. Serum levels of leptin (A), glucose (B), insulin (C), and corticosterone (D) in nonfasted GHR<sup>-/-</sup> and +/+ littermate control mice. Results are expressed as means  $\pm$  SE. Student's  $t$ -test (\*\*\*)  $P < 0.001$ , \*\*  $P < 0.01$ .

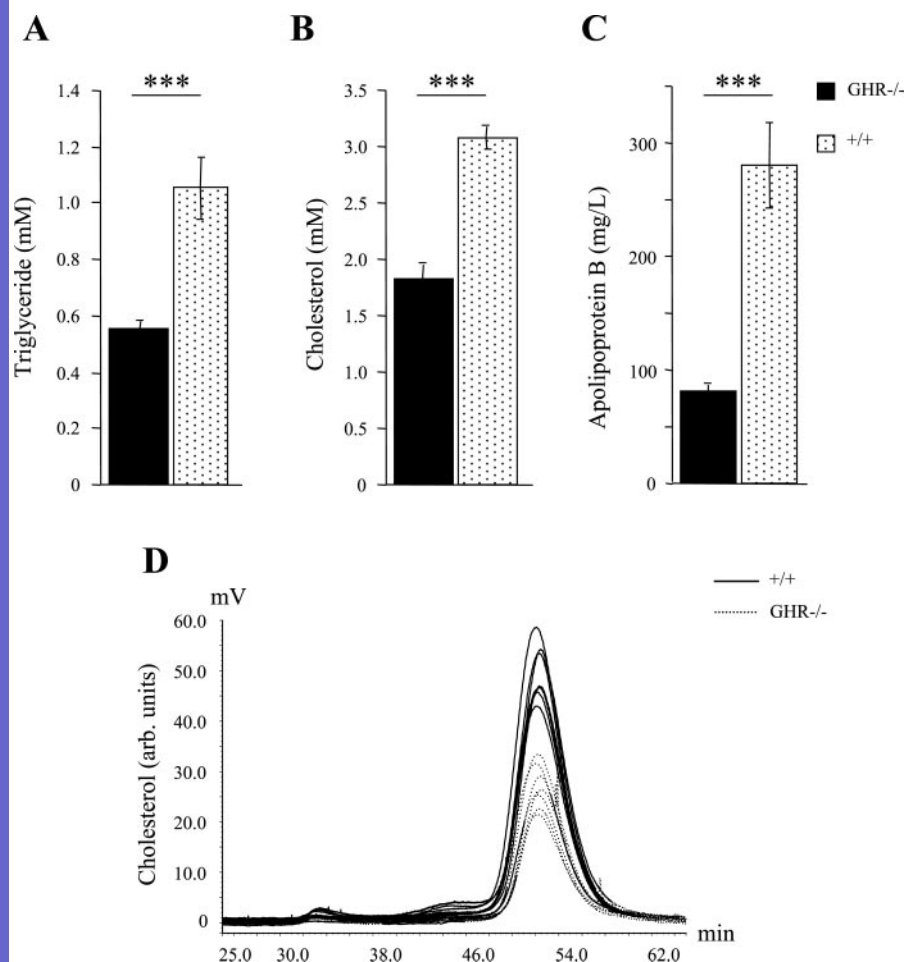


Fig. 6. GHR<sup>-/-</sup> are hypolipidemic. Triglyceride levels (A) and total cholesterol (B) in GHR<sup>-/-</sup> and +/+ littermate control mice. C: serum apolipoprotein (apo)B levels were determined by electroimmunoassay. D: serum lipoprotein size distribution was determined in individual mice with the size exclusion high performance liquid chromatography system SMART. Values are expressed as means  $\pm$  SE. Student's *t*-test (\*\*\**P* < 0.001, \*\**P* < 0.01).

insulinemia and hypoglycemia (see reviews in Refs. 32 and 51). Although GHR<sup>-/-</sup> mice are insulin sensitive, they had increased plasma glucocorticoid levels, as also shown by others (21). Glucocorticoid treatment can increase food intake and promote fat accumulation in mice (13, 50). It is therefore possible that the increased food intake and body fat observed in GHR<sup>-/-</sup> mice, at least partly, is due to increased serum levels of corticosterone. However, the fact that patients with isolated GH deficiency without changed glucocorticoid levels are also obese mitigates the assumption that the increased plasma corticosteroid levels are involved.

In line with our observation, fasting serum ghrelin levels in GHR<sup>-/-</sup> mice have been reported to be unchanged (38). Furthermore, we did not see any change in GHSR mRNA expression in the hypothalamus, suggesting that the higher food intake in GHR<sup>-/-</sup> mice is not due to increased ghrelin sensitivity. Moreover, we show that the acute effect of ghrelin was not markedly changed by nutritional status even though ghrelin responsiveness has previously been shown to increase in fasting animals (22). However, we cannot exclude the possibility that the preexisting hyperphagia in the GHR<sup>-/-</sup> mice, due to undefined mechanisms, might have blunted an additional appetite-stimulatory effect of ICV ghrelin.

In line with the findings in GHR<sup>-/-</sup> mice, visceral fat is increased and lean mass is decreased in humans with GH deficiency (5, 6). GH deficiency has been ascribed as an additional factor apart from body composition (and sex) in

regulating leptin (16). Thus the markedly enlarged retroperitoneal fat depot, the low lean mass, and the impaired GH signaling in the GHR<sup>-/-</sup> mice might explain the high levels of leptin. Because food intake was increased at baseline, it can be concluded that the high circulating levels of leptin could not have acted to suppress food intake in GHR<sup>-/-</sup> mice. In contrast to us, Berryman et al. (7) did not see increased serum leptin levels in 5-mo-old obese GHR<sup>-/-</sup> mice, probably because of large variations in serum leptin levels within the groups.

Despite their obesity, GHR<sup>-/-</sup> mice showed a marked decrease in both apoB-containing lipoproteins and HDL levels. In contrast, a previous study, using mixed groups of female and male mice, showed normal serum lipid levels in GHR<sup>-/-</sup> mice (34). It is possible that the discrepant data could be due to other effects of GHR deficiency in females and males. GH-deficient humans and hypophysectomized rats have higher LDL cholesterol and apoB levels than normal controls (4, 17, 40, 41). However, GH-deficient humans (44), hypophysectomized rats (40), and GHR<sup>-/-</sup> mice have in common lower levels of HDL cholesterol than controls. Thus it seems that mice might respond differently from humans and rats to GH deficiency with respect to apoB-containing lipoproteins but not with respect to HDL levels.

In summary, we show that GHR deficiency in mice is associated with marked changes in food intake, body composition, leptin levels, and lipoprotein metabolism. Moreover, our



data suggest that ghrelin's acute central actions to increase food intake require functionally intact GHR signaling.

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DISCLAIMER

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REFERENCES

1. Al-Regaiey KA, Masternak MM, Bonkowski M, Sun L, and Bartke A. Long-lived growth hormone receptor knockout mice: interaction of reduced insulin-like growth factor I/insulin signaling and caloric restriction. *Endocrinology* 146: 851–860, 2005.
2. Amador-Noguez D, Yagi K, Venable S, and Darlington G. Gene expression profile of long-lived Ames dwarf mice and Little mice. *Aging Cell* 3: 423–441, 2004.
3. Azain MJ, Roberts TJ, Martin RJ, and Kasser TR. Comparison of daily versus continuous administration of somatotropin on growth rate, feed intake, and body composition in intact female rats. *J Anim Sci* 73: 1019–1029, 1995.
4. Barreto-Filho JA, Alcantara MR, Salvatori R, Barreto MA, Sousa AC, Bastos V, Souza AH, Pereira RM, Clayton PE, Gill MS, and Aguiar-Oliveira MH. Familial isolated growth hormone deficiency is associated with increased systolic blood pressure, central obesity, and dyslipidemia. *J Clin Endocrinol Metab* 87: 2018–2023, 2002.
5. Bengtsson BA, Brummer RJ, Eden S, Rosen T, and Sjöström L. Effects of growth hormone on fat mass and fat distribution. *Acta Paediatr Suppl* 383: 62–66, 1992.
6. Bengtsson BA, Eden S, Lonn L, Kvist H, Stokland A, Lindstedt G, Bosaeus I, Tolli J, Sjöström L, and Isaksson OG. Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. *J Clin Endocrinol Metab* 76: 309–317, 1993.
7. Berryman DE, List EO, Coschigano KT, Behar K, Kim JK, and Kopchick JJ. Comparing adiposity profiles in three mouse models with altered GH signaling. *Growth Horm IGF Res* 14: 309–318, 2004.
8. Bjursell M, Egecioglu E, Gerdin AK, Svensson L, Oscarsson J, Morgan D, Snaith M, Tornell J, and Bohlooly YM. Importance of melanin-concentrating hormone receptor for the acute effects of ghrelin. *Biochem Biophys Res Commun* 326: 759–765, 2005.
9. Blissett J, Harris G, and Kirk J. Effect of growth hormone therapy on feeding problems and food intake in children with growth disorders. *Acta Paediatr* 89: 644–649, 2000.
10. Bohlooly YM, Olsson B, Bruder CE, Linden D, Sjogren K, Bjursell M, Egecioglu E, Svensson L, Brodin P, Waterton JC, Isaksson OG, Sundler F, Ahren B, Ohlsson C, Oscarsson J, and Tornell J. Growth hormone overexpression in the central nervous system results in hyperphagia-induced obesity associated with insulin resistance and dyslipidemia. *Diabetes* 54: 51–62, 2005.
11. Chan YY, Steiner RA, and Clifton DK. Regulation of hypothalamic neuropeptide-Y neurons by growth hormone in the rat. *Endocrinology* 137: 1319–1325, 1996.
12. Chandrasekar V, Bartke A, Coschigano KT, and Kopchick JJ. Pituitary and testicular function in growth hormone receptor gene knockout mice. *Endocrinology* 140: 1082–1088, 1999.
13. Chen HL and Romsos DR. A single intracerebroventricular injection of dexamethasone elevates food intake and plasma insulin and depresses

- metabolic rates in adrenalectomized obese (ob/ob) mice. *J Nutr* 125: 540–545, 1995.
14. Coschigano KT, Clemmons D, Bellush LL, and Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. *Endocrinology* 141: 2608–2613, 2000.
15. Coschigano KT, Holland AN, Riders ME, List EO, Flyvbjerg A, and Kopchick JJ. Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor I levels and increased life span. *Endocrinology* 144: 3799–3810, 2003.
16. de ABES, Gill MS, De Freitas ME, Magalhaes MM, Souza AH, Aguiar-Oliveira MH, and Clayton PE. Serum leptin and body composition in children with familial GH deficiency (GHD) due to a mutation in the growth hormone-releasing hormone (GHRH) receptor. *Clin Endocrinol (Oxf)* 51: 559–564, 1999.
17. de Boer H, Blok GJ, Voerman HJ, Phillips M, and Schouten JA. Serum lipid levels in growth hormone-deficient men. *Metabolism* 43: 199–203, 1994.
18. Frick F, Bohlooly YM, Linden D, Olsson B, Tornell J, Eden S, and Oscarsson J. Long-term growth hormone excess induces marked alterations in lipoprotein metabolism in mice. *Am J Physiol Endocrinol Metab* 281: E1230–E1239, 2001.
19. Gossard F, Dihl F, Pelletier G, Dubois PM, and Morel G. In situ hybridization to rat brain and pituitary gland of growth hormone cDNA. *Neurosci Lett* 79: 251–256, 1987.
20. Hahn TM, Breininger JF, Baskin DG, and Schwartz MW. Coexpression of Agrp and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci* 1: 271–272, 1998.
21. Hauck SJ, Hunter WS, Danilovich N, Kopchick JJ, and Bartke A. Reduced levels of thyroid hormones, insulin, and glucose, and lower body core temperature in the growth hormone receptor/binding protein knockout mouse. *Exp Biol Med (Maywood)* 226: 552–558, 2001.
22. Hewson AK, Tung LY, Connell DW, Tookman L, and Dickson SL. The rat arcuate nucleus integrates peripheral signals provided by leptin, insulin, and a ghrelin mimetic. *Diabetes* 51: 3412–3419, 2002.
23. Hojvat S, Baker G, Kirsteins L, and Lawrence AM. Growth hormone (GH) immunoreactivity in the rodent and primate CNS: distribution, characterization and presence posthypophysectomy. *Brain Res* 239: 543–557, 1982.
24. Kamegai J, Minami S, Sugihara H, Hasegawa O, Higuchi H, and Wakabayashi I. Growth hormone receptor gene is expressed in neuropeptide Y neurons in hypothalamic arcuate nucleus of rats. *Endocrinology* 137: 2109–2112, 1996.
25. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, and Wakabayashi I. Central effect of ghrelin, an endogenous growth hormone secretagogue, on hypothalamic peptide gene expression. *Endocrinology* 141: 4797–4800, 2000.
26. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, and Wakabayashi I. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. *Diabetes* 50: 2438–2443, 2001.
27. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, and Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402: 656–660, 1999.
28. Lai Z, Roos P, Zhai O, Olsson Y, Fohlenhag K, Larsson C, and Nyberg F. Age-related reduction of human growth hormone-binding sites in the human brain. *Brain Res* 621: 260–266, 1993.
29. Lai ZN, Emtner M, Roos P, and Nyberg F. Characterization of putative growth hormone receptors in human choroid plexus. *Brain Res* 546: 222–226, 1991.
30. Lall S, Tung LY, Ohlsson C, Jansson JO, and Dickson SL. Growth hormone (GH)-independent stimulation of adiposity by GH secretagogues. *Biochem Biophys Res Commun* 280: 132–138, 2001.
31. Leonsson M, Oscarsson J, Bosaeus I, Lundgren BK, Johannsson G, Wiklund O, and Bengtsson BA. Growth hormone (GH) therapy in GH-deficient adults influences the response to a dietary load of cholesterol and saturated fat in terms of cholesterol synthesis, but not serum low density lipoprotein cholesterol levels. *J Clin Endocrinol Metab* 84: 1296–1303, 1999.
32. Levin BE, Routh VH, Kang L, Sanders NM, and Dunn-Meynell AA. Neuronal glucosensing: what do we know after 50 years? *Diabetes* 53: 2521–2528, 2004.

33. Linden D, Alsterholm M, Wennbo H, and Oscarsson J. PPARalpha deficiency increases secretion and serum levels of apolipoprotein B-containing lipoproteins. *J Lipid Res* 42: 1831–1840, 2001.
34. Liu JL, Coschigano KT, Robertson K, Lipsett M, Guo Y, Kopchick JJ, Kumar U, and Liu YL. Disruption of growth hormone receptor gene causes diminished pancreatic islet size and increased insulin sensitivity in mice. *Am J Physiol Endocrinol Metab* 287: E405–E413, 2004.
35. Lobie PE, Garcia-Aragon J, Lincoln DT, Barnard R, Wilcox JN, and Waters MJ. Localization and ontogeny of growth hormone receptor gene expression in the central nervous system. *Brain Res Dev Brain Res* 74: 225–233, 1993.
36. Mustafa A, Nyberg F, Bogdanovic N, Islam A, Roos P, and Adem A. Somatogenic and lactogenic binding sites in rat brain and liver: quantitative autoradiographic localization. *Neurosci Res* 20: 257–263, 1994.
37. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, and Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 409: 194–198, 2001.
38. Nass R, Liu J, Hellmann P, Coschigano KT, Gaylinn B, Berryman DE, Kopchick JJ, and Thorner MO. Chronic changes in peripheral growth hormone levels do not affect ghrelin stomach mRNA expression and serum ghrelin levels in three transgenic mouse models. *J Neuroendocrinol* 16: 669–675, 2004.
39. Nikkila EA and Pelkonen R. Serum lipids in acromegaly. *Metabolism* 24: 829–838, 1975.
40. Oscarsson J, Olofsson SO, Bondjers G, and Eden S. Differential effects of continuous versus intermittent administration of growth hormone to hypophysectomized female rats on serum lipoproteins and their apoproteins. *Endocrinology* 125: 1638–1649, 1989.
41. Oscarsson J, Olofsson SO, Vikman K, and Eden S. Growth hormone regulation of serum lipoproteins in the rat: different growth hormone regulatory principles for apolipoprotein (apo) B and the sexually dimorphic apo E concentrations. *Metabolism* 40: 1191–1198, 1991.
42. Oscarsson J, Wiklund O, Jakobsson KE, Petruson B, and Bengtsson BA. Serum lipoproteins in acromegaly before and 6–15 months after transphenoidal adenomectomy. *Clin Endocrinol (Oxf)* 41: 603–608, 1994.
43. Peng XD, Park S, Gadelha MR, Coschigano KT, Kopchick JJ, Frohman LA, and Kineman RD. The growth hormone (GH)-axis of GH receptor/binding protein gene-disrupted and metallothionein-human GH-releasing hormone transgenic mice: hypothalamic neuropeptide and pituitary receptor expression in the absence and presence of GH feedback. *Endocrinology* 142: 1117–1123, 2001.
44. Rosen T, Eden S, Larson G, Wilhelmson L, and Bengtsson BA. Cardiovascular risk factors in adult patients with growth hormone deficiency. *Acta Endocrinol (Copenh)* 129: 195–200, 1993.
45. Rowland JE, Lichanska AM, Kerr LM, White M, d’Aniello EM, Maher SL, Brown R, Teasdale RD, Noakes PG, and Waters MJ. In vivo analysis of growth hormone receptor signaling domains and their associated transcripts. *Mol Cell Biol* 25: 66–77, 2005.
46. Schulz C, Wiczorek I, Reschke K, and Lehnert H. Effects of intracerebroventricularly and intraperitoneally administered growth hormone on body weight and food intake in fa/fa Zucker rats. *Neuropsychobiology* 45: 36–40, 2002.
47. Sjogren K, Bohlooly YM, Olsson B, Coschigano K, Tornell J, Mohan S, Isaksson OG, Baumann G, Kopchick J, and Ohlsson C. Disproportional skeletal growth and markedly decreased bone mineral content in growth hormone receptor  $-/-$  mice. *Biochem Biophys Res Commun* 267: 603–608, 2000.
48. Snel YE, Brummer RJ, Doerga ME, Zelissen PM, and Koppeschaar HP. Energy and macronutrient intake in growth hormone-deficient adults: the effect of growth hormone replacement. *Eur J Clin Nutr* 49: 492–500, 1995.
49. Tschop M, Smiley DL, and Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 407: 908–913, 2000.
50. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* 21: 697–738, 2000.
51. Wynne K, Stanley S, McGowan B, and Bloom S. Appetite control. *J Endocrinol* 184: 291–318, 2005.
52. Zhou Y, Xu BC, Maheshwari HG, He L, Reed M, Lozykowski M, Okada S, Cataldo L, Coschigano K, Wagner TE, Baumann G, and Kopchick JJ. A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). *Proc Natl Acad Sci USA* 94: 13215–13220, 1997.