Chapter 1

General introduction and outline of the thesis
The pituitary gland

The human pituitary gland is a small gland located within the sella turcica, a bony structure at the base of the brain. Although the pituitary gland only has the size of a pea, it plays a major role in the regulation of many functions throughout the body. The importance and functions of the pituitary gland were not well understood until the latter half of the nineteenth century and the beginning of the twentieth century. Before that time, in early centuries, the gland was thought to be the source or site of drainage of phlegm from the brain to the nose. The name of the pituitary gland is probably derived from the Latin word 'pituita', which means phlegm.

Nowadays, the prominent role of the pituitary gland in the hormonal regulation of critical processes such as growth, reproduction, metabolism, development and stress homeostasis, is well-recognized. Therefore, the pituitary gland has also been referred to as the ‘master gland’, ‘queen of the glands’ or ‘conductor of the endocrine orchestra’.

The pituitary gland is composed of two functionally and anatomically distinct parts: the anterior and the posterior pituitary. The anterior pituitary, also known as the adenohypophysis, contains specialized hormone producing cells that synthesize and secrete the following hormones: growth hormone (GH), prolactin (PRL), thyrotrophin (TSH), adrenocorticotropic hormone (ACTH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (figure 1). These trophic pituitary hormones stimulate their respective target organs, such as the thyroid gland or the adrenal glands, to secrete hormones, which in their turn will exert effects on peripheral tissues as well as inhibit pituitary hormone secretion by negative feedback.

The posterior pituitary, also known as the neurohypophysis, contains the axons of neurons originating in the hypothalamus. These axons, terminating in the posterior pituitary via the pituitary stalk, store and secrete oxytocin and antidiuretic hormone (ADH).

Pituitary adenomas

In the pituitary gland, tumors may arise. Pituitary tumors represent approximately 10% of all diagnosed primary brain and central nervous system tumors. The most common tumors of the pituitary gland are pituitary adenomas (PAs). PAs are benign neoplasms derived from adenohypophysial cells. The overall estimated prevalence rate of PAs in the general population is 16.7%, ranging from 14.4% in autopsy studies to 22.5% in radiological studies. In a recent study from Finland,
the overall standardized annual incidence rate was 4 per 100,000 persons. Moreover, an increasing trend in the incidence of PAs was observed throughout the study period. This was due to an increased incidence of incidentally discovered asymptomatic PAs (incidentalomas) on neuroradiological imaging. Pituitary carcinomas and distant metastases are extremely rare.

While a minority of PAs are associated with hereditary syndromes, the majority of PAs are sporadic tumors. To date, the exact pathogenetic mechanisms causing these sporadic PAs are unclear. In PAs, mutations in classic oncogenes and tumor suppressor genes that are usually encountered in other, non-endocrine tumors, are rarely found. It is thought that hypothalamic, intrapituitary and peripheral factors involved in the regulation of normal pituitary functioning and development, play a role in the initiation and promotion of PA growth.

Despite the benign nature of PAs, these adenomas can cause considerable morbidity due to compression of vital adjacent structures and/or hypersecretion of pituitary hormones. PAs may present with a wide variety of symptoms and signs, depending on the size and hormonal activity of the tumor. These symptoms and signs may be related to pituitary tumor mass expansion, pituitary hormone insufficiency or pituitary hormone excess.

The classification of PAs may be performed in several ways. Based on tumor size, PAs can be divided into microadenomas (maximum diameter < 10mm) and...
macroadenomas (maximum diameter ≥ 10mm). Based on hormonal activity in vivo, PAs can be divided into clinically nonfunctioning and functioning PAs. The latter group includes PAs secreting GH, PRL, TSH, ACTH and/or LH/FSH. In the following section, TSH-secreting, ACTH-secreting, GH-secreting and nonfunctioning PAs will shortly be discussed as some of the effects of these tumors and/or their treatments will be further addressed in this thesis.

**TSH-secreting pituitary adenomas**
TSH-secreting PAs (TSH-omas) are very rare tumors, representing < 2% of all PAs. In a recent Swedish study, the national prevalence and annual incidence of TSH-omas were 2.8 and 0.15 per million persons, respectively. Hypersecretion of TSH by these tumor causes central hyperthyroidism, but the severity of clinical symptoms of hyperthyroidism may vary per patient. As TSH-omas are usually large at the time of diagnosis, symptoms and signs of tumor mass effects are frequently present. Biochemically, TSH-omas are classically characterized by elevated thyroid hormone concentrations in combination with nonsuppressed TSH concentrations, reflecting the dysregulation of the negative feedback loop.

**ACTH-secreting pituitary adenomas**
ACTH-secreting PAs account for approximately 10 - 15% of all PAs, but lower rates have also been reported. The estimated prevalence of ACTH-secreting PAs is nearly 40 per million persons, whereas the reported incidence ranges between 1.2 and 2.4 per million persons per year. The overproduction of ACTH by these tumors leads to chronic hyperstimulation of the adrenal glands and subsequent hypersecretion of cortisol, resulting in Cushing’s disease (CD). Clinical manifestations of CD are predominantly related to hypercortisolism and include metabolic, cardiovascular, cognitive and psychological alterations. In addition, CD is associated with impaired bone health, resulting in osteoporosis and increased fracture risk. Moreover, excess mortality, mainly due to cardiovascular diseases, has also been reported.

**GH-secreting pituitary adenomas**
Of all PAs, GH-secreting PAs, have been estimated to represent 9% to 20%. GH-secreting PAs arising during childhood clinically present with gigantism, whereas those arising during adulthood lead to acromegaly. The prevalence and annual incidence of acromegaly are 40 to 60 and 3 to 4 per million persons, respectively. Acromegaly is characterized by somatic disfigurements, mostly due to enhanced bone growth and soft tissue swelling, and a variety of systemic manifestations, including cardiovascular, respiratory, musculoskeletal, skin and metabolic complications.
In addition, patients with macroadenomas may have symptoms and signs of local tumor mass effects, such as optic chiasm compression or hypopituitarism. Skeletal disorders, particularly arthropathy, occur in the majority of patients with acromegaly and are the most important cause of functional disability. Also, there are indications that acromegaly may be related to increased tumor risk of the colon. Moreover, acromegaly is associated with increased mortality.

Nonfunctioning pituitary adenomas
Of all patients presenting with a PA, approximately 25% to 37% has a clinically nonfunctioning pituitary adenoma (NFPA). As NFPAs range from completely asymptomatic incidentalomas discovered on neuroradiological imaging to symptomatic macroadenomas, reported frequency rates vary, depending on the surveillance method used. In a community-based study from the United Kingdom (UK), the prevalence of NFPAs was 22.2 per 100,000 persons. In another study, the annual incidence rate was 1.0 per 100,000 persons. NFPAs are characterized by the absence of clinical symptoms and signs of pituitary hormone overproduction. Therefore, these tumors may present in a late stage as pituitary macroadenomas. The most common presenting clinical features of NFPAs include visual field disturbances, headaches and hypopituitarism, which are mostly due to tumor mass effects on surrounding structures. For instance, suprasellar extension may cause pressure on the optic chiasm and subsequent visual field defects, whereas compression of the pituitary stalk or of normal pituitary tissue may result in hypopituitarism.

Treatment of pituitary adenomas
The optimal treatment strategy for a PA depends on several factors such as tumor type, tumor size and patient characteristics. For instance, the presence of hypopituitarism or optic chiasm compression may influence the treatment decision. Therefore, careful assessment of each patient should lead to an individualized treatment approach. In general, the goals of PA treatment include tumor removal, relief of tumor mass effects, reversal of comorbidities, long-term disease control without recurrences, and in case of hormone secreting PAs, long-term normalization of hormonal excess. To achieve these ambitious goals, several treatment modalities are available, including transsphenoidal surgery, radiotherapy and medical therapy. Transsphenoidal surgery is recommended as the primary treatment in the majority of PA patients. However, as PAs may be large and expansive, achievement of complete tumor removal can be difficult and adjuvant therapy may be necessary. Pituitary radiotherapy is usually considered in case of large residual tumor after surgery, in case of recurrent or persistent disease, in candidates unsuitable for surgery.
or after failure of medical therapy. Despite the efficacy of radiotherapy in promoting tumor control, its widespread use, especially prophylactically, has declined throughout the years and is currently a matter of debate. Radiotherapy is associated with serious long-term side effects such as hypopituitarism, which has been reported in more than 50% of the cases. Moreover, concerns have been raised about possible increased risks of secondary brain tumors and cerebrovascular disease following pituitary radiotherapy.

Medical therapy represents another treatment option. In patients with prolactinomas, treatment with dopamine agonists is even the therapy of first choice. The development of relatively new drugs, such as somatostatin analogues (SSAs) and GH-receptor antagonists, has advanced the medical treatment of other types of PAs. Pasireotide, an SSA, has recently been approved for treatment of CD when surgery has failed or is not considered an option. In patients with acromegaly, SSAs are currently the most widely used drugs. SSAs are able to induce biochemical disease control and, in some cases, tumor shrinkage. Similarly, in TSH-omas SSA treatment has shown to be able to normalize TSH and thyroid hormone concentrations in the majority of the patients. Despite these promising findings, the role and place of SSAs in the management of TSH-omas have not yet been firmly established. In chapter 2 the management of TSH-omas, with a focus on SSA treatment, will be further addressed.

**Growth hormone**

As mentioned above, one of the hormones produced and secreted by the pituitary gland is GH, a single-chain polypeptide of 191 amino acids. GH-producing cells, also called somatotrophs, account for approximately 50% of the cell population of the anterior pituitary. Somatotrophs secrete GH in a pulsatile fashion, with a major secretory pulse at the onset of early, slow-wave, sleep. The secretion of GH by somatotrophs is regulated by complex mechanisms, involving various central and peripheral signals. GH-releasing hormone (GHRH) and somatostatin, both hypothalamic hormones, are two major central regulators. GHRH stimulates, whereas somatostatin inhibits GH secretion. Although GH secretion is also influenced by other factors and conditions, it is further regulated by its target hormone, insulin-like growth factor 1 (IGF-1), which exerts negative feedback on GH synthesis and release (figure 2).

GH is a key regulator of longitudinal bone growth during childhood. In addition, GH has a wide variety of effects, mainly of anabolic nature, in adulthood such as stimulation of lipolysis and promotion of protein synthesis. The GH receptor is expressed in
many tissues including liver, bone, adipose tissue, kidney, muscle and intestine. GH has both direct and indirect effects on peripheral tissues. Indirect effects of GH are predominantly mediated by IGF-1, which is synthesized in the liver, but also in other tissues, in response to GH stimulation. Indeed, the major effector of GH action is IGF-1. IGF-1 may act as a circulating hormone as well as a local growth factor with paracrine and/or autocrine effects. Circulating IGF-1, mainly of hepatic origin, is bound to IGF-binding proteins (IGFBPs), of which IGFBP-3 is the predominant circulating binding protein.

**Growth hormone deficiency**

The most common causes of GH deficiency (GHD) in adults are PAs and/or their treatments, which can cause one or more pituitary hormone deficiencies. In contrast, in children GHD is mostly due to idiopathic isolated GHD (IGHD), meaning that the etiology of GHD is unknown and that the GHD is not associated with other pituitary hormone deficiencies. Consequently, GHD may be divided into adult-onset GHD (AO-GHD) and childhood-onset GHD (CO-GHD). Additionally, GHD may also be categorized as IGHD or multiple pituitary hormone deficiency, the latter including patients with GHD as well as other pituitary hormone deficiencies.
The secretion of GH appears to be particularly vulnerable to pathological insults of the pituitary gland. Therefore, GH is usually the first hormone to be affected following pituitary damage, in a review, it was reported that approximately 85% of patients with nonfunctioning macroadenomas have GHD. The prevalence of GHD in adults has been estimated to be approximately 20 per 100,000 persons in the UK. The annual incidence has been suggested to be 1 per 100,000 persons. In the Netherlands, approximately 220 adults with severe GHD were registered each year in the time period between 1999 and 2008.

The clinical features of GHD are heterogeneous. CO-GHD is mainly characterized by impaired longitudinal growth and short stature, whereas AO-GHD is characterized by altered body composition, including increased fat mass and decreased lean body mass, unfavorable lipid profile, reduced muscle mass and strength, decreased exercise capacity, impaired psychological well-being and reduced quality of life. In addition, adult GHD is associated with decreased bone mineral density (BMD) and possibly increased fracture risk. Moreover, there are indications that cardiovascular morbidity and mortality are increased in patients with adult GHD.

**Growth hormone replacement therapy**

**History**
The history of GH replacement therapy (GH-RT) begins in the 1940s - 1950s, when methods were developed to purify bovine and porcine GH. However, treatment of the first child with presumed GHD with bovine GH in 1956 disappointingly showed no metabolic effects. In 1958, the first study showing a positive effect of human GH, derived from cadaveric human pituitaries, on growth in a patient with dwarfism, was published. Soon afterwards, it became clear that severe GHD children benefited from therapy with human GH. Unfortunately, the supply of human GH was limited, because more than 365 human pituitaries were necessary to treat one patient for one year. Therefore, GH treatment was restricted to children with short stature and proven severe GHD. In 1985, the worldwide use of human pituitary-derived GH came to an abrupt end after the appearance of several reports of Creutzfeldt-Jakob disease in patients previously treated with human GH. Fortunately, in the meanwhile, a new methodology, recombinant technology, had been developed, enabling the production of enormous amounts of synthetic recombinant human GH. The availability of recombinant human GH in 1985 led to renewed scientific and clinical interest into the effects of GH in health and disease. Also, as more indications for GH treatment
were explored, both the physiological significance of and the possible therapy for adult GHD were investigated \(^{50,61}\).

**Efficacy and safety**

In 1989, the results of first placebo-controlled trials investigating effects of GH-RT in GHD adults were published \(^{65,66}\). These and subsequent, mostly short-term, studies consistently demonstrated that adult GHD was associated with adverse symptoms and signs, as described above, which could be reversed or attenuated by GH-RT \(^{65-71}\). GH-RT was shown to be able to improve body composition, by reducing fat mass and increasing lean body mass, bone health, physical performance, cardiovascular risk factors, well-being and quality of life. This led to the increasing recognition of adult GHD as a clinical syndrome that could possibly be treated with GH-RT \(^{55,59}\). In 1995 and 1996, GH-RT for GHD adults was approved in Europe and the United States of America (USA), respectively \(^{72}\). Nowadays, approximately twenty years later, data on long-term effects of adult GH-RT are only gradually emerging in literature. In a recent review, evaluating effects of GH-RT with a duration of at least five years, beneficial effects on quality of life, body composition, BMD, carotid intima media thickness and lipid profile were found. On the other hand, effects on several other cardiovascular and metabolic parameters were variable \(^{73}\). More studies with a longer follow-up duration are needed to evaluate the long-term effects of GH-RT in GHD adults \(^{73,74}\).

The most frequently reported side effects of GH-RT in adults, occurring in 2 - 18% of patients, arise from fluid retention and include peripheral edema, joint and muscle stiffness, paresthesias, arthralgias, and carpal tunnel syndrome \(^{53,55,59,72}\). Most of these adverse reactions present in the early phase of GH-RT and resolve either spontaneously or after dose reduction \(^{55}\). In addition, fewer side effects have been reported since GH dosing plans in GHD adults have evolved from a weight-based strategy to an individualized dose-titration strategy, resulting in lower dosages \(^{75-77}\).

Concerns have been raised about the safety of GH-RT with regard to the development of new malignancies or regrowth or recurrence of treated PAs, because GH and IGF-1 are potentially growth stimulating hormones. Experimental studies suggest that GH and IGF-1 may play a role in the genesis and growth of tumors \(^{78-80}\). In addition, acromegaly has been associated with an increased prevalence of colonic polyps and, less frequently, with other tumors \(^{31}\). Moreover, some, but not all, epidemiological studies indicate that high-normal IGF-1 concentrations may increase cancer risk \(^{81}\). As NFPA cells have been shown to express GH receptors, the possible influence of GH on tumor recurrence or regrowth has been questioned \(^{82}\). Although relatively few reported studies have investigated recurrence or regrowth of PAs in
adult GHD patients using GH-RT, results so far seem reassuring. However, as study populations sizes were small, further and larger studies are needed.

**The Dutch National Registry of Growth Hormone Treatment in Adults**

The Dutch National Registry of Growth Hormone Treatment in Adults (in Dutch: de Landelijke Registratie Groei hormoonbehandeling (LRG) Volwassenen), a nationwide surveillance database including GHD adults, was founded on the initiative of the Dutch Ministry of Health in 1998. At that time, shortly after the approval of GH-RT for GHD adults in Europe, long term follow-up data in large patient groups were not available. In addition, as virtually unlimited amounts of recombinant GH suddenly had become available, illegitimate use of GH, for instance by athletes, and the rise of a black market for GH were feared. Therefore, the Dutch National Registry of Growth Hormone treatment in Adults was established with the aim of regulating GH use and gaining more insight into the long-term efficacy, safety and costs of adult GH-RT. Only adults with severe GHD, diagnosed according to the Growth Hormone Research Society consensus guidelines, were eligible for reimbursement of GH-RT costs by the health insurers. Moreover, approval of the indication by an independent board of endocrinologists as well as inclusion of anonymized patient data into the registry were mandatory for reimbursement. A wide variety of data from all included patients, collected (bi-) annually from medical records by trained monitors, were registered. Data collection and test procedures have previously been described in more detail elsewhere. Until data closure in 2009, 2891 adults with severe GHD were enrolled in the registry.

As the registry was financed by the Dutch Health Care Insurance Board, it was financially independent of pharmaceutical companies. Furthermore, the large group of adult GHD patients enrolled and the thorough collection of data over a long period of time, provided excellent research opportunities. Therefore, the database is of considerable additional value for investigations into adult GHD, causes of adult GHD (e.g. PAs), and GH-RT. Chapters 3, 4 and 5 of this thesis are based on data from the Dutch National Registry of Growth Hormone treatment in Adults.

**The GH-IGF-1 axis and aging**

In healthy persons, the concentrations of GH and IGF-1 vary with age. Shortly after birth, the GH secretion rate is high. Thereafter, the GH secretion rate falls and remains relatively stable throughout the prepubertal period. During puberty, a period characterized by fast somatic growth, the concentrations of GH and IGF-1 rapidly
increase. After achievement of full adult height and maturation, serum GH and IGF-1 concentrations gradually begin to decline. This decline continues throughout adult life and eventually results in significantly lower GH and IGF-1 concentrations in healthy older persons than in healthy young persons. It has been estimated that, starting from young adult life, the secretion of GH decreases with 14% per decade. The natural decline in the activity of the GH-IGF-1 axis with advancing age has also been termed the ‘somatopause’, in analogy to the menopause, which refers to the age-related decline in gonadal function. Although the exact mechanisms behind the ‘somatopause’ are unclear, it has been postulated that changes in the hypothalamic control of GH secretion, in particular reduced GHRH and increased somatostatin release, play an important role.

Normal aging is associated with several clinical features that are similar to those encountered in severe adult GHD, e.g. decreased lean body mass, increased fat mass, reduced BMD, decreased physical performance, reduced energy and impaired cognitive functioning. These similarities in combination with the concurrence of the ‘somatopause’ with the appearance of features of normal aging, have led to the hypothesis that the ‘somatopause’ may account for, at least in part, age-related changes and diseases.

The GH-IGF-1 axis, aging and bone

One of the features of normal aging is a decline in BMD, which may lead to the more severe condition of osteoporosis. In a recent study, it was estimated that in 2010 approximately 54% of the US adult population aged 50 years or older had either low bone mass or osteoporosis. Osteoporosis is an important risk factor for fractures. In the elderly population, osteoporosis-related fractures are an important public health issue with devastating consequences, as they are linked with disability, institutionalization, morbidity, high economic costs, and even increased mortality. It is therefore important to clarify the pathogenesis of osteoporosis, which could lead to improvement of prevention and treatment. Because GH and IGF-1 are known key regulators of bone metabolism and considered to be essential for longitudinal bone growth, skeletal maturation and bone mass maintenance, it has been postulated that the GH-IGF-1 axis plays a role. Nevertheless, the possible relationship between the ‘somatopause’ and osteoporosis in healthy older persons remains to be established.

The GH-IGF-1 axis, aging and mood

In the older population, depression and depressive symptoms are common. Although the prevalence of major depression (MDD), diagnosed according to Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria, is relatively low with 1.8%,
the prevalence of all clinically relevant depressive symptoms, also called minor depression, is higher, being approximately 13.5% in community-dwelling older persons. Both major and minor depression are relevant conditions in older persons, as they are associated with decreased quality of life, increased use of health services, higher costs, worse outcomes of somatic illnesses, and increased mortality.

Although the etiology of depression in older persons is complex and presumably multifactorial, it has been hypothesized that the GH-IGF-1 axis may be involved. Both GH excess, i.e. acromegaly, and severe GHD have been associated with disturbances in cognition, mood and quality of life. Furthermore, many brain structures, including those associated with mood such as the hippocampus and the amygdala, express IGF-1 receptors. Moreover, late-life depression often occurs in the context of cognitive decline, which has been associated with lower IGF-1 concentrations. Despite these findings, population-based epidemiological studies exploring the possible relationship between the GH-IGF-1 axis and depression in older persons are rare. Therefore, it remains to be clarified whether the ‘somatopause’ contributes to the development of MDD or minor depression in otherwise healthy older persons.

The Longitudinal Aging Study Amsterdam

In the late 1980s, the increasing aging population was recognized as an emerging demographic driving force in the Netherlands, influencing Dutch society in general and health care use in particular. Therefore, in 1992 the Longitudinal Aging Study Amsterdam (LASA) was initiated by the Dutch Ministry of Health, with the aim of investigating predictors and consequences of aging in the Netherlands. The LASA is an ongoing, prospective, multidisciplinary cohort study, focusing on physical, emotional, cognitive and social functioning in community-dwelling older persons. For the LASA, persons aged 55 - 85 years were randomly selected from the population registries of 11 municipalities in three different geographical regions in the Netherlands, i.e. area’s in the vicinity of Amsterdam, Oss and Zwolle, respectively. In this manner, a nationally representative sample of the older Dutch population was formed. At the LASA baseline, in 1992/1993, 3107 persons were included. New additional cohorts were started in 2002 and 2012.

LASA measurements were performed at baseline and subsequently every following three years. Data collection and sampling procedures have previously been described in detail elsewhere. A wide variety of parameters were measured, including serum IGF-1 concentration in a subsample of persons (n = 1319) participating in the second data collection cycle (1995/1996). These unique data were used for chapters 6 and 7 of this thesis.
Chapter 1

Aims and outline of the thesis

In the preceding paragraphs, several conditions involving the pituitary gland, i.e. PAs, adult GHD, GH-RT and the GH-IGF-1 axis in older persons, have been described. Although the pituitary gland has received more scientific attention throughout the years, much is still unknown. Especially long-term effects of rare disorders of the pituitary gland, such as TSH-omas, adult GHD and their associated treatments, as well as the role of the GH-IGF-1 axis in the aging process, remain to be elucidated. Therefore, the following subjects will be addressed in this thesis: in chapter 2, long-term follow-up data, including clinical, diagnostic, treatment and follow-up characteristics, of patients with TSH-omas are described. In chapters 3, 4 and 5, data from the Dutch National Registry of Growth Hormone Treatment in Adults are presented. Chapter 3 describes the characteristics of and fracture occurrence in adults with severe GHD due to NFPA, CD and acromegaly, respectively, at commencement of and during long-term GH-RT. Chapter 4 investigates the risk of cerebrovascular events, secondary intracranial tumors and mortality after radiotherapy for a NFPA. Chapter 5 evaluates the occurrence of tumor regrowth or recurrence in adult GHD patients with a (previous) NFPA using GH-RT. In chapters 6 and 7, data from the LASA study are presented. Chapter 6 studies cross-sectional associations of serum IGF-1 concentration with bone related parameters, as well as prospective associations of serum IGF-1 concentration with BMD and fracture risk, in community-dwelling older persons. Chapter 7 explores the cross-sectional and longitudinal relationships between serum IGF-1 concentration and minor and major depression in the same large population of older persons. Finally, in chapter 8, a summary of the main findings, a general discussion, including possible directions for future research, and concluding remarks are provided.
References


Chapter 1


