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

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1Department of Physiology, Göteborg University, Gothenburg; 2AstraZeneca Research and Development, Molndal; 3Wallenberg Laboratory, Göteborg University, Gothenburg, Sweden; and 4Edison Biotechnology Institute and Department of Biomedical Sciences, Ohio University, Athens, Ohio

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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−/− and littermate control (+/+ ) mice to determine whether ghrelin’s acute effects on appetite are dependent on GHR signaling. GHR−/− 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−/− mice. After ghrelin injection, AgRP mRNA expression in the hypothalamus was higher in +/+ littersmates than in GHR−/− mice, indicating a possible importance of AgRP in the GHR-mediated effect of ghrelin. Compared with controls, GHR−/− 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−/− 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 GHS 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 fibrillic acid 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−/−) 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−/− 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−/− 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−/− mice (43). Moreover, gene expression analysis in liver of GHR−/− mice, mice with truncated GHR intracellular domains, and other GHR-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-
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, Västena, 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 obturater (Eicom) was inserted into guide to maintain cannula patency. The animals were allowed 4 days of postoperative recovery. ICV injections (1 μl) 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-μl 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 μg 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 μg) 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 μg 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°C until assayed. Hypothalamus, the remaining brain, and various peripheral organs were dissected and stored at −80°C.

RNA extraction, DNase treatment, and cDNA synthesis. Total RNA preparation from dissected organs was performed using TRIzol Reagent kit (Invitrogen, Life Technologies, Carlshad, CA) according to the manufacturer’s protocol. The RNA pellet was dissolved in RNase-free H2O, 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− Reverse Transcriptase and random hexamer primers (Life Technologies, Frederick, MD), according to manufacturer’s protocol. After the synthesis, cDNA samples were stored at −20°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 P (MRP6) 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 μl of master mix and 3 μl 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 (DXA). DXA 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°C until assayed. Blood was collected and serum separated for later analysis, meanwhile stored at −80°C.

Table 1. Sequences for Taqman PCR primers and probes

<table>
<thead>
<tr>
<th>Gene</th>
<th>FP 5′-3′</th>
<th>RP 5′-3′</th>
<th>Probe 5′-3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPY precursor</td>
<td>CTCGAGGCGGCAGCAATTCC</td>
<td>GAACGGAATGATATCTGGGCTAC</td>
<td>CCTCTGCTGGCGCCTTCTG</td>
</tr>
<tr>
<td>AgRP precursor</td>
<td>TTGGGGGAGGCTCATAGTCC</td>
<td>GACTCTGTCAGCGCTTACAGT</td>
<td>GGAAGCGCCACTGAGCTG</td>
</tr>
<tr>
<td>Pro-MCH precursor</td>
<td>GAGATGCGCTACCGCTGAGTCCA</td>
<td>ACAGATACCATCAGGCGTCAAAAT</td>
<td>TCCCTTTGAGAATCTGGAGCTTCTC</td>
</tr>
<tr>
<td>CART precursor</td>
<td>AGTTGGGAGAGTGGCCGAAA</td>
<td>GAGAGGAAAGATGTGGCAAGATGCTT</td>
<td>AGACTGACAGAGTTGCCCCGAGCTT</td>
</tr>
<tr>
<td>POMC precursor</td>
<td>CGGACGAGGCGGCGGCGGAGAAGA</td>
<td>TCCTTTGGATCCTAGGCGGAA</td>
<td>AGGGGGCTTCCATTGAGAGGAGTTCG</td>
</tr>
<tr>
<td>Orexin precursor</td>
<td>GCCGCTCTCGGAAAGGAGCTG</td>
<td>GGCTTGGCAGAGCTGAGATA</td>
<td>CGGCGTGGCAAGCAGCTG</td>
</tr>
<tr>
<td>GHSR precursor</td>
<td>CTGAAGATGGAGATCGAGTGTC</td>
<td>CTTGAGGAGTTCAGCGTCA</td>
<td>TCCCTTGAAGGAGCTGAGTC</td>
</tr>
</tbody>
</table>

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 Eruomedecine, 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-μl 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 littermate controls. 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, 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.
levels in either GHR$^{-/-}$ 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$^{-/-}$ and littermate control mice given vehicle. However, when given ghrelin, littermate control mice had significantly higher AgRP mRNA expression than GHR$^{-/-}$ 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$^{-/-}$ 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).

**Table 2. Expression levels of hypothalamic genes in freely fed GHR$^{-/-}$ and their +/- littermates**

<table>
<thead>
<tr>
<th>Gene</th>
<th>GHR$^{-/-}$</th>
<th>GHR$^{+/+}$</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Ghrelin</td>
</tr>
<tr>
<td>CART</td>
<td>0.0029±0.0003</td>
<td>0.0029±0.0003</td>
</tr>
<tr>
<td>MCH</td>
<td>2.1±0.19</td>
<td>1.72±0.21</td>
</tr>
<tr>
<td>Orexin</td>
<td>2.44±0.19</td>
<td>3.11±1.18</td>
</tr>
<tr>
<td>POMC</td>
<td>0.020±0.003</td>
<td>0.022±0.006</td>
</tr>
<tr>
<td>GHSR</td>
<td>0.65±0.07</td>
<td>0.47±0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE and presented as $2^{ΔCT}$, where ΔCT is the mean of triplicates normalized using mouse acidic ribosomal phosphoprotein PO (M36B4). GHR$^{-/-}$, 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.
Body weight and body composition. Because food intake was altered in GHR−/− mice, GHR−/− 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−/− mice compared with their littermate controls (Table 3). To determine whether deletion of GHR affected body composition, DEXA analysis was preformed. 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−/− 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−/− 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 intrascapular brown adipose tissue were disproportionally larger in GHR−/− mice compared with +/+ littermates (76 ± 3.6%, P < 0.001) in the GHR−/− mice compared with littermate controls.

Serum hormones and lipoproteins. In accordance with the increased body fat mass in the GHR−/− mice, serum leptin levels were increased 4.8-fold (P < 0.001) in GHR−/− 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−/− 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−/− mice and their littermates. Serum cholesterol and triglyceride levels were lower in GHR−/− 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−/− 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−/− 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−/− 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−/− mice, indicating that GHR−/− mice are less responsive to ghrelin than +/+ mice.

It is not clear why the GHR−/− 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−/− mice compared with the +/+ mice given vehicle, whereas +/+ mice given ghrelin had significantly higher AgRP mRNA expression than GHR−/− 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...
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⁻/⁻ 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. 4. GHR⁻/⁻ 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⁻/⁻ (n = 7) and +/+ littermate control mice (n = 7). Values are given as means ± SE. Student’s t-test (***P < 0.001).

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Fig. 5. Serum chemistry analysis. Serum levels of leptin (A), glucose (B), insulin (C), and corticosterone (D) in nonfasted GHR⁻/⁻ and +/+ littermate control mice. Results are expressed as means ± 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 and body fat observed in 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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