Diagnostic and clinical aspects of growth hormone- and adrenocorticotrophic hormone deficiency

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Summary

This thesis focuses on some diagnostic and clinical aspects of growth hormone (GH) and adrenocorticotropic hormone (ACTH) deficiency in adults.

It is now widely recognised that GH has an important role in the health of adults as well as in the development of children. The availability of biosynthetic GH made it possible to investigate the effects of GH in adults with GH deficiency more extensively.

In **chapter 1** (general introduction) several features of GH and insulin-like growth factor-I (IGF-I) are discussed. GH is synthesised in the anterior lobe of the pituitary and its secretion is dependent of various physiological factors as well as hormones from the hypothalamus, like growth hormone releasing hormone (GHRH) and somatostatin. GH has a biphasic effect on glucose, free fatty acids (FFA) and amino acid levels in plasma: a lowering effect like insulin, followed by increased mobilisation of FFA (lipolysis) and diminished glucose utilisation. Many effects of GH are mediated by IGF-I, which is mainly synthesised in the liver. IGF-I has a potent mitogenic action, especially on immature differentiating cells. IGF-I is bound to specific carrier proteins (IGF binding proteins, IGFBP), which are considered to modulate IGF action *in vivo*.

GH deficiency in adults is recognised as a distinct clinical entity. The most prominent features of GH deficiency in adults are altered body composition ('more fat, less muscle'), impaired quality of life and reduced physical strength. There is also an increase in cardiovascular risk factors, such as an unfavourable lipid profile and central obesity.

The biochemical diagnosis of GH deficiency in adults can be assessed by various (mostly non-physiologic provocative) tests. The insulin tolerance test (ITT) is nowadays still considered to be the golden standard. This test and others are briefly discussed, including the promising GH Releasing Peptide 6-GHRH test.

The beneficial effect of GH in children with GH deficiency is known since the late 1950s. Due to the limited supply, it was not possible to treat adults until biosynthetic human GH could be manufactured around 1985. GH substitution in GH deficient adults has a positive effect on body composition due to the anabolic and lipolytic action, leading to reduction of fat mass and an increase in total body water content (fat free mass). Exercise capacity increases too after GH replacement therapy. Adults with GH deficiency have both a reduced rate of bone formation and reduced mineral mass. GH replacement therapy initially reduces bone mineral mass slightly, due to remodelling, but after approximately one year bone mineral mass increases to levels above baseline.

Adults with GH deficiency suffer from increased morbidity and mortality, in part as a result of cardiovascular events. These patients show adverse lipid profiles, which are supposed to contribute to morbidity. GH replacement therapy has been shown to have favourable effects on total cholesterol and LDL cholesterol, but the effect on HDL cholesterol seems to be variable.

Among the stimulation tests to evaluate GH secretion capacity, the GH-releasing hormone stimulation (GHRH) test has the advantage above the insulin tolerance test (ITT) of being less cumbersome. However, these two tests have never been compared extensively. Furthermore, pituitary GH release is directly stimulated by GHRH, whereas GH stimulation after hypoglycaemia is considered to be the result of inhibition of the hypothalamic somatostatin secretion, making the equality between these tests not self-evident. Therefore, in **chapter 2** the GH responses to the insulin tolerance test (ITT) and growth hormone-releasing hormone (GHRH) test are evaluated in 34 adults with untreated non-functioning pituitary macroadenomas. Comparisons between these two tests are made after stratifying the patients for serum prolactin level as an indication of hypothalamic-pituitary dysfunction and for the

number of other pituitary hormone deficiencies. In the whole group the median peak GH level to GHRH was higher than to ITT, but this difference was only observed in the group with hyperprolactinaemia. Among the patients with hyperprolactinaemia, an insufficient GH peak after ITT occurred more often than after GHRH stimulation. Peak GH to ITT was also lower in patients with other pituitary hormone deficiencies than in patients without other deficiencies. In contrast, GHRH-stimulated GH was not related to the number of other pituitary hormone deficiencies. These results show that the ITT and GHRH-test are not equivalent in assessing the somatotroph axis in patients with non-functioning pituitary macroadenoma and hyperprolactinaemia. It is stated that these test results differ due to a different mechanism of action of these stimuli. Hyperprolactinaemia may be associated with a lower somatostatin tone, elevating both basal and GHRH stimulated GH, with no influence on peak GH to ITT.

Acromegalic patients harbour an increased risk of developing colorectal cancer. It is unknown whether prolonged GH replacement therapy in GH deficient adults is a risk factor for acquiring such a malignancy. In **chapter 3** the results of a randomised, placebo controlled study in 16 GH deficient adults are presented, in which the colonic epithelial proliferation rate, as a biomarker of the risk of developing colorectal cancer, was measured before and after 6 and 12 months treatment. This proliferation rate was similar before and after GH replacement therapy for 6 months (n=8). After 12 months treatment, no changes compared to baseline were observed either (n=9). Separate evaluation of the proliferation rate at the basal, mid and luminal portions of the colonic crypts also failed to show any effect of GH treatment. It was concluded that 6 to 12 months of GH replacement therapy with IGF-I levels in the high physiological range does not adversely affect colonic epithelial cell proliferation as a biomarker for the risk of developing colorectal cancer.

Both GH deficiency and acromegaly are associated with an increased cardiovascular risk, which may in part be attributable to lipid and lipoprotein abnormalities. Little is known about High Density Lipoprotein (HDL) metabolism in both conditions. **Chapter 4** deals with the question whether GH deficiency and acromegaly are associated with abnormalities in HDL cholesteryl ester and free cholesterol concentrations, and whether possible changes in plasma lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP), factors involved in HDL metabolism, would affect plasma cholesterol esterification and cholesteryl ester transfer in these conditions. Twelve untreated GH deficient men, 12 acromegalic patients and 24 healthy males were investigated. Compared to healthy subjects, HDL cholesterol and HDL cholesteryl ester were lower in both GH deficient and acromegalic patients. Plasma LCAT, CETP and PLTP activity levels were lower in acromegalic patients and CETP activity was lower in the GH deficient patients compared to healthy subjects. Both plasma cholesterol esterification (EST) and cholesteryl ester transfer (CET) were decreased in acromegalic as well as in GH deficient patients. Independent negative relationships were found between plasma IGF-I and plasma LCAT, CETP and PLTP activity levels. Thus, GH deficient and acromegalic patients show abnormalities in HDL metabolism, consistent with impaired LCAT action. The concomitant decrease in plasma EST and CET suggests that reverse cholesterol transport may be diminished, contributing to increased cardiovascular risk.

In **chapter 5** the influence of GH replacement therapy on LCAT, CETP and PLTP was studied in 24 GH deficient adults. In the first 6 months, these patients were randomised to placebo (n=8), low dose GH (1 U daily, n=8) and high dose GH (2 U daily, n=8), followed by a 6 months open extension study with high dose GH. After 6 months of GH replacement, the levels of plasma VLDL+LDL cholesterol and apolipoprotein B decreased, whereas HDL cholesterol and HDL cholesteryl ester increased. Plasma LCAT and CETP activity levels as well as plasma EST and CET decreased after 12 months treatment. Plasma PLTP

remained unchanged.
The data support the notion that GH replacement therapy is beneficial to the lipoprotein profile in GH deficient adults. Prolonged replacement therapy decreases plasma LCAT and CETP activity levels, contributing to a decrease in plasma cholesterol esterification and cholesteryl ester transfer.

The hypothalamus, pituitary and adrenal form a neuroendocrine axis, which regulates cortisol production and release in the adrenal cortex. Methods of evaluating the HPA-axis in (suspected) cortisol deficiency include measurement of basal serum cortisol and plasma ACTH concentrations, the ACTH stimulation test and dynamic tests to assess ACTH reserve (e.g. ITT). Patients can show variable test results due to variation in site of the primary lesion (adrenal or central), but also due to the duration and extent of the disease. The latter issue explains a normal cortisol response in the ACTH test in recent-onset ACTH deficiency or partial primary adrenal insufficiency.

In assessing the integrity of the hypothalamic-pituitary-adrenal axis (HPA-axis), the ITT is widely used and considered as the golden standard by many investigators. However, this test has the disadvantage of being cumbersome and dangerous for certain patients as well. A screening test would be beneficial to limit the amount of patients that needs the ITT. Therefore, in **chapter 6** the performance of screening tests (serum cortisol at 08^{00} h and 16^{00} h, 24-h urinary free cortisol) and the human-corticotrophin releasing hormone test is demonstrated in 80 patients with hypothalamic-pituitary disorders and compared with the results of the ITT. When comparing the screening tests, it was found that the diagnostic yield for the 08^{00} h serum cortisol was significantly larger than for the 16^{00} h serum cortisol and 24-h urinary free cortisol. No difference was observed in the pre- and postoperative evaluation of 08^{00} h serum cortisol. The human-Corticotrophin Releasing Hormone test did not perform better as compared with the 08^{00} h serum cortisol. It was concluded that the 0800 h serum cortisol measurement serves as the best screening test to evaluate the adrenal function in hypothalamic-pituitary disorders with the ITT as the reference test.

As there are no data with respect to the effect of glucocorticoid replacement therapy on HDL metabolism in hypopituitary subjects, the possible influence of conventional glucocorticoid replacement therapy on plasma lipids, plasma LCAT, CETP and PLTP activity levels, as well as on plasma cholesterol esterification (EST) and cholesteryl ester transfer (CET) was evaluated in 24 hypopituitary patients. These results are described in **chapter 7**. Plasma total and VLDL+LDL cholesterol were lower in patients on glucocorticoid therapy, but there were no differences in HDL cholesterol and triglycerides. Plasma LCAT was 45% lower and CETP 34% lower in patients on glucocorticoid therapy. These effects were independent of gender and fat mass. Plasma EST and CET were also decreased in glucocorticoid-receiving patients, and these changes are at least partly attributable to lower LCAT and CETP activity levels. There was no difference found in plasma PLTP activity between patients with and without glucocorticoid treatment, suggesting a different regulatory effect on plasma CETP compared to PLTP. It is concluded that parameters of reverse cholesterol transport are impaired in patients receiving conventional glucocorticoid replacement therapy. Such disturbances of HDL metabolism could be involved in increased cardiovascular risk in glucocorticoid-treated hypopituitary patients, despite the lack of an unfavourable plasma lipid profile.

The conversion of cortisone to cortisol by 11ß hydroxysteroid dehydrogenase type 1 ($11\beta HSD_1$) may be diminished as a result of GH replacement therapy. As a consequence, this effect would lower the bioavailability of cortisone acetate in patients with adrenal insufficiency, who are treated with cortisone acetate in stead of hydrocortisone tablets. **Chapter 8** describes the results of a randomised, placebo-controlled GH replacement study in 24 patients (17 subjects on cortisone acetate) during 6 months (0 to 2 U GH/day), followed by

a 6 months open extension study (2 U GH/day). The influence of GH replacement therapy on the setpoint of the cortisol to cortisone interconversion was evaluated. After 6 months placebo no changes in urinary cortisol metabolites were observed. The urinary (THF + alloTHF)/THE ratio was unaltered in cortisone acetate treated patients and in patients with intact adrenal function after 6 months GH, whereas after 12 months GH the (THF + alloTHF)/THE ratio decreased only in cortisone acetate treated patients. Urinary THF and alloTHF were higher in cortisone acetate treated patients than in patients with intact adrenal function before GH and remained so after 12 months GH. The sum of cortisol + cortisone metabolites did not change after GH in either group. These results support the possibility that GH replacement decreases $11\beta HSD_1$ activity, which becomes manifest in patients receiving a conventional dose of cortisone acetate. The $11\beta HSD_2$ activity does not decrease. The observation that cortisone acetate treatment is disadvantageous in adrenal insufficiency, or that the bioavailability of cortisone acetate tablets is impaired during GH replacement, is not supported by this study.