The impact of glucocorticoid replacement therapy on bone mineral density in patients with hypopituitarism before and after growth hormone replacement therapy

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Abstract

Introduction
Growth hormone increases bone formation and linear growth while glucocorticoids inhibit bone formation. Growth hormone modulates the metabolism of glucocorticoids by inhibiting the activity and expression of 11-beta-hydroxysteroid dehydrogenase type 1, the enzyme that converts the inactive metabolite cortisone to the active hormone cortisol. The aim of this project was to study bone mineral density before and after two years on growth hormone replacement therapy in patients with hypopituitarism. The main hypothesis was that patients on glucocorticoid replacement demonstrate greater improvement in bone mineral density when treated with growth hormone than glucocorticoid sufficient patients.

Material and methods
This was a retrospective study on 175 adult patients with hypopituitarism due to non-functioning pituitary adenoma. All patients were growth hormone deficient. Bone mineral density was measured before growth hormone replacement therapy started and after two years of therapy. Of 175 patients, 77 (44%) were glucocorticoid sufficient and 98 (56%) were glucocorticoid insufficient, receiving a mean ± SD hydrocortisone equivalent dose of 20.9 ± 5.0 mg/day.

Results
Bone mineral density at baseline did not differ between glucocorticoid sufficient and insufficient patients, neither at lumbar spine nor proximal femur neck. Both groups improved their bone mineral density significantly after two years on growth hormone replacement. However, no significant difference between the two groups regarding change in bone mineral density was observed.

Discussions
Growth hormone replacement therapy for 2 years increases bone mineral density in hypopituitary patients, but the treatment response is not influenced by glucocorticoid insufficiency and its replacement therapy.
Abbreviations

ACTH = adenocorticotrophic hormone
BMD = Bone mineral density
CRH = Corticotrophic-releasing hormone
FSH = follicle stimulating hormone
GH = Growth hormone
GHRH = Growth hormone-releasing hormone
GnRH = Gonadotropin-releasing hormone
11-β-HSD = 11β-Hydroxysteroid dehydrogenase
IGF-1 = insulin-like growth factor 1
LH = Lutenizing hormone
MRI = Magnetic resonance imaging
POMC = Pro-opimelanocortin
SD = Standard deviations
TRH = Thyrotropin-releasing hormone
TSH = thyroid stimulating hormone
T_3 = Triiodothyronine
T_4 = Thyroxine
Introduction

1 The pituitary
Virtually all physiologic activities are regulated by the actions and interactions between the endocrine and nervous systems whereby the nervous system regulates the endocrine system which in turn modulates the activity of the central nervous system.

The pituitary is the organ that, alongside the hypothalamus, controls the function of several endocrine glands; the thyroid, the adrenals and the gonads.

1.1 Anatomy & histology
The human pituitary gland consists of two separate lobes, the anterior and the posterior pituitary. The lobes have different embryological origins. The posterior lobe, also known as the neurohypophysis, is of neural origin and consists of the axons and nerve endings of neurons originating from the supraoptic and paraventricular nuclei of the hypothalamus and supporting tissues. The anterior pituitary, or adenohypophysis, originates from the Rathke’s pouch and migrates to join the neurohypophysis.

The pituitary gland lies at the base of the skull in a portion of the sphenoid bone called the sella turcica and is surrounded by dura. The roof is formed by the diaphragma sellae, a reflection of the dura attached to the clinoid processes of the sphenoid bone. 5-10 mm above the diaphragma sellae lies the optic chiasm.

The size of the pituitary gland varies. On average it measures 15 x 10 x 6 mm and weighs 500-900 mg. During pregnancy the pituitary gland can double in size. The main cell types of the pituitary is as follows; somatotrophs, lactotrophs, thyrotrophs, corticotrophs and gonadotrophs.

1.1.1 Somatotrophs
Somatotrophs secrete growth hormone (GH) and are usually located in the lateral portions of the anterior lobe, they account for approximately half of the adenohypophysial cells.

1.1.2 Lactotrophs
Lactotrophs are responsible for the secretion of prolactin and are randomly distributed in the anterior pituitary. They account for 10-25% of the anterior pituitary cells and proliferate rapidly during pregnancy, accounting for the twofold increase in gland size.

1.1.3 Thyrotrophs
Those are the cells that secrete thyroid stimulating hormone (TSH). As the least common of the pituitary cell types, they only account for about 10% of
adenohypophysial cells. They are usually located in the anteromedial and anterolateral portions of the gland and demonstrate marked hypertrophy during states of primary thyroid failure, increasing the size of the pituitary gland.

1.1.4 Corticotrophs
Corticotrophs are located in the anteromedial portion of the gland and make up about 15-20% of adenohypophysial cells. They secrete adenocorticotropic hormone (ACTH).

1.1.5 Gonadotrophs
They secrete luteinizing hormone (LH) and follicle stimulating hormone (FSH) and are scattered throughout the entire anterior lobe. These cells constitute 10-15% of the cells of the anterior pituitary.

1.2 Anterior pituitary hormones
The anterior pituitary gland secretes six major hormones; ACTH, GH, TSH, prolactin, LH and FSH.

1.2.1 ACTH
ACTH is a peptide derived from a larger precursor molecule, pro-opimelanocortin (POMC). POMC is processed in corticotrophs and divided into smaller biologically active fragments, including melanocyte-stimulating hormone, ACTH and β-endorphin.

ACTH stimulates the adrenal cortex to secret glucocorticoids by binding to receptors on the adrenal cortex and inducing steroidogenesis. The main glucocorticoid in humans is cortisol.

ACTH secretion is stimulated by corticotropin-releasing hormone (CRH) from the hypothalamus. ACTH is released in a pulsatile and diurnal manner, with a peak before awakening and a decline as the day progresses. The body also responds to all kinds of stress by releasing CRH and subsequently ACTH and cortisol, be it emotional, physical or chemical.

There are two mechanisms for negative feedback on ACTH secretion, both at the hypothalamic and pituitary levels. The “fast feedback” is sensitive to the rate of change in cortisol levels, while “slow feedback” responds to the absolute cortisol level. ACTH also has inhibiting effect on its own secretion.
1.2.2 Growth hormone
As evidenced by its name, GH induces linear growth via insulin-like growth factor 1 (IGF-1) and direct effect on the skeletal tissue. IGF-1 enhances amino acid uptake and accelerates the transcription and translation of mRNA that results in increasing protein synthesis. GH also mobilizes fat as a fuel source thus decreasing protein catabolism during states of starvation. GH replacement therapy has been shown to increase bone mineral density (BMD) in people with adult-onset growth hormone deficiency (1).

GH impairs glucose uptake into cells and decreases carbohydrate utilization resulting in glucose intolerance and secondary hyperinsulinism.

GH secretion is stimulated by growth hormone-releasing hormone (GHRH) and inhibited by somatostatin, both of which are secreted by the hypothalamus. Peak GH levels occur 1-4 hours after the onset of sleep (2). Furthermore stress has been shown to provoke GH release. GH secretion is also controlled by metabolic factors as evidenced by how glucose lowers GH and amino acids stimulate GH secretion.

1.2.3 Prolactin
Prolactin secretion stimulates lactation after childbirth and is elevated during pregnancy as a major factor in preparation for milk production. Lactation is inhibited by high estrogen levels during pregnancy and is only made possible when both progesterone and estrogen levels drop after partus.

The hypothalamic hormone, dopamine, has inhibitory effects on prolactin secretion.

1.2.4 Thyroid-stimulating hormone
TSH stimulates iodide uptake, production and secretion of thyroid hormones (T4 and T3) from the thyroid gland. The hypothalamus has inhibitory as well as stimulating function in the secretion of TSH from the pituitary with two separate hormones. Somatostatin is the inhibitory hormone and thyrotropin-releasing hormone (TRH) stimulates TSH release from the pituitary. Other factors include glucocorticoids, who impair the sensitivity of the pituitary to TRH, and estrogens who increase the sensitivity of the pituitary to TRH.

1.2.5 Gonadotropins
LH and FSH are secreted by the same cell type and are both composed of alpha and beta subunits, with the beta subunit giving them their unique biological activity. Both
LH and FSH bind to receptors in the ovary and testis and regulate the function of the gonads by promoting sex steroid production.

LH stimulates testosterone production from the Leydig cells of the testis while FSH stimulates testicular growth and enhances the production of androgen-binding protein by the Sertoli cells which are necessary for sustaining the maturing sperm cell. Thus both LH and FSH are required for the maturation of the spermatozoa.

LH stimulates estrogen and progesterone production from the ovary in women while the development of the ovarian follicle is largely under the control of FSH.

LH and FSH secretion is controlled by gonadotropin-releasing hormone (GnRH) which is responsible for the phasic release of gonadotropins for ovulation, and determines the onset of puberty.

2 Pituitary adenomas

Pituitary adenomas can be classified according to their size and whether or not they are clinically functional or nonfunctional, i.e. whether they secrete hormones or not. Adenomas larger than 1 cm are called macroadenomas and adenomas smaller than 1 cm are called microadenomas. Microadenomas are split evenly between functional and nonfunctional adenomas (3-4), while macroadenomas consists more often of nonfunctional adenomas (5-7). This is explained by the fact that functional adenomas are more often discovered before they have grown large enough to present with symptoms while nonfunctional adenomas are clinically silent until they have grown enough to present themselves with symptoms such as headache or visual field impairments.

Microadenomas present with manifestations of hormonal excess without sellar enlargement or extrasellar extensions, whilst macroadenomas cause generalized sellar enlargement. Tumors that are smaller than 2 cm in diameter can usually be successfully treated surgically as long as they are confined to the sella turcica. Tumors larger than 2 cm are more difficult to manage, especially those with suprasellar, sphenoid sinus or lateral extensions into the sinus cavernosus. Panhypopituitarism normally occurs only in macroadenomas and the frequency increases in correlation with the tumor size.
Since nonfunctional tumors are usually large when the diagnosis is established, patients often present with headaches and visual field defects and endocrine manifestations that have possibly been present for months to years before the diagnosis is confirmed (8). The most common hormonal deficiency is gonadal and GH deficiency but hypothyroidism and hypoadrenalism are relatively common as well (8, 9). Patients should be evaluated by using magnetic resonance imaging (MRI) and visual field testing. Also assessment of pituitary hormones and end-organ function should be performed to determine whether hormonal replacement is needed.

The primary treatment of most pituitary adenomas is surgery. Second line treatment is pituitary irradiation or medical treatment to suppress hypersecretion of hormonally active adenomas. The aim of all treatment forms is to maintain normal secretion and correct hypersecretion of the pituitary hormones and to remove or reduce the volume of the adenoma itself. While transsphenoidal surgery is the most effective treatment and is indicated in patients experiencing visual field defects, there are cases when a non-functioning adenoma does not reach the optic chiasm and a wait-and-see-approach is more suitable (10).

3 Hypopituitarism

Hypopituitarism is defined as the inability of the pituitary gland to produce sufficient amounts of pituitary hormones. The cause can either be an inability of the gland itself or lack of releasing hormones from the hypothalamus. Either way the effects are lifelong unless successful treatment can restore the pituitary function. Studies have shown that patients with hypopituitarism have increased mortality (11-13).

Hypopituitarism, with the prevalence of 45.5 cases per 100,000 and incidence of 4.2 cases per 100,000 per year, is mainly caused by benign pituitary adenomas (14). Recently traumatic brain injuries and subarachnoidal hemorrhages (15) have also been shown to be a relatively common cause of hypopituitarism.

Hypopituitarism is diagnosed through measurements of hormones concentrations in serum. Symptoms due to hypopituitarism can be overt or subclinical as well as having a sudden and acute clinical onset. Shortages of ACTH (16) and TSH (17) can be life-threatening while shortages of gonadotropins and GH are not fatal.
3.1 Treatment for hypopituitarism
Hypopituitarism is treated with appropriate pituitary hormone substitution. Since glucocorticoid deficiency is fatal, substitution should begin immediately after deficiency has been confirmed. Thyroxin substitution should not begin until after glucocorticoid replacement has begun since thyroxine increases the metabolic rate of glucocorticoids and can induce acute glucocorticoid deficiency (18).

Sex hormone replacement can return bone mass in both men and women while also returning sexual function and libido. Due to increased risk of cardiovascular and neoplastic diseases sex hormone substitution in women should be discontinued after menopause (19, 20). GH deficiency is assumed to be a factor in the excess cardiovascular mortality seen in patients suffering from hypopituitarism (21). GH substitution has positive effects on body composition, lipid profile and quality of life (22). GH replacement therapy is therefore considered beneficial for patients with confirmed deficiency.

Patients with pituitary insufficiency should be regularly re-evaluated and their hormone levels be monitored. Hormone replacement therapy can greatly increase the quality of life and reduce morbidity and mortality.

4 Glucocorticoids and their effects on the body
All human tissue is affected by glucocorticoids. The proteins which are synthesized in response to glucocorticoids-receptor activation can vary greatly, thus explaining the variation in physiological response to glucocorticoids. This is due to the difference in specific gene expression in different cell types.

4.1 Metabolic effects
Generally speaking, glucocorticoids inhibit DNA and, in most tissues, RNA and protein synthesis while accelerating protein catabolism. In the liver, RNA and protein synthesis are actually stimulated. Glucocorticoids increase gluconeogenesis in the liver and increase the release of substrates for gluconeogenesis, especially from muscle tissue. Lipolysis is stimulated in adipose tissue resulting in the release of glycerol and free fatty acids.

4.2 Effects in various tissues
Glucocorticoid excess inhibits fibroblasts, leading to loss of collagen and connective tissue. This can lead to thinning of the skin, easy bruising and poor wound healing.
Glucocorticoids also directly inhibit bone formation and stimulate bone resorption by activating osteoclasts and inhibiting osteoblast proliferation. Glucocorticoids also indirectly affect bone formation by reducing calcium absorption in the intestines, resulting in increased parathyroid hormone secretion which stimulates bone resorption to maintain normal serum calcium levels.

Glucocorticoids inhibit the immunologic response by reducing the number of circulating lymphocytes, monocytes and eosinophils. They can also decrease the migration of inflammatory cells to sites of injury, explaining their anti-inflammatory action as well as the increased susceptibility to infections.

Glucocorticoids enter the brain and can significantly alter behavior and cognitive function. In excess they initially cause euphoria but with prolonged exposure the patient becomes irritable and depressed. Glucocorticoid excess may lead to decreased libido, increased appetite and insomnia.

4.3 Effects on other hormones
Glucocorticoid excess inhibits ovulation and causes amenorrhea in females by suppressing the responsiveness of LH to GnRH. This causes suppression of both estrogens and progestins with aforementioned results. Gonadotropin secretion is inhibited in men, causing low plasma testosterone levels, decreased libido and impotence.

By lowering TSH responsiveness to TRH, glucocorticoids inhibit the synthesis and secretion of TSH. Glucocorticoid excess decreases the conversion of triiodothyronine (T\(_3\)) to thyroxine (T\(_4\)) resulting in low T\(_3\) concentration levels.

5 The cortisone-to-cortisol-shuttle
Two types of 11\(^\beta\)-Hydroxysteroid dehydrogenase (11\(^\beta\)-HSD) are found in the human body. Type 1 11\(^\beta\)-HSD converts the inactive cortisone to cortisol (23) while type 2 11\(^\beta\)-HSD metabolizes active cortisol to the inactive cortisone (24). Studies have shown that GH inhibits type 1 11\(^\beta\)-HSD and therefore decreases the amount of circulating cortisol in the body (25-27).
Figure 1. A The effect of 11-β-HSD on circulating cortisol in the body. B The activity of 11-β-HSD is increased in GH deficiency and decreased by GH (© 2007 Filipsson & Johannsson (28))

6  Osteopenia
Osteoporosis and osteopenia are condition where BMD is lower than normal.

Osteoporosis is defined as a T – score lower than 2.5, i.e. BMD is at least 2.5 standard deviations (SD) less than the normal mean value for young adults. Osteopenia is defined as a T – score lower than -1 and higher than -2.5, i.e. BMD is 1 – 2.5 SD less than the normal mean value for young adults. It has been estimated that 33.6 million Americans fit this criteria. Osteopenia is a useful diagnosis as it is a precursor to osteoporosis, so when a diagnosis has been made steps can be taken to prevent further bone loss. Fracture-related morbidity is a serious risk, so to be able to identify patients with higher risk of osteoporosis could prove crucial in lowering that risk. Lifestyle modification and reassessment in 2 or 3 years is a reasonable strategy for patients diagnosed with osteopenia. A number of drugs are available for prevention of osteoporosis, e.g. biphosphonates. Patients are also encouraged to exercise regularly (29), quit smoking, avoid excessive alcohol intake and increasing their intake of calcium (30) and Vitamin D (31, 32).

7  Hypopituitarism and osteopenia

7.1  Glucocorticoids and osteopenia
Glucocorticoids have direct inhibiting effects on bone formation through various effects, the most important being their inhibiting effects on osteoblasts (33), their enhancing effects on osteoclasts (34) and their apoptosis inducing effects on osteocytes (33). Glucocorticoids also have extraskeletal effects on bone formation, they decrease calcium reabsorption in the gut while increasing calcium loss in the kidney, decrease sex hormone and growth hormone action and production (34) which
leads to decreased muscle strength. All these factors contribute to decreased bone formation and increased bone resorption, resulting in loss of bone mass and decreased bone quality. Even low doses of glucocorticoids can have significant effects on the risk of vertebral fractures (35).

Patients with hypopituitarism have reduced BMD, increased fracture risk and osteoporosis (36, 37). In a previous study, it was demonstrated that women with hypopituitarism receiving glucocorticoid replacement therapy have lower BMD compared to women with intact hypothalamus-pituitary-adrenal axis (38).

7.2 Growth hormone and osteopenia
Growth hormone stimulates the production of IGF-1 which in turn stimulates bone formation through osteoblastic functions (39, 40). Studies have shown that patients with growth hormone deficiency are three times more likely to suffer osteoporotic fractures (40).

7.3 Sex hormones and osteopenia
Estrogen reduces the lifespan of osteoclasts by increasing apoptosis while also decreasing their formation and activity (41). Estrogen also extends osteoblast lifespan by antagonizing their glucocorticoid-induced apoptosis (42, 43). Estrogen therefore acts to conserve bone mass and maintains balance between bone formation and bone resorption.

As in women, androgens are vital for bone growth and maintenance in men (44-46). Hypogonadism results in rapid bone loss and is a major risk factor for osteoporotic fractures (45). Testosterone affects apoptosis of both osteoblasts and osteoclasts and has a modest effect on osteoblast proliferation. The main result of testosterone action is the reduction of bone resorption (46).

7.4 Thyroid hormone and osteopenia
Osteoblasts and osteoclasts both have a specific receptor for thyroid hormone which, when excessively activated, results in enhanced bone resorption and bone loss (47).
Aim of the study

The aim of this project was to study BMD before and after two years on GH replacement therapy in patients with hypopituitarism and to analyze whether patients receiving glucocorticoid replacement have different therapeutic response to GH compared to glucocorticoid sufficient patients.

GH modulates the metabolism of glucocorticoid by inhibiting the activity and expression of 11-β-HSD type 1, the enzyme that converts the inactive metabolite cortisone to the active hormone cortisol. Therefore, the main hypothesis is that patients on glucocorticoid replacement demonstrate greater improvement in BMD when treated with GH than glucocorticoid sufficient patients.
Material and methods

Patients and study design
This was a retrospective study on 201 adult patients with hypopituitarism due to non-functioning pituitary adenoma. All patients had GH deficiency, which was confirmed by insulin tolerance test (82%) and Arginine-GHRH test (10%) in majority of the patients. Data on physical and laboratory examinations and anthropometric and BMD measurements were analyzed for patients diagnosed with GH deficiency between 1992 and 2006, before any GH replacement was commenced, and after two years on GH treatment. Patients who were receiving glucocorticoid treatment due to other diseases than glucocorticoid insufficiency (sarcoidosis n = 2, polymyalgia rheumatica n = 1, temporal arteritis n = 1 and Crohn’s disease n = 1), and one patient with childhood-onset disease, were excluded from the analysis. Additionally, twenty patients were excluded because of incomplete data. In total, complete baseline data was available for 175 patients, 64 (37%) women and 111 (63%) men, with mean age 55.5 ± 11.6 years.

Of 175 patients, 77 (44%) were glucocorticoid sufficient and 98 (56%) were glucocorticoid insufficient receiving treatment with cortisone acetate (60%) or hydrocortisone (40%). The mean hydrocortisone equivalent dose was 20.9 ± 5.0 mg/day. Seventy-six per cent (n = 133) had central hypothyroidism and were treated with L-thyroxine. The L-thyroxine dose was titrated to attain a euthyroid state and/or free T4 (fT4) levels above 15 pmol/L (reference range 12-22 pmol/L). All patients with hypogonadotropic hypogonadism (n = 127; 73%) received sex steroid replacement with the exception of women of post-menopausal age (>50–55 years).
Table 1. Baseline characteristics of 175 patients with non-functioning pituitary adenoma.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n=175)</th>
<th>GC sufficient (n = 77)</th>
<th>GC insufficient (n = 98)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (year, mean ± SD)</td>
<td>55.5 ± 11.6</td>
<td>55.8 ± 11.5</td>
<td>55.3 ± 11.7</td>
<td>0.787</td>
</tr>
<tr>
<td>Female/male</td>
<td>64/111</td>
<td>22/55</td>
<td>42/56</td>
<td>0.051</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>85.3 ± 14.1</td>
<td>87.6 ± 15.2</td>
<td>83.6 ± 12.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.3 ± 9.8</td>
<td>176.1 ± 9.3</td>
<td>172.8 ± 9.9</td>
<td>0.26</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.1 ± 3.9</td>
<td>28.2 ± 3.9</td>
<td>28.0 ± 4.0</td>
<td>0.772</td>
</tr>
<tr>
<td>Hormone deficiency (%)</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TSH</td>
<td>133 (76)</td>
<td>40 (52)</td>
<td>93 (95)</td>
<td></td>
</tr>
<tr>
<td>LH/FSH</td>
<td>127 (73)</td>
<td>46 (60)</td>
<td>81 (83)</td>
<td></td>
</tr>
<tr>
<td>ADH</td>
<td>21 (12)</td>
<td>2 (3)</td>
<td>19 (19)</td>
<td></td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>n = 160</td>
<td></td>
<td></td>
<td>0.297</td>
</tr>
<tr>
<td>Yes</td>
<td>20 (11)</td>
<td>6 (8)</td>
<td>14 (16)</td>
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</tr>
<tr>
<td>No</td>
<td>105 (66)</td>
<td>51 (69)</td>
<td>54 (63)</td>
<td></td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>35 (22)</td>
<td>17 (23)</td>
<td>18 (21)</td>
<td></td>
</tr>
<tr>
<td>Treatment (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operation</td>
<td>139 (79)</td>
<td>69 (90)</td>
<td>70 (71)</td>
<td>0.002</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>2 (1)</td>
<td>0</td>
<td>2 (2)</td>
<td></td>
</tr>
<tr>
<td>Operation + Radiotherapy</td>
<td>27 (15)</td>
<td>8 (10)</td>
<td>19 (19)</td>
<td>0.045</td>
</tr>
<tr>
<td>No treatment</td>
<td>7 (4)</td>
<td>0</td>
<td>7 (7)</td>
<td></td>
</tr>
<tr>
<td>Free T4</td>
<td>15.6 ± 3.9</td>
<td>14.5 ± 3.0</td>
<td>16.4 ± 4.3</td>
<td>0.001</td>
</tr>
<tr>
<td>IGF</td>
<td>102.5 ± 59.6</td>
<td>113.6 ± 58.7</td>
<td>93.6 ± 59.1</td>
<td>0.28</td>
</tr>
</tbody>
</table>

**Ethical considerations**
Informed written consent was obtained from all patients. The local ethical committee of the University of Gothenburg, Göteborg, Sweden, approved the study. The study was conducted according to the Declaration of Helsinki.

**Methods**
Height was measured with an accuracy of 0.5 cm and weight (in kilograms) to one decimal place; body mass index (BMI) was calculated as weight/height² (kilograms/m²).

BMD at lumbar spine and proximal femur neck were measured by dual-energy X-ray absorptiometry (DSEA; Lunar DPX-L, Lunar Corporation, Madison, WI, USA; Software 1.1 to 8.70). Throughout the study period, a phantom (BONA SIDE, Ltd 313, West Beltline HWY, Madison, WI, USA) was used for calibration.
T-score was used to interpret the BMD data. T-score indicates how much the patients BMD varies from the young normal reference mean, i.e. it compares the patients BMD to a healthy 30-year old of the same sex and ethnicity. A negative score indicates lower BMD and a positive score indicates higher BMD. T-score of -1 or higher is considered normal, score between -1 and -2.5 is defined as osteopenia and scores lower than -2.5 indicates osteoporosis.

Between 1992 and November 2002, serum IGF-I was measured with RIA after acid/ethanol precipitation of IGF binding proteins (Nichols Institute, San Juan Capistrano, CA). Thereafter a chemiluminescence immunoassay was used (Nichols Advantage system). The currently used method (since September 2006) is Immulite 2500 (Diagnostic Products Corp. Siemens, Deerfield, IL).

Statistical methods
All statistical analyses were performed using SPSS 17.0 for windows. Data are presented as mean ± SD or median (25-75 percentiles). For comparisons between groups, unpaired t-test was used for normally distributed data and Mann-Whitney U-test for non-normally distributed data. For within group comparisons, a paired t-test was used for normally distributed data and Wilcoxon signed rank tests for non-normally distributed data. For proportions, Pearson Chi-square or Fishers exact test were used as appropriate. In the multiple linear regression analysis, the dependent variables were studied with backward elimination. The dependent variables were BMD at proximal femur neck and lumbar spine. The potential explanatory variables were age, weight, gender, treatment with glucocorticoids, treatment with sex hormones, and serum levels of free T₄ and IGF-I. The presence of osteopenia and osteoporosis (binary, dependent variables) was studied by logistic regression analysis, with the same explanatory variables as for the multiple linear regression analysis. A *P*-value of < 0.05 was considered statistically significant.
Results

Baseline characteristics for glucocorticoid sufficient and glucocorticoid insufficient patients

The groups were similar in terms of age (table 1). There tended to be more women in the glucocorticoid insufficient group, although the difference did not reach statistical difference. Glucocorticoid insufficient patients were significantly more likely to suffer from other pituitary hormone deficiencies (table 1). Of patients with glucocorticoid insufficiency, 95% were TSH deficient, 83% had hypogonadotrophic hypogonadism and 19% had diabetes insipidus, compared with 52%, 60% and 3%, respectively, of glucocorticoid sufficient patients. Glucocorticoid sufficient patients tended to weigh more while there was no significant difference between the two groups regarding BMI. Glucocorticoid insufficient patients had higher levels of free T4 but no difference in IGF levels between the groups was observed.

Out of 77 glucocorticoid sufficient patients, 69 (90%) had underwent surgery and 8 patients (10%) required additional radiotherapy. In the glucocorticoid insufficient group 70 patients (71%) out of 98 underwent surgery while 19 (19%) required additional radiotherapy.

Records about smoking status were available for 160 patients, showing no significant difference between the two groups regarding smoking habits.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>BMD - lumbar</th>
<th>T - score lumbar</th>
<th>BMD - femur</th>
<th>T score femur</th>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
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<tr>
<td>GC sufficient</td>
<td>1.17 ± 0.18</td>
<td>-0.45 ± 1.45</td>
<td>0.93 ± 0.13</td>
<td>-0.92 ± 1.06</td>
</tr>
<tr>
<td>GC insufficient</td>
<td>1.17 ± 0.19</td>
<td>-0.40 ± 1.54</td>
<td>0.94 ± 0.14</td>
<td>-0.82 ± 1.10</td>
</tr>
<tr>
<td>P - value</td>
<td>0.93</td>
<td>0.83</td>
<td>0.51</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>2 years</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC sufficient</td>
<td>1.23 ± 0.19</td>
<td>0.00 ± 1.61</td>
<td>0.95 ± 0.13</td>
<td>-0.75 ± 1.02</td>
</tr>
<tr>
<td>GC insufficient</td>
<td>1.21 ± 0.23</td>
<td>0.08 ± 1.62</td>
<td>0.95 ± 0.14</td>
<td>-0.71 ± 1.04</td>
</tr>
<tr>
<td>P - value</td>
<td>0.67</td>
<td>0.76</td>
<td>0.95</td>
<td>0.82</td>
</tr>
<tr>
<td><strong>Change</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC sufficient</td>
<td>0.05 ± 0.06</td>
<td>0.42 ± 0.52</td>
<td>0.02 ± 0.05</td>
<td>0.19 ± 0.43</td>
</tr>
<tr>
<td>GC insufficient</td>
<td>0.04 ± 0.12</td>
<td>0.48 ± 0.79</td>
<td>0.01 ± 0.06</td>
<td>0.09 ± 0.46</td>
</tr>
<tr>
<td>P - value</td>
<td>0.43</td>
<td>0.6</td>
<td>0.18</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 2. BMD and T – score figures for both groups at baseline, after 2 years of therapy and the difference between them.
Bone mineral density at baseline
BMD at baseline did not differ between the two groups, neither at lumbar spine nor proximal femur neck (table 2). Similarly, no differences in T-scores were observed between the groups.
In a regressions analysis, after adjustment for weight, age, gender, free T₄ levels, IGF-I levels and sex hormone treatment, glucocorticoid insufficiency was not found to be associated with BMD or T-score at any site.

Bone mineral density after two years on GH replacement therapy
Both groups improved their BMD and T – score after 2 years of GH replacement therapy (table 2). No significant difference was between the two groups regarding change in BMD and T – score levels.
Discussions

In this study, BMD was not found to be lower in patients with glucocorticoid insufficiency compared to sufficient patients, neither before nor after 2 years on GH replacement. Although both groups increased their BMD after two years on GH replacement therapy there was no significant difference in change between the groups. The results are therefore in contrast to our hypothesis that glucocorticoid insufficient patients would demonstrate greater increase in BMD.

In earlier studies on patients with GH deficiency it was shown that female patients who were glucocorticoid insufficient had lower BMD and had more frequently osteoporosis than female patients who were glucocorticoid sufficient (37). It has also been shown that glucocorticoid treatment causes a decrease in BMD (48, 49) while studies on bone metabolism in patients with primary adrenal insufficiency show conflicting results. One study reported decreased BMD in men only (50), another study reported decreased BMD in women only (51), yet another study reported no change in BMD (52). In patients with ACTH insufficiency, receiving glucocorticoid replacement therapy, decreased BMD has been reported (53) while another study did not show an association with increased fracture risk (36). The impact of glucocorticoid replacement therapy on bone metabolism is therefore not fully understood.

Glucocorticoid insufficient patients in our study were suffering from more hormone deficiencies than the glucocorticoid sufficient group and were, in addition, more likely to have undergone both radiotherapy as well as pituitary operation. Although not statistically significant, the glucocorticoid insufficient group did weigh less than the glucocorticoid sufficient group at baseline, possibly explained by the fact that there was a larger proportion of females in that group. Also, free T₄ levels were significantly higher in the glucocorticoid insufficient group, explained by the fact that glucocorticoid insufficient patients suffered more often from hypothyroidism and were being treated with somewhat supraphysiological doses of L-thyroxin. All these factors, the difference in free T₄ levels, weight and gender distribution potentially affects the results concerning BMD. However, after adjustment for these and other explanatory variables in a regression analysis, no difference was observed between the groups.
The main strength of our study was the large number of patients included in the study, and the fact that they were all suffering from the same disease, i.e. non-functioning pituitary adenoma. The variation in weight between the two groups must be considered a disadvantage as well as the difference between the two groups regarding free T₄ levels. The uneven sex ratio between the glucocorticoid sufficient and glucocorticoid insufficient groups must also be considered a disadvantage. Our patients were all receiving similar amounts of glucocorticoids so it was impossible to infer whether BMD could have been differently influenced by larger doses of glucocorticoids.

In conclusion, patients with glucocorticoid insufficiency receiving a mean hydrocortisone dose of 20 mg do not have lower BMD compared to glucocorticoid sufficient patients. Our results suggest that the BMD of patients receiving GH replacement therapy is not affected by the inhibiting effects of GH on the enzyme 11-β-HSD type 1. Therefore patients on glucocorticoid replacement do not demonstrate greater improvement in BMD when treated with GH than glucocorticoid sufficient patients.
Acknowledgements

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References

Books and brochures:


Scientific journals:


