It is well known that peripherally administered growth hormone (GH) results in decreased body fat mass. However, GH-deficient patients increase their food intake when substituted with GH, suggesting that GH also has an appetite stimulating effect. Transgenic mice with an overexpression of bovine GH in the central nervous system (CNS) were created to investigate the role of GH in CNS. This study shows that overexpression of GH in the CNS differentiates the effect of GH on body fat mass from that on appetite. The transgenic mice were not GH-deficient but were obese and showed increased food intake as well as increased hypothalamic expression of agouti-related protein and neuropeptide Y. GH also had an acute effect on food intake following intracerebroventricular injection of C57BL/6 mice. The transgenic mice were severely hyperinsulinemic and showed a marked hyperplasia of the islets of Langerhans. In addition, the transgenic mice displayed alterations in serum lipid and lipoprotein levels and hepatic gene expression. In conclusion, GH overexpression in the CNS results in hyperphagia-induced obesity indicating a dual effect of GH with a central stimulation of appetite and a peripheral lipolytic effect. Diabetes 54:51–62, 2005

From the 1Department of Physiology, Göteborg University, Göteborg, Sweden; the 2Department of Internal Medicine Research Centre for Endocrinology and Metabolism, Sahlgrenska University Hospital, Göteborg, Sweden; 3AstraZeneca Research and Development, Mölndal, Sweden; 4Wallenberg Laboratory for Cardiovascular Research, Sahlgrenska University Hospital, Göteborg, Sweden; 5AstraZeneca Research and Development, Alderley Park, Macclesfield, Cheshire, U.K.; and the Departments of 6Physiology and 7Medicine, Lund University, Lund, Sweden.

Address correspondence and reprint requests to Mohammad Bohlooly, AstraZeneca Transgenics and Comparative Genomics, AstraZeneca Research and Development, 43183 Mölndal, Sweden. E-mail: mohammad.bohlooly@astrazeneca.com.

Received for publication 23 April 2004 and accepted in revised form 28 September 2004.

AGRP, agouti-related protein; apo, apolipoprotein; bGH, bovine growth hormone; CNS, central nervous system; GFAp, glial acid fibrillary protein; GH, growth hormone; ICV, intracerebroventricular; MC4-R, melanocortin receptor-4; MCH, melanin-concentrating hormone; MCH-R, melanin-concentrating hormone receptor; NPY, neuropeptide Y; POMC, proopiomelanocortin; RER, respiratory exchange ratio.

© 2005 by the American Diabetes Association.

The costs of publication of this article were deferred in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
develop obesity with hyperleptinemia due to antagonism of the MC4-R by ectopic expression of the agouti protein (34,35). Whether AGRP neurons express GH receptors is still unknown. Also, neuropeptide Y (NPY) neurons in the arcuate nucleus are involved in energy homeostasis (36). Chronic administration of NPY to the hypothalamus of normal animals mimics the phenotype of leptin deficiency, including obesity, hyperphagia, and inhibition of GH production (37), and furthermore, leptin administration reduces NPY expression (38). NPY neurons in the arcuate nucleus have been shown to express GH receptors, while no expression was found in NPY neurons outside the arcuate nucleus (39). Whether the effects of GH on food intake, as seen in GH-deficient patients, are mediated by these central processes is not known.

We have generated a mouse model in which bovine GH (bGH) is overexpressed in the CNS using the promoter of glial acid fibrillary protein (GFAP). The GFAP-bGH mice were shown to have a CNS-specific expression of bGH (40). bGH could be detected in the serum of two lines of GFAP-bGH mice (line 2: 207 ± 21 ng/ml; line 3: 334 ± 53 ng/ml, which have been used in this study), but serum IGF-I levels were similar in GFAP-bGH mice and littermate wild-type control mice. These findings show that the GFAP-bGH mice are not GH-deficient in the peripheral circulation, and that the circulating levels of bGH are much lower than metallothionein-I transgenic mice with a general overexpression of bGH (41). In addition, both Mt-bGH and GFAP-bGH had threefold elevated corticosterone levels (40). In contrast to the Mt-bGH mice, the GFAP-bGH mice did not display enhanced spontaneous locomotor activity, indicating that this behavior involves peripheral effects (40).

The aim of this study was to investigate the effect of overproduction of GH in the CNS on food intake, body fat mass, insulin sensitivity, and subsequent alterations in lipoprotein metabolism. These are known effects of GH (3,42–44), but the importance of GH production in the CNS for these effects in an animal model that is not GH deficient is not known.

RESEARCH DESIGN AND METHODS

To generate GFAP-bGH transgenic animals, 110 injected C57 BL/6xCBA embryos were implanted into 5 C57 BL/6xCBA foster mothers. Out of 22 newborn mice, 5 mice were identified as carrying the GFAP-bGH transgene (founder animals) using Southern blot analysis (40). In this study, the founder line with highest bGH expression in the brain (line 3) was used in most experiments, but to confirm the GFAP-bGH phenotype, additional experiments were performed in a second founder line (line 2) (40). The environment of the animal room was controlled with a 12-h light-dark cycle (7:30 a.m. to 7:30 p.m., with a 1-h dawn/sunset function), a relative humidity between 52% and 70% (founder animals), and immediately frozen in liquid nitrogen for further storage at −150°C.

Intracerebroventricular cannulation. Normal male C57BL/6 mice were anesthetized with initial 4% isoflurane (Baxter, Kista, Sweden) followed by a maintenance dose of 2% isoflurane and placed in a stereotaxic frame (Stoeling, Wood Dale, IL) to implant a permanent 31-gauge stainless steel guide cannula (Eicom, Kyoto, Japan) above the third ventricle (0.94 mm posterior to the bregma, 1.0 mm below the surface of the skull). Guide cannulas were held in position by dental cement (Heraeus Kulzer, Hannau, Germany) attached to two stainless steel screws driven into the skull. A stainless steel obturater (Eicom) was inserted into the guide to maintain cannula patency. The animals were allowed 4 days postoperative recovery. ICV injections (1 μl) were carried out after a short period of anesthesia with 2% isoflurane. bGH was injected by a stainless steel injector, inserted in and projected 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. Food was deprived from the mice 18 h before measurement of food intake. Measurements started at 10:00 a.m. Cages (37 × 10−3 m2) were prepared with normal diet and aspen chip at the bottom. The animals were then incubated at 80°C for 1 h to correct for any differences in humidity. After the cage had reached room temperature, they were accurately weighted. The mice were weighed and immediately put in the preweighed cage with free access to food and water. Measurement of feeding was done for 48 continuous hours. The mice were then returned to their original cages. All excrements were removed, and the cages were reincubated at 80°C for 1 h in order to dry out water spill and urine, and finally weighed at room temperature. In a separate experiment, ad libitum–fed 3-month-old male C57BL/6 mice (randomized by body weight) were ICV injected with vehicle (Ringer, n = 4), or GH (10.6 μg, n = 5), and food intake was measured for 3 h postinjection as described above.

Indirect calorimetry. Oxygen consumption (vO2) and carbon dioxide production (vCO2) were measured using an open circuit calorimetry system (Oxymax; Columbus Instruments International, Columbus, OH). The animals were placed in calorimeter chambers with ad libitum access to normal lab diet and water for 48 h. An air sample was withdrawn for 75 s every 20 min. The O2 and CO2 content were measured by a paramagnetic oxygen sensor and a spectrophotometric CO2 sensor. These values were used to calculate vO2 and vCO2. Data from the first 24 h were not used in the analysis. Data from corresponding hours during the second 12-h light period were used in 2-h bins. We calculated energy expenditure (kcal/h) from the following equation: (3.815 + 1.232 RER) × vO2, where RER is the respiratory exchange ratio (volume of CO2 produced per volume of O2 consumed [both ml/kg·min−1]) and vO2 is the volume of O2 consumed per hour per kilogram of mass of animal. The value of metabolic rate was correlated to individual body weights. The resting metabolism was analyzed by using the lowest value of RER during light period and the value of metabolic rate at that exact time for calculations.

Peripheral quantitative computerized tomography. Computerized tomography was performed with the STRATEC pQCT XCT (version 5.4B; Norland Medical Systems) operating at a resolution of 70 μm as previously described (45,46). Sections were made at the same level in all mice (i.e., 5 mm proximally to the right iliac crest). Magnetic resonance imaging. At 6–7 months of age, four male GFAP-bGH transgenic mice and five male littermate controls were killed. We used a magnetic resonance imaging system (Varian, Palo Alto, CA) incorporating a 4.7 T magnet (Oxford Instruments, Oxford, U.K.) and pulsed field gradients capable of 200 mT m−1 with a rise time of 0.3 ms. A quadrate birdcage radiofrequency transceiver with a 10-mm internal diameter and sufficient radiofrequency homogeneity to encompass the entire cadaver was used. Image acquisition used a multislice two-dimensional spin-echo technique (TR = 5 s; TE = 11 ms; 41 contiguous transverse slices; 2-mm slice thickness; matrix 128 × 128; field of view 50 mm × 50 mm × 82 mm). Both fat (CH3)2 and water (H2O)-suppressed magnetic resonance images were obtained. This image matrix included the entire animal. Two phantoms were also included in the image field. These were two 4.2-mm inner diameter tubes containing water and olive oil. The fat- and water-suppressed images were obtained by applying a Gaussian saturation pulse applied on the fat and water resonances.

Image analysis. Segmentation was performed manually using software written in-house in interface definition language (Research Systems, Boulder, CO). Segmentation of both subcutaneous adipose tissue and abdominal/thoracic adipose tissue was obtained from the water-suppressed images. The segmentations were performed from the cranial end of the thoracic cavity to the caudal end of the abdomen. Thus, the body was included but limbs and head excluded from the segmentation. The subcutaneous adipose tissue in this region was defined as adipose tissue present outside the abdominal and thoracic cavities. The volumes were computed using programs written in-house in interface definition language. The segmented regions were subsequently rendered to colored image representations of the different tissues of the visceral and subcutaneous adipose tissues. Brown fat was identified from signal intensity.

Magnetic resonance spectroscopy. Magnetic resonance spectra were also obtained without the phentoms present using the same coil. Eight averages
were acquired, and the repetition time was 17 s. Tissue water was used as an internal reference at a chemical shift of 4.8 ppm. Spectra were integrated using VNMR software (Varian, Palo Alto, CA). The integrals of the water signal (I_{H2O}) and the charger (CH2) and CH3 signals from fat (I_{fat}) were used in the calculation. The integrals were normalized to the total area to account for variations in sample thickness.

**Analysis of hormones and glucose in serum/plasma.** Serum leptin levels were measured by an ELISA assay (Crystal Chem, Downers Grove, IL). Serum insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, 125I-labeled human insulin as tracer, and antirabbit Ig as secondary antibody. Details of the antibodies and methods are previously described (40). For each hormone, five sections taken at different levels of each pancreas (n = 6) were examined.

**Analysis of glucose and serum/plasma.** Serum leptin levels were measured by an ELISA assay (Crystal Chem, Downers Grove, IL). Plasma insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, 125I-labeled human insulin as tracer, and antirabbit Ig as secondary antibody (Linco Research, St. Charles, MO). Serum triglycerides were measured using enzymatic colorimetric assays (CH/CHOD-PAP and TG/GPO-PAP; Roche Diagnostics, Mannheim, Germany). Serum apolipoprotein (apo) B was measured by a radioimmunoassay (RPA 548) from rat insulin as standard (Linco Research, St. Charles, MO). Plasma corticosterone was measured by a radioimmunoassay (RPA 548) from rat insulin as standard (Linco Research, St. Charles, MO). Plasma cortisol was measured by a radioimmunoassay (RPA 548) from rat insulin as standard (Linco Research, St. Charles, MO). Plasma glucose was measured by an electroimmunoassay as previously described (3,47). The cholesterol, triglyceride, and LDL levels were measured using the EukGE-WS2 protocol (Affymetrix). The chips were then directly hybridized against the MG-U74Av2 mouse genome array (Affymetrix) using protocols supplied by the manufacturer. Washing and staining were done with a Fluidics Station 400 using the EukGE-WS2 protocol (Affymetrix). Microarray analysis was performed as described before (48) using enhanced chemiluminescence (ECL) detection (Amersham Pharmacia Biotech, Uppsala, Sweden) and a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The results were analyzed using the GeneSpring (Silicon Genetics, Redwood City, CA) program. Initially, genes that were scored as absent on at least 22 of 24 arrays were sorted out. We then filtered out genes with statistically significant differences using the ANOVA test. Genes with a p value of 0.01 or lower were then analyzed using a parametric test, without the assumption of equal variance (Welch ANOVA) with a P value cutoff to 0.05. This analysis was followed by multiple testing correction analysis. We used the Benjamini and Hochberg test, which controls the false discovery rate defined as the proportion of genes expected to be identified by chance relative to the total number of genes called significant. Genes with significant changes of expression between the transgenic group and the wild-type group were further filtered using a ratio of ≥1.25 (25%) or ≤0.75 (100%) as threshold. The results were then sorted according to function, protein family, or metabolic pathway.

**Real-time PCR.** Hypothalami from six transgenic males, five transgenic females, and seven control mice (6–7 months old) from each sex were dissected. Total RNA was extracted by a TRI reagent (Sigma Aldrich St. Louis, MO, USA). First-strand cDNA was synthesized from total RNA using Superscript preamplification system (Invitrogen LifeTechnologies, Carlsbad, CA). Real-time PCR analysis was performed with an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) using FAM-, VIC-, and TAMRA-labeled fluorogenic probes. Threshold cycle (CT) was defined as the cycle number at which the fluorescence value crosses the threshold value. Sequences for the primers and probes are presented in Table 1.

**Statistics.** Comparisons between two groups were made by unpaired Student’s t test. Two-way ANOVA was used to analyze glucose tolerance test and weight change in the transgenic mice and their controls. Welch ANOVA followed by Benjamini and Hochberg false discovery rate test was used to analyze the DNA array data. Values are presented as mean ± SE. P < 0.05 was considered significant.

**RESULTS**

**Body weight.** The generation of GFAP-bGH animals was previously described (40). To investigate the relationship between GH overexpression in the CNS and postnatal growth, the body weights of the GFAP-bGH line with highest GH expression (line 3) and control littermates were followed in more detail. A difference in weight was seen in female mice at ~20–25 days of age, when the transgenic females gained more weight than controls. A weight difference between transgenic male mice and male controls was detected at 55–60 days of age (male P < 0.01 and female P < 0.001, n = 7–12) (Fig. 1A). At 130 days (18 weeks) of age, transgenic females were 31% (P < 0.05) heavier and transgenic males 19% (P < 0.05) heavier than their littermate controls. Similar results were seen in a second GFAP-bGH transgenic line (line 2) derived from a different founder. At 130 days (18 weeks) of age, the transgenic females from this founder line were 42% (P < 0.001) heavier than their littermate controls (GFAP-bGH 38.6 ± 1.8 g vs. wild type 27.1 ± 1.4 g), confirming an increased body weight as a result of overexpression of bGH in the CNS.

**Body fat mass.** To investigate whether the increased body weight in GFAP-bGH mice was accompanied with an altered body composition, abdominal computer tomography scans were performed in vivo. Interestingly, both the visceral and subcutaneous fat deposits were increased in the GFAP-bGH mice compared with the control mice when they were 6 months old (Fig. 1B). The body composition was also analyzed in vivo using MR spectroscopy (Fig. 1C). The integrals of the water signal and the CH2 + CH3 signal from fat measured from the nuclear magnetic resonance spectra showed a 100% increase in fat integral/total body weight ratio.
15.7 ± 1.7 units; \( P < 0.01 \) Student’s \( t \) test), a 152% increase in estimated mass of fat in gram (GFAP-bGH 16.2 ± 0.3 g vs. wild type 6.4 ± 0.9 g; \( P < 0.01 \), Student’s \( t \) test), and a 101% increase in estimated mass proportion of fat per mouse (GFAP-bGH 30.5 ± 0.9% vs. wild type 15.2 ± 1.7%; \( P < 0.01 \), Student’s \( t \) test) in GFAP-bGH mice compared with control mice. Segmentation volumes of the abdominal/thoracic compartment, measured using magnetic resonance imaging, showed a 159% increase in total adipose volume/ml (GFAP-bGH 10.3 ± 0.5 volume/ml vs. wild type 4.0 ± 0.4 volume/ml; \( P < 0.01 \), Student’s \( t \) test), a 169% increase in subcutaneous adipose volume/ml, a 146% increase in visceral adipose volume/ml, and a 123% increase in interscapular brown fat pad/ml (\( n = 4–5 \); Fig. 1C).

Dissection of fat depots confirmed that the GFAP-bGH mice were obese. The absolute as well as the relative weights (in percentage of body weight) of dissected retroperitoneal, reproductive, and brown adipose tissue depots were markedly increased in both male and female GFAP-bGH mice compared with wild-type littermate controls (Table 2). Similar results on adipose tissue weights were found in a second GFAP-bGH transgenic line (line 2) derived from a different founder. The absolute as well as the relative weights of dissected retroperitoneal (GFAP-bGH 0.41 ± 0.02 g vs. wild type 0.21 ± 0.03 g, \( P < 0.01 \) and GFAP-bGH 1.0 ± 0.04% bw vs. wild type 0.8 ± 0.07% bw, \( P < 0.05 \), Student’s \( t \) test) and reproductive (GFAP-bGH 3.4 ± 0.15 g vs. wild type 1.4 ± 0.3 g, \( P < 0.01 \) and GFAP-bGH 9.0 ± 0.7% bw vs. wild type 5.0 ± 0.9% bw, \( P < 0.05 \), Student’s \( t \) test) adipose tissue depots were markedly increased in females of this second line of GFAP-bGH mice compared with their wild-type littermate controls, confirming the obese phenotype of GFAP-bGH mice.

In addition, the weights of several other organs, including brain, spleen, heart, kidney, lung, testis, and liver, were changed in absolute or relative terms in the transgenic mice (Table 2). Leptin levels have been shown to correlate to the amount of fat (51,52), and as expected, serum levels of leptin were increased in both female (58%) and male (44%) GFAP-bGH mice compared with littermate controls (Fig. 1D).

**Food intake and indirect calorimetry.** To test the possibility that the difference in weight gain between GFAP-bGH transgenic mice and wild-type mice could be due to a difference in food intake, we measured the food intake over 48 h after an 18-h fasting period in male GFAP-bGH mice and littermate male controls. During the fasting period, there was no difference in weight loss between the GFAP-bGH transgenic and wild-type mice (\( P = 0.8 \); data not shown). GFAP-bGH mice had increased accumulated food intake (+60%; \( P < 0.0001 \)) and relative food intake (+32%; \( P < 0.05 \)) compared with wild-type littermate controls (Fig. 2A and B). Using indirect calorimetry, no significant changes in RER or energy expenditure were observed between GFAP-bGH mice and wild-type littermate controls (Fig. 2, C and D).

A single ICV injection of dexamethasone has been shown to increase food intake in adrenalectomized obese leptin-deficient ob/ob mice (53). Thus, the increased food intake in GFAP-bGH mice could be due to the increased serum corticosterone levels found in these mice (40). To investigate whether GH has a direct effect in the CNS on

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer 1</th>
<th>Primer 2</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGRP</td>
<td>TGGGCGGAGGTGCTAGATC</td>
<td>GAGGAATCAGATGAGGATATGGGA</td>
<td>TCGGTCTCTTCGACTAATCCCGCCAAMC4-R</td>
</tr>
<tr>
<td>M36B4</td>
<td>GAGGAATCAGATGAGGATATGGGA</td>
<td>AAGCAGGCTGACTTGGTTGC</td>
<td>TCGGTCTCTTCGACTAATCCCGCCAAMC4-R</td>
</tr>
<tr>
<td>MCH-R</td>
<td>GAGGAATCAGATGAGGATATGGGA</td>
<td>TTGGCGGAGGTGCTAGATC</td>
<td>TCGGTCTCTTCGACTAATCCCGCCAAMC4-R</td>
</tr>
<tr>
<td>MCH</td>
<td>GAGGAATCAGATGAGGATATGGGA</td>
<td>TTGGCGGAGGTGCTAGATC</td>
<td>TCGGTCTCTTCGACTAATCCCGCCAAMC4-R</td>
</tr>
<tr>
<td>NP1</td>
<td>GAGGAATCAGATGAGGATATGGGA</td>
<td>TTGGCGGAGGTGCTAGATC</td>
<td>TCGGTCTCTTCGACTAATCCCGCCAAMC4-R</td>
</tr>
<tr>
<td>PomC</td>
<td>GAGGAATCAGATGAGGATATGGGA</td>
<td>TTGGCGGAGGTGCTAGATC</td>
<td>TCGGTCTCTTCGACTAATCCCGCCAAMC4-R</td>
</tr>
</tbody>
</table>

The expression of AGRP and MCH-R were detected using SYBR Green I dye, whereas all other transcripts were detected using Taqman probes labelled with FAM in the 5' end and TAMRA in the 3' end.
food intake, a separate experiment was conducted where food intake was measured following ICV injection of bGH in ad libitum–fed C57BL/6 mice (Fig. 2E). ICV injection of bGH increased food intake (46%; *P* < 0.05). In addition, no change in serum corticosterone level was observed in the bGH-treated mice compared with vehicle-treated animals (bGH [n = 5] 219.3 ± 25.8 ng/ml vs. vehicle [n = 4] 232.8 ± 13.7 ng/ml; *P* = 0.68, Student’s *t* test).

**Hypothalamic gene expression.** Several hypothalamic genes, known to regulate food intake, were measured to determine the mechanism behind the increased food intake in GFAP-bGH mice. The hypothalamic AGRP mRNA levels were increased by more than twofold in both female and male transgenic mice compared with wild-type mice (Fig. 2F). Furthermore, NPY mRNA levels were significantly increased in female GFAP-bGH transgenic mice (Fig. 2G). In contrast, the hypothalamic mRNA levels of POMC, melanin-concentrating hormone (MCH), melanin-concentrating hormone receptor (MCH-R), and MC4-R were not significantly different between control mice and GFAP-bGH transgenic mice (data not shown).

**Glucose tolerance.** GFAP-bGH transgenic mice at 6–7 months of age had severely increased basal insulin levels compared with littermate controls (Fig. 2A). Furthermore, this was also observed during a glucose tolerance test where the insulin levels of the GFAP-bGH transgenic mice were markedly higher at each time point (*P* < 0.001, *n* = 6) (Fig. 3B). However, there was no difference in the basal glucose levels or glucose tolerance between the transgenic mice and littermate controls (*P* = 0.12). Similar results were observed when these mice were 9 months old (data not shown).

**Islet morphology.** Because the GFAP-bGH mice were hyperinsulinemic, we investigated whether this was reflected in the morphology of the pancreatic islets. Pancreatic sections from GFAP-bGH transgenic mice and littermate controls were examined by immunohistochemistry with proinsulin and glucagon antibodies. A marked islet hyperplasia was observed in the pancreas from GFAP-bGH mice, and the islet architecture was disturbed with an α-cell disorganization (Fig. 3C). Thus, in GFAP-bGH transgenic mice, glucagon cells were regularly observed in the central portion of the islets, whereas in the littermates, the normal mantle zone of α-cells was observed. In the embryonic pancreas (stage E19 examined), cords and clusters of proinsulin and glucagon immunoreactive cells were seen, with no distinct formation of mantle-type islets in either transgenic or littermate controls. Furthermore, there was no overt difference in the amount of endocrine tissue between the two genotypes at this developmental stage (not shown).

**Serum lipoproteins.** To investigate whether the alterations in body composition and insulin sensitivity were accompanied by changes in serum lipoprotein levels, total serum levels of cholesterol and triglycerides as well as serum lipoprotein profiles at 6 months of age were measured. The effects were similar in male and female mice; therefore, only data from male mice are given in Fig. 4A–E.
Serum cholesterol levels were higher in both male and female transgenic mice by 49 and 89%, respectively, while the serum levels of triglycerides were 52 and 43% lower in female transgenic mice by 49 and 89%, respectively, while serum cholesterol levels were higher in both male and female transgenic mice compared to wild-type littermate controls (data not shown).

The hepatic triglyceride content was lower in male transgenic mice compared to wild-type littermate controls (data not shown). The hepatic triglyceride content was lower in male transgenic mice compared to wild-type littermate controls (data not shown).

**Table 2**
The effect of brain-specific bGH overexpression on body and organ weight

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th>Absolut weight (mg)</th>
<th>Relative weight (% body wt)</th>
<th>Males</th>
<th>Absolut weight (mg)</th>
<th>Relative weight (% body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body (g)</td>
<td>Tg</td>
<td>47 ± 2*</td>
<td>—</td>
<td>Tg</td>
<td>51 ± 2†</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>37 ± 2</td>
<td>—</td>
<td>Wt</td>
<td>42 ± 2</td>
<td>—</td>
</tr>
<tr>
<td>Brain (hypothalamus)</td>
<td>Tg</td>
<td>504 ± 5†</td>
<td>1.07 ± 0.04*</td>
<td>Tg</td>
<td>508 ± 6*</td>
<td>1.01 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>479 ± 3</td>
<td>1.31 ± 0.07</td>
<td>Wt</td>
<td>479 ± 8</td>
<td>1.16 ± 0.04</td>
</tr>
<tr>
<td>Heart</td>
<td>Tg</td>
<td>173 ± 5†</td>
<td>0.37 ± 0.01</td>
<td>Tg</td>
<td>214 ± 10†</td>
<td>0.42 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>137 ± 6</td>
<td>0.37 ± 0.02</td>
<td>Wt</td>
<td>181 ± 9</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>Tg</td>
<td>948 ± 111*</td>
<td>4.1 ± 0.14</td>
<td>Tg</td>
<td>2716 ± 234*</td>
<td>5.34 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>1487 ± 179</td>
<td>3.92 ± 0.22</td>
<td>Wt</td>
<td>1897 ± 197</td>
<td>4.52 ± 0.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>Tg</td>
<td>328 ± 14</td>
<td>0.7 ± 0.03</td>
<td>Tg</td>
<td>471 ± 29</td>
<td>0.93 ± 0.03†</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>297 ± 8</td>
<td>0.81 ± 0.05</td>
<td>Wt</td>
<td>471 ± 8</td>
<td>1.14 ± 0.04</td>
</tr>
<tr>
<td>Spleen</td>
<td>Tg</td>
<td>125 ± 7</td>
<td>0.26 ± 0.01</td>
<td>Tg</td>
<td>163 ± 12†</td>
<td>0.32 ± 0.02†</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>107 ± 9</td>
<td>0.3 ± 0.04</td>
<td>Wt</td>
<td>96 ± 3</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>Lung</td>
<td>Tg</td>
<td>164 ± 4</td>
<td>0.35 ± 0.01*</td>
<td>Tg</td>
<td>204 ± 4*</td>
<td>0.41 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>160 ± 3</td>
<td>0.44 ± 0.03</td>
<td>Wt</td>
<td>184 ± 5</td>
<td>0.45 ± 0.02</td>
</tr>
<tr>
<td>Testis</td>
<td>Tg</td>
<td>—</td>
<td>—</td>
<td>Tg</td>
<td>215 ± 11</td>
<td>0.43 ± 0.02*</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>—</td>
<td>—</td>
<td>Wt</td>
<td>218 ± 9</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td>Retroperitoneal adipose tissue</td>
<td>Tg</td>
<td>963 ± 75†</td>
<td>2.02 ± 0.08†</td>
<td>Tg</td>
<td>929 ± 69†</td>
<td>1.85 ± 0.15†</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>519 ± 58</td>
<td>1.37 ± 0.08</td>
<td>Wt</td>
<td>503 ± 47</td>
<td>1.21 ± 0.1</td>
</tr>
<tr>
<td>Reproductive adipose tissue</td>
<td>Tg</td>
<td>6430 ± 507†</td>
<td>13.49 ± 0.74†</td>
<td>Tg</td>
<td>2772 ± 230†</td>
<td>5.5 ± 0.44*</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>3701 ± 562</td>
<td>9.62 ± 0.78</td>
<td>Wt</td>
<td>1738 ± 158</td>
<td>4.16 ± 0.31</td>
</tr>
<tr>
<td>Brown adipose tissue</td>
<td>Tg</td>
<td>462 ± 34†</td>
<td>0.98 ± 0.07†</td>
<td>Tg</td>
<td>408 ± 17†</td>
<td>0.81 ± 0.03†</td>
</tr>
<tr>
<td></td>
<td>Wt</td>
<td>192 ± 29</td>
<td>0.5 ± 0.04</td>
<td>Wt</td>
<td>231 ± 18</td>
<td>0.56 ± 0.04</td>
</tr>
</tbody>
</table>

Data are means ± SE. Adipose tissue, “reproductive” refers to the epididymal or parametrial adipose tissue of male and female mice, respectively. Wild-type (Wt) males (n = 7) and GFAP-bGH transgenic (Tg) males (n = 7) and GFAP-bGH transgenic females (n = 7) were compared and wild-type female mice (n = 7), and GFAP-bGH transgenic females (n = 7) were compared separately. Littermates at the age of 6–7 months were used. *P < 0.05 and †P < 0.01 vs. corresponding littermate controls (unpaired Student’s t test). Genome-wide expression analysis of the liver. To bring further understanding to the metabolic changes in the liver introduced by the overexpression of bGH in the CNS, we performed gene expression analysis of livers from six transgenic and six wild-type male mice using the Affymetrix MG-u74Av2 chip. Applying the statistical restrictions mentioned in RESEARCH DESIGN AND METHODS, we filtered out 781 genes and expressed sequence tags with an up or downregulation of 25% (1.25 fold change). Using an expression ratio change of 100% (twofold) or more resulted in 176 genes being differentially expressed between GFAP-bGH mice and littermate controls. Further selection was done using the Incyte hierarchies of protein function and enzymes as well as the enzyme commission number. In Table 3, the difference in gene expression between GFAP-bGH transgenic mice and littermate controls is exemplified by the prolactin receptor, major urinary proteins, and carbonic anhydrase III (Table 3). In addition, a few other genes involved in different metabolic pathways were also differentially expressed and further discussed below.
DISCUSSION

In this study, we demonstrate that overexpression of GH in the CNS results in hyperphagia-induced obesity. The increased food intake was associated with a marked increase in AGRP and a slight increase in NPY levels in the hypothalamus. GFAP-bGH mice also had elevated serum insulin and cholesterol levels. In spite of the insulin resistance, they had normal glucose tolerance because they exhibited an adequate and exaggerated insulin response. Thus, GH overexpression in the CNS results in changed glucose and lipid metabolism.

The increased leptin levels in the GFAP-bGH mice, together with the increased food intake, indicate an impaired hypothalamic response to leptin, which is further supported by a clear upregulation of hypothalamic AGRP mRNA levels. AGRP is known to functionally antagonize leptin action in the hypothalamus (32,33). NPY mRNA levels were slightly elevated in female GFAP-bGH mice, which also indicates decreased leptin action. These results support that increased levels of GH in the CNS induce alteration in the hypothalamic systems controlling satiety and orexic behavior. In line with our findings, hypothalamic expression of rats significantly decreased NPY mRNA levels in the arcuate nucleus, and GH treatment restored these levels to those of intact rats (39). We could not detect any significant changes in the expression of MC4-R, MCH, MCH-R, and POMC, indicating that the alterations in gene expression of peptides involved in appetite control are specific and limited to only certain peptides. Therefore, CNS-acting GH may stimulate appetite by increasing AGRP expression. Similarly, rats injected intracerebroventricularly with the endogenous GH secretagogue, ghrelin also develop hyperphagia-induced obesity, which is suggested to be mediated via an induction of AGRP and NPY (59,60). Thus, one may speculate that the effects of ghrelin on feeding behavior could involve downstream local GH signaling in the CNS. However, ICV injections of ghrelin increased food intake in GH-deficient rats (60), arguing that ghrelin can have GH-independent effects on food intake.

Berryman et al. have shown that mice with a general overexpression of GH have unchanged relative food intake (13). This finding is in accordance with our own Mt-bGH
mice (60a). The reason for the different effect of general GH overexpression versus CNS-specific overexpression of GH on food intake is unclear. We show in this study that GH has an acute effect on food intake following an ICV injection. Thus, the local GH concentration in the CNS may be different between GFAP-bGH and Mt-bGH mice, or peripheral effect(s) may counteract the CNS effect of GH on food intake.

In two other studies overexpressing human GH in the cerebral cortex and hypothalamus, respectively, the mice were shown to exhibit a dwarf phenotype (61,62). These mice showed reduced GH and IGF-I mRNA levels in the pituitary and liver, respectively, and circulating levels of IGF-I (61,62), suggesting that the growth retardation in these mice is due to disruption of mouse GH synthesis and release from the pituitary. Other groups have reported obesity after expressing GH and GH-releasing hormone in the CNS, which also has been attributed to lowering of endogenous GH levels (63,64), because GH deficiency leads to increased fat depots in man and experimental animals (6,9,10,65). In our model, however, the serum levels of GH were not reduced. In fact, both lines of GFAP-bGH mice used in this study had a leakage of bGH produced in the CNS to the circulation (40). Interestingly, we found that the hepatic gene expression of the GH receptor was increased in the transgenic mice, but no change in IGF-1 mRNA was observed, indicating that the total GH secretion is not markedly changed in these animals. The liver expression profile in GFAP-bGH mice is somewhat similar to that observed in Mt-bGH transgenic male mice (66) with respect to the effect on the GH receptor mRNA and the genes that are regulated by the sexually dimorphic secretory pattern of GH as exemplified by carbonic anhydrase III. However, most of the changes observed in Mt-bGH mice with respect to regulation of genes involved in lipid and carbohydrate metabolism are not observed in the GFAP-bGH mice (66). Together, these results indicate a change from pulsatile to continuous secretion of GH in male mice as indicated by the change in gene expression of the prolactin receptor, testosterone 16α-hydroxylase, 3-ketosteroid reductase, major urinary proteins, and carbonic anhydrase III (55–58,67). Thus, the changed plasma pattern of GH seems to feminize the hepatic gene expression of male mice. However, in terms of obesity, insulin resistance, and serum lipoproteins, we did not observe different effects in males and females. Therefore, the possibility of feminization of the male liver gene expression did not have any impact on our major findings regarding the phenotype of these mice. Moreover, we can conclude from the results of measurements of circulating bGH in the two lines of GFAP-bGH mice (40) and the gene expression data that there may be a small increase in peripheral GH but no GH deficiency.

It is well known that insulin resistance is linked to obesity in man and experimental animals (68,69). In spite of the marked hyperinsulinemia that reached levels similar to those seen in the severely insulin-resistant ob/ob mouse, GFAP-bGH mice did not develop type 2 diabetes during the observation period. Indeed, fasting glucose levels were normal and so was glucose elimination during a glucose tolerance test. This suggests that the islet response to glucose challenge is adequate for the ambient insulin resistance, fully compensating for the reduced insulin action.

The islet morphology revealed a marked hyperplasia of the pancreatic islets, which is expected following a long-standing insulin resistance. An interesting observation was the disturbance in islet cytoarchitecture, in which the α-cells were disorganized and partially dispersed among the centrally located β-cells. A similarly disturbed cytoar-

FIG. 3. GFAP-bGH mice are insulin resistant. Basal insulin levels in GFAP-bGH mice (Tg) and wild-type mice (Wt) are shown (A). Serum insulin and glucose levels were measured before and 1, 5, 10, 20, 30, and 50 min after intravenous injection of glucose (1g/kg) in male mice (B). Pancreatic islet morphology and proinsulin and glucagon immunoreactivity in male GFAP-bGH and wild-type mice as studied by immunohistochemistry are shown (C). Littermates at the age of 6–7 months were used. Values are means ± SE (n = 7). *P < 0.05 and **P < 0.01 vs. wild type (Student’s t test). The glucose tolerance test was analyzed by two-way ANOVA using genotype and time as factors.
Architecture has been observed in several genetic mouse models of insulin resistance and hyperinsulinemia and is thought to be one manifestation of a type 2 diabetes phenotype (70,71). At the embryonic stage examined (E19), the amount of endocrine cells in the pancreas did not differ between the transgenic mice and littermate controls. Therefore, the islet hyperplasia seems to commence postnatally, as do the islet cytoarchitecture changes, possibly in response to the hyperphagia-induced obesity.

The transgenic mice showed a decreased level of gene expression of hepatic lipase and scavenger receptor class type I, which may indicate decreased turnover of HDL (72). ApoC-II is an important lipoprotein lipase activator. An upregulation of apoC-II may result in increased turnover of triglyceride-rich lipoproteins, leading to decreased triglyceride and apoB levels. Moreover, the mRNA level for the enzyme responsible for apoB mRNA editing, apobec-1, was increased. This finding indicates increased editing of apoB mRNA and therefore a decreased production of apoB 100 (73). Because apoB 48–containing lipoproteins have a higher turnover than apoB 100, this finding may help to explain the lower apoB levels in the GFAP-bGH mice. The observed increase in lecithin cholesterol acyltransferase gene expression together with the possibility of increased turnover of triglyceride-rich lipoproteins may contribute to increased production of HDL cholesterol in the transgenic animals. Thus, both increased production and decreased turnover of HDL may help to explain the changed lipoprotein profile in these mice.

We have previously shown that the GFAP-bGH mice used in this study have threefold elevated corticosterone levels compared with wild-type animals (40). Glucocorticoid treatment has been shown to increase food intake in humans (74) and mice (53) and promote fat accumulation and insulin resistance in mice (75,76). These effects could possibly be due to an increased hypothalamic AGRP and NPY expression (77). It is therefore possible that the increased food intake observed in GFAP-bGH mice is at least partly due to increased serum levels of corticosterone (40). However, Mt-bGH mice with similarly increased corticosterone levels as GFAP-bGH mice are lean and have unchanged food intake compared with wild-type mice (3, 13) (60a). It can therefore be argued that peripheral GH overexpression counteracts the effect of central GH overexpression on food intake and obesity. Moreover, it is possible that peripheral GH overexpression counteracts the effect of high corticosterone levels on food intake and obesity. To investigate whether GH has a direct effect in the CNS on food intake without changing corticosterone levels, we injected C57BL/6 mice with bGH ICV. Using this model, we could show that GH increased food intake without any effect on plasma corticosterone levels. This finding argues against a major role of changed corticosterone levels for the observed effect of central GH overexpression on food intake. However, we cannot rule out the

![FIG. 4. GFAP-bGH mice are hypercholesterolemic. Total cholesterol (A) and triglyceride levels (B) in GFAP-bGH (Tg) and wild-type (Wt) mice. Serum apoB levels were determined with an electroimmunoassay (C), and hepatic LDL receptor protein levels were identified with Western blot (D). Serum lipoprotein size distribution was determined in individual mice using size exclusion high performance liquid chromatography system, SMART (E). Littermate male GFAP-bGH and wild-type mice at the age of 6–7 months were used. Values are means ± SE (n = 7). *P < 0.05 and **P < 0.01 vs. wild type (Student’s t test).]
The data indicate that the effect of GH in the CNS can increase food intake and produce leptin resistance without peripheral GH deficiency. Thus, if increased GH production in the CNS is not balanced by an increased peripheral metabolic effect of GH, obesity will develop.

### Acknowledgments

This study was supported by the European Commission (MitAge QLRT 2000-00054), the Swedish Medical Research Council (grants 4250, 4499, 6834, and 14291), the Swedish Cancer Foundation, the Swedish Foundation for Strategic Research, the Lundberg Foundation, the Swedish Medical Society, AstraZeneca Research and Development, Pharmacia-Upjohn, Novo Nordisk Foundation, Albert Pahlsson Foundation, Swedish Diabetes Association, King Gustav V’s and Queen Victoria’s Foundation, Sahlgrenska University Foundation, the Swedish Heart and Lung Foundation, and the Swedish Association Against Rheumatic Disease.

We thank Maud Pettersson, Lena Kvist, Doris Persson, Anna Karin Gerdin, Lena Amrot Fors, and Anne-Cristine Carlsson for excellent technical assistance and Jean Tessier and Rod Pickford for help with the magnetic resonance imaging/magnetic resonance spectra analysis. We also thank Kerstin Enquist, Mona Byström, and Eva Jonsson for help with the real-time PCR analysis. Dr. Stefan Pierru for careful revision of this manuscript, and SWEGENE Centre for Bio-Imaging (CBI), Göteborg University for technical support regarding Image analysis (pQCT).

### References


17. Gossard F, Dihl F, Pelletier G, Dubois PM, Morel G: In situ hybridization to the cDNA for the rat growth hormone receptor at different ages. *Brain Res* 239:543–557, 1982


26. van Driel IR, Davis CG, Goldstein JL, Brown MS: Self-association of the receptor for human growth hormone (MCH), neurotensin (NT), proopiomelanocortin (POMC) and neuropeptide Y (NPY) are targets of leptin signaling in the hypothalamus. *Endocrinology* 139:795–798, 1998


29. Sahu A: Evidence suggesting that galanin (GAL), melanin-concentrating hormone (MCH), neurotensin (NT), proopiomelanocortin (POMC) and
dyslipidemia and diabetes on a high fat diet. Endocrinology 2004 [Epub ahead of print]