Acromegaly and Cushing's syndrome:

LIFE AFTER CURE

MARGREET WAGENMAKERS

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Colofon

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”Ieder mens is anders dan anderen.
Altijd verschillen arts en patiënt van elkaar:
in geografische herkomst, religie, opleiding
of (medische) kennis, en macht.
Goede zorgverleners erkennen deze
verschillen, en schenken aandacht aan
de individuele context van de patiënt.”

M.E.T.C. van den Muijsenbergh, NTVG 2013

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General introduction and outline of this thesis

General Introduction and Outline of this Thesis

In 1905 Ernest Starling used the word "hormone" (derived from the ancient Greek word ὁρμαω, "to set in motion") for the first time to describe the recently discovered "internal chemical messengers that are carried from the organ they are produced to the organ they affect by the blood stream" [1]. Nowadays we know that hormones are essential for the wellbeing of every human being. Moreover, human life would be impossible without hormones.

This thesis focuses on the treatment and long-term effects of two diseases that are caused by overproduction of two very important hormones: acromegaly caused by growth hormone (GH) overproduction and Cushing's syndrome (CS) caused by cortisol overproduction. In both diseases the pituitary gland plays a key role.

The pituitary gland

Anatomy and function

The pituitary gland (or hypophysis) is an endocrine gland, about the size of a pea and approximately 0.6 grams in weight, which is located in the midline of the base of the skull [2]. It is frequently called "the master gland" as it orchestrates the complex regulatory functions of many other endocrine glands.

The pituitary gland (Figure 1) consists of 2 lobes: the anterior pituitary gland (or adenohypophysis) and the posterior pituitary gland (or neurohypophysis). The anterior pituitary gland, which is of endodermal origin, produces six major hormones: 1) prolactin, 2) GH, 3) adrenocorticotropic hormone (ACTH), 4) luteinizing hormone (LH), 5) follicle-stimulating hormone (FSH), and 6) thyroid-stimulating hormone (TSH). These hormones are secreted in a pulsatile manner, in response to specific hypothalamic releasing and inhibiting factors and each of these hormones exerts specific responses in its peripheral target tissues. The hormonal products of peripheral target glands, in turn, have feedback control at the level of the hypothalamus and pituitary gland.
The posterior pituitary gland consists of pituicytes, modified glial cells, and axons whose cell bodies are located in the hypothalamus. It stores and releases hormones such as antidiuretic hormone (ADH) and oxytocin.

The pituitary gland lies outside the dura, below the optic chiasm and the hypothalamus (Fig 3). It rests in the sella turcica, which is part of the sphenoid bone.

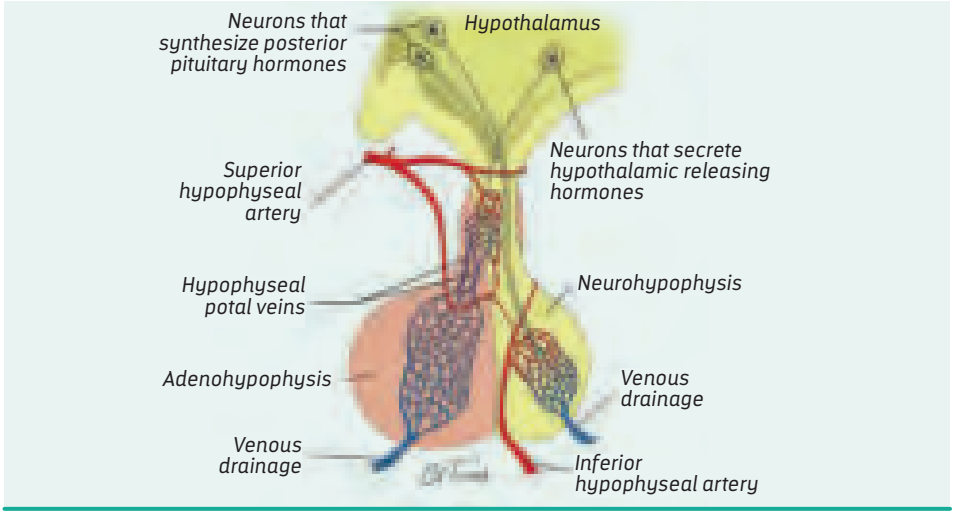

Figure 1) Anatomy of the pituitary gland. *(www.medscape.com)*

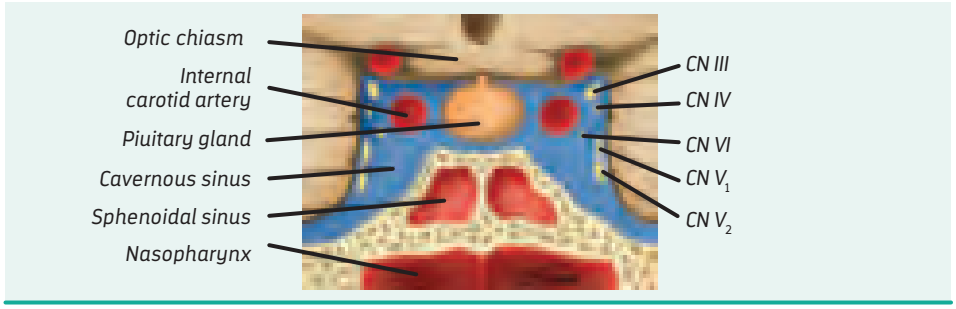

Figure 2) Anatomy of structures surrounding the pituitary gland. *(www.medscape.com)*

Anteroinferior to the sella turcica lies the sphenoid sinus. Bilateral to the sella turcica (thus in the parasellar region) lie the cavernous sinuses (Fig 2). The cavernous sinuses are built up by a multi-loculated venous structure containing a part of the internal carotid artery, the oculomotor nerve (cranial nerve III), the trochlear nerve (cranial nerve IV), the abducens nerve (cranial nerve VI) and the V1 and V2 branches of the trigeminal nerve. Thus, one can imagine that large expanding pituitary tumors may cause significant morbidity.

The blood supply of the pituitary gland originates from the superior and inferior hypophyseal arteries (Figure 1). The hypothalamic-pituitary portal plexus provides the major blood source for the anterior pituitary gland, so pulses of hypothalamic hormones can directly be transmitted without significant systemic dilution.

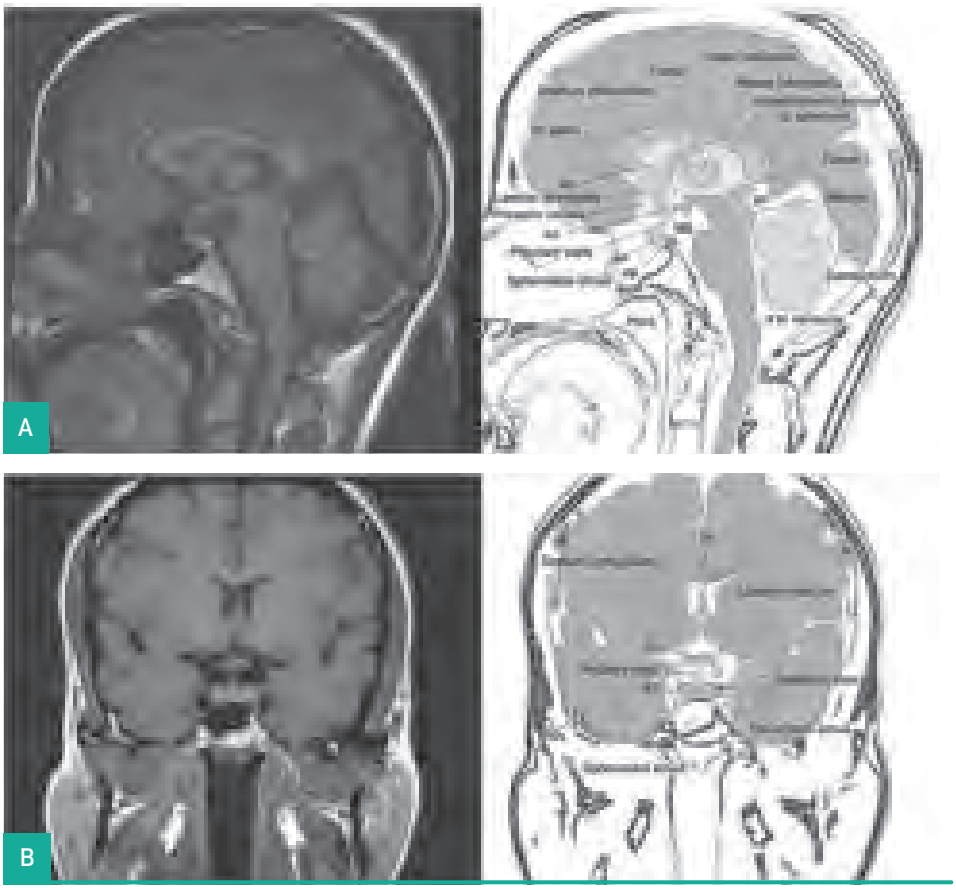

Figure 3) Anatomy of the normal pituitary gland.

(From Williams Textbook of endocrinology, Melmed et al, 2011, Figure 7,2., published with permission from Elsevier) PP, posterior pituitary gland; AP, anterior pituitary gland; oc, optic chiasm; cc, corpus callosum; MB, mammillary body; pc, posterior commissure; ac; anterior commissure.

The somatotropic axis

GH is the most abundant anterior pituitary hormone. It is secreted by somatotroph cells, which account for up to 50% of the total anterior pituitary cell population[2]. GH secretion is controlled by both hypothalamic and peripheral factors (Figure 4). In short, GH is secreted under dual hypothalamic control: GH–releasing hormone (GHRH) induces and somatostatin suppresses GH secretion. In healthy individuals GH is released in approximately 10 intermittent pulses per 24 hours, mostly during the night, with GH levels undetectable during most of the day [3].

The peripheral action of GH is mediated via the GH receptor, which is expressed mainly in the liver and cartilage. GH induces the production of insulin-like growth factor type-1 (IGF-1) in the liver. IGF-I directly causes proliferation and inhibition of apoptosis of virtually all cell types and has negative feedback control on GHRH and GH production. Besides GHRH and IGF-1 many other factors have a significant influence on the somatotropic axis. For example: secretion of both GH and IGF-1 is influenced by available nutrients: GH production increases during fasting and IGF-1 levels are suppressed in malnourished patients. Furthermore GH production diminishes during aging and in obesity and IGF-I is suppressed in patients with liver disease, hypothyroidism, or poorly controlled diabetes [4].

Hypothalamic–pituitary–adrenal (HPA) axis

ACTH producing cells (corticotropic cells) account for about 20% of the pituitary cell population[2]. Corticotropin-releasing hormone (CRH), which is produced in the hypothalamus, is the most important stimulator of ACTH production. ACTH stimulates the adrenal glands to produce cortisol. In healthy individuals ACTH secretion has a classic circadian rhythm: levels peak early in the morning and reach a nadir at midnight. Furthermore cortisol inhibits the synthesis of CRH and ACTH via negative feedback [5].

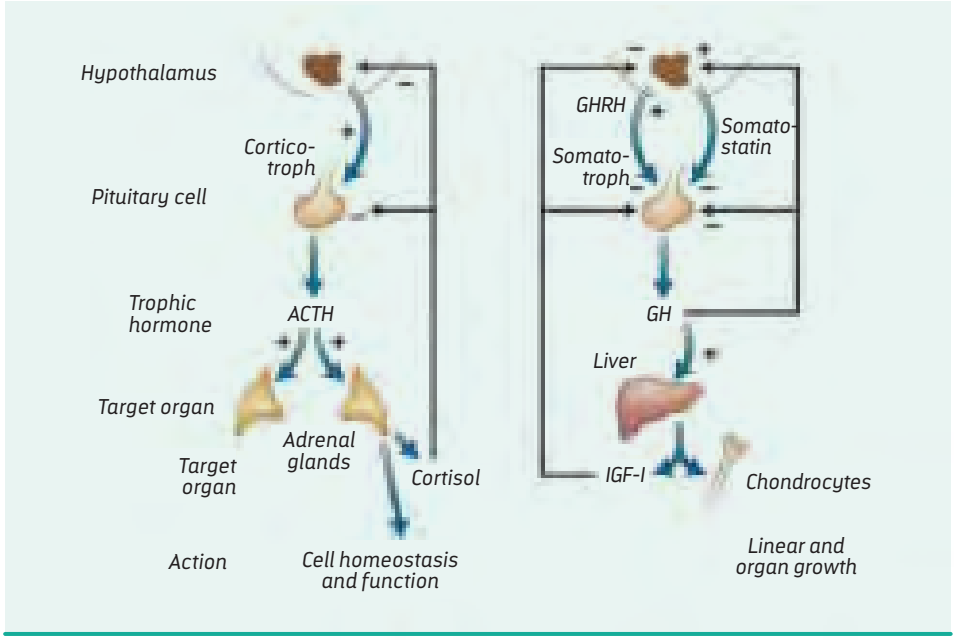

Figure 4) The Hypothalamic–pituitary–adrenal axis and somatotropic axis.
(Adapted from Williams Textbook of endocrinology, Melmed et al, 2011, Figure 8,4., published with permission from Elsevier)

The major function of the HPA axis is to mediate the neuroendocrine stress response and maintain metabolic homeostasis and it has important anti-inflammatory effects and catabolic effects to provide substrates for gluconeogenesis. In conditions of stress the secretion of CRH and thus also of cortisol are stimulated. The peripheral actions of cortisol are mediated via glucocorticoid receptors (GR) and mineralocorticoid receptors (MR). GRs are ubiquitously expressed, but MRs are expressed only in selected tissues [6].

Disorders of the pituitary gland

Disorders of the pituitary gland have 3 classical manifestations: **1)** symptoms related with pituitary insufficiency, **2)** symptoms related with hormonal excess, and **3)** symptoms associated with mass effects of the pituitary lesion. Although especially pituitary insufficiency can have multiple causes (for example genetic or congenital disorders, infiltrative diseases, infection, trauma, surgery or irradiation[7]) all three manifestations of pituitary gland disorders are most frequently caused by a pituitary tumor.

Pathological processes arising from both the gland itself or from the surrounding structures are relatively common, but often asymptomatic. In adults who undergo cranial imaging studies for reasons other than suspected pituitary disease, an unsuspected pituitary lesion (a so called incidentaloma) can be found on CT-scans in 4–20% of all patients and on MRI-scans in 10–38% of all patients [8]. During autopsy an unsuspected pituitary adenoma is found in ›10%. Indeed, pituitary adenomas, which are benign neoplasms that arise from adenohypophysial cells [2], are by far the most prevalent pituitary lesions (± 85% of all pituitary lesions[9]) and they account for 6-10% of all symptomatic intracranial tumors[10]. Pituitary adenomas are classified according to size: they are called a microadenoma if they are ‹ 10 mm in diameter and a macroadenoma if they are ≥ 10 mm in diameter[9]. Although they are benign they can invade surrounding structures relatively easily. Pituitary carcinomas are rare. About one third of all pituitary adenomas are clinically non-functioning and do not cause a clinically distinct hypersecretory syndrome [2]. The majority of these adenomas arise from gonadotroph cells. All other types of pituitary adenomas can produce one or more pituitary hormones that cause clinical signs and symptoms of hormonal excess. The most common hormone producing adenoma is the prolacting (PRL)-producing adenoma (or prolactinoma) that arises from lactotroph cells. TSH-producing adenomas are rare. GH-producing adenomas (which arise from somatotroph cells and cause acromegaly) and ACTH-producing adenomas (which arise from corticotroph cells and cause Cushing's syndrome) account for 10-15 % of all adenomas each [2].

Adenomas of the adenohypophysis are monoclonal neoplasms, which suggests that somatic mutations precede clonal expansion[11]. However pituitary adenomas rarely occur in the setting of hereditary syndromes (like multiple endocrine neoplasia type 1 and Carney's complex) or as familial isolated pituitary adenomas (FIPAs). More than 95% of pituitary adenomas are sporadic, meaning there is no family history of pituitary adenomas [11]. Over the past decade, due to technical advances in genetic research, a number of mutations that are associated with pituitary adenomas have been found. For example germline mutations in the aryl-hydrocarbon receptor-interacting protein (AIP, a tumor suppressor gene that acts via the cAMP pathway) have been identified in 20% of FIPA families. Germline AIP mutations have also been found in 8-18% of selected seemingly sporadic cases of young adults with macroadenomas or gigantism[11]. Microduplication on chromosome Xq26.3 has been found in a number of children with GH secreting macroadenomas, causing X-linked acrogigantism [12,13]. Furthermore somatic mutations in the ubiquitin-specific protease USP8 gene have been found in 36% of patients with an ACTH-producing adenoma [14,15]. Although much progress has been made in the understanding of the pathophysiology of pituitary adenomas, many factors still need to be elucidated.

Acromegaly

Acromegaly is the rare clinical syndrome (incidence of 3-4 cases/1 million people/year, prevalence of 60 per million) that is caused by prolonged exposure to excessive amounts of GH [3]. The GH excess causes proliferation of many tissues including connective tissue, cartilage and bone. This causes characteristic changes of the face (e.g frontal bossing, mandibular prognatism, hypertrophy of the tongue, lower lip and nose) and enlargement of the hands and feet (the acra). Furthermore, if the GH excess is already present in childhood or adolescence, before the closure of the epiphyseal growth plates, it causes gigantism. The term "acromegaly" (from ancient Greek: ακροσ = extremity and μεγαλοσ = large) was used for the first time in 1886 by the French neurologist Dr. Pierre Marie, although he was not the first to give an accurate clinical description of this disease.

Most people without a medical training will probably never have heard of the term "acromegaly", but everybody will, at least unconsciously, know certain cases. Especially acromegalic giants have been famous since ancient times: Goliath probably suffered from acromegaly, as did the Egyptian Pharaoh Akhenaten (± 1358 BC, Fig. 5). During the 17th century giants were recruited for the Prussian army for the "Potsdammer Riesengarde" (2 battalions of 600 men each!, Fig. 6) and as bodyguards for the king of England, solely because of their height. From the 18th century until the Second World War it was common that people with physical abnormalities were used as show objects in circus shows and expositions and giants were a popular attraction [16,17]. Even nowadays, people

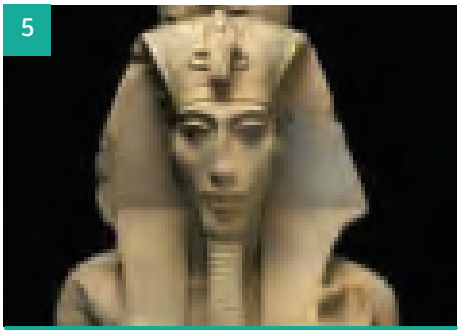

Figure 5) Statue of the Egyptian Pharaoh Akhenaten.
Figure 6) Drawing of the "Potsdammer Riesengarde".

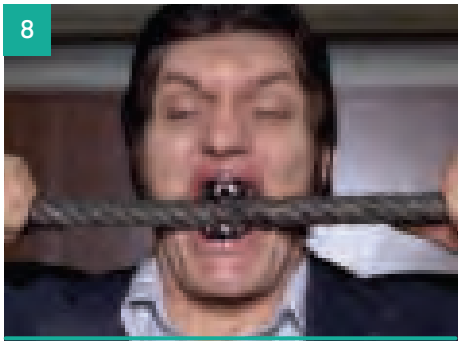

Figure 7) Carel Struyken as "Lurch" in the "Adams Family".
Figure 8) Richard Kiel as "Jaws" in the "James Bond"series.

who suffer(ed) from acromegaly can still become very popular because of their appearance: nearly everybody knows the famous actors Carel Struyken ("Lurch" in the "Adams Family", Fig. 7) and Richard Kiel ("Jaws" in the "James Bond" series, Fig. 8).
Besides the typical changes in appearance, acromegaly also influences many other organ systems. At diagnosis the majority of the patients have arthropathy, with joint complaints in up to 70% of patients [18]. This may cause major functional disability. Many patients have respiratory dysfunction due to soft tissue swelling, with obstructive sleep apnea syndrome (OSAS) documented in more than 50% of patients at diagnosis. Furthermore the excess amount of GH can cause growth of many organs including the heart, resulting in major structural and functional alterations. This may cause arrhythmias, cardiac valve disease and myocardial hypertrophy, which may eventually result in congestive heart failure. Furthermore GH has many metabolic effects which may result in overt diabetes mellitus (DM, present in 10-25% of all patients at diagnosis), hypertension (present in 25-35% of all patients at diagnosis) and dyslipidaemia [19]. Thus, it is not

surprising that acromegaly is associated with a markedly decreased quality of life (QoL) [20-22] and an increased overall mortality rate, with an overall standardized mortality rate of more than 3.0 in inadequately treated patients [23,24], mainly because of cardiovascular disease.

Acromegaly is caused by a GH secreting pituitary adenoma (of which 25% co-secrete PRL) in nearly all cases. Mean age at diagnosis is 45 years and males and females seem to be affected equally [25]. Overall, younger patients have more aggressive tumors that secrete higher amounts of GH. Because the signs and symptoms of acromegaly develop insidiously there is often a delay in diagnosis for up to 10 years. At diagnosis more than 70% of all GH secreting adenomas are macrodenomas. Acromegaly is rarely caused by a pituitary carcinoma, a hypothalamic tumor secreting GHRH or ectopic GH or GHRH secretion from a neuroendocrine tumor [3].

Cushing's syndrome

Cushing's syndrome (CS) is caused by prolonged exposure to supraphysiological levels of glucocorticoids. Although iatrogenic CS, caused by the use of exogenous glucocorticoids, is quite common, endogenous CS, like acromegaly, is a rare disorder with a reported incidence of 2-3 cases per 1.000.000 people per year [26]. CS is named after the American neurosurgeon Harvey Cushing. Cushing's life-long interest in CS was triggered by his encounter in 1910 with a patient who has become known as Minnie G (Fig 9.) [27,28]. Twenty-two years later Cushing published his seminal report "The basophil adenomas of the pituitary gland and their clinical manifestations (pituitary basophilism)". In this classic work, he reported in detail the cases of 12 patients with CS, resulting in a description of CS that has not yet been improved upon. He believed that an abnormality in the pituitary gland was the cause of the syndrome.

Unlike in acromegaly, none of the signs and symptoms of CS is pathognomonic. Common symptoms of CS are weight gain (the most common symptom, present in 95% of all patients) fatigue, depression, insomnia, irritability, decreased memory and concentration, decreased libido, menstrual irregularities and back pain. However, these symptoms are also common in the general population and are not very discriminatory. The same accounts for common signs of CS: obesity (mostly centripetal), a dorsocervical fat pad (the so called buffalo hump), supraclavicular fat pads, facial fullness (or moon face), peripheral edema, acne, hirsutism or balding in females, a thin skin and poor skin healing, hypertension (present in up to 90% of all patients!), DM (present in up to 50%), dyslipidaemia, osteoporosis, hypokalemia, kidney stones and recurrent infections [26, 29]. Many of these symptoms and signs are also present in people with morbid obesity or

Figure 9) Harvey Cushing and "Minnie G."

the metabolic syndrome [30], conditions that are very common in our present society where bad eating habits and a sedentary lifestyle are the norm. Signs that are more discriminatory for CS (thus having a higher sensitivity) are peripheral muscle atrophy or muscle weakness, easy bruising, striae (especially when reddish/purple in color and > 1 cm wide), facial plethora and, in children, weight gain with decreasing growth velocity [26]. When CS is full blown, the diagnosis is straightforward. However, because none of the signs and symptoms are pathognomonic the diagnosis may be very challenging in milder cases. This often causes a delay in diagnosis. This delay is problematic since CS causes a significantly impaired quality of life [20, 31-33] and, in untreated cases, the 5-year survival has been reported to be as low as 50% [24, 34, 35]. Cardiovascular disease is the major cause of mortality.

CS can be either ACTH dependent, with high levels of plasma ACTH that stimulate the adrenal glands to secrete excessive amounts of cortisol, or ACTH independent, with excessive autonomous production of cortisol by abnormal adrenocortical tissue which suppresses CRH and ACTH secretion. ACTH dependent causes of CS account for 80-85% of all cases of endogenous CS. An ACTH secreting pituitary adenoma (also called

Cushing's disease (CD), usually caused by a microadenoma) is the cause of CS in 80% of the patients with ACTH dependent CS. In the remaining 20% an ectopic ACTH secreting tumour (frequently a neuroendocrine tumor of the lung) is the cause. ACTH independent CS is most frequently caused by a unilateral adrenal tumour, which is a benign adenoma in 60% of the cases and a carcinoma in 40% of the cases [36]. Both CD and cortisol producing adrenal adenomas are more frequently present in women (male to female ratio 1:3). Mean age at diagnosis is 40 years [34].

PART I
Treatment of acromegaly and Cushing's disease by transsphenoidal surgery

Up till now transsphenoidal surgery (TS) of the pituitary gland is the treatment of choice for virtually all patients with acromegaly and CD [37-40].
The transsphenoidal route to access the brain leads through the sphenoid sinus (either via the nose or upper lip) within the constraints of nasal/sinus anatomy, which provides a narrow but relatively noninvasive corridor to the skull base and has been known since ancient times. Egyptian embalmers used this route during the mummification process to remove the brain without disfiguring the face and head [41].

Until the end of the 19th century the transsphenoidal route was not used. At that time pituitary tumors were already recognized, but were considered rare and the function of the pituitary gland was still completely unknown (although it had been noticed that patients with acromegaly almost always had an enlarged sella). Attempts to remove pituitary tumors had been made, but were always performed transcranially [42].
The transsphenoidal route to access the sella turcica was rediscovered by Giordano during anatomical studies with cadavers at the end of the 19th century. At the beginning of the 20th century a number of European and American investigators, including Schloffer (who performed the first transsphenoidal operation on a large pituitary tumor in 1906) and Harvey Cushing, found that TS was feasible in living patients and it was successfully performed in a few patients with nonfunctional macroadenomas or acromegaly (which ended the long debate about the etiology of acromegaly [42]). However, at that time the illumination of the operating field during TS was inadequate and the risk of meningitis was high since there were no antibiotics, so nearly all neurosurgeons abandoned the transsphenoidal route in favor of the transcranial route. Pituitary surgery was restricted for patients with mass effect of the pituitary tumor who needed decom-

pression. Patients with acromegaly without mass effects were treated with radiation therapy or sometimes with medical therapy [43].

It took until the second half of the 1950s before the French neurosurgeon Guiot introduced the use of intraoperative fluoroscopy and reinstated TS as an important therapeutic option for patients with pituitary tumors. When Jules Hardy (a Canadian neurosurgeon who learned TS from Guiot) added the use of the operating microscope, which provided state of the art optics in combination with a binocular vision [44], in 1963, TS with the aid of a microscope became the gold standard for pituitary surgery.
Because of the excellent visualization of the pituitary gland with the microscope Hardy could recognize adenomas within the pituitary gland, enabling him to perform a selective adenomectomy.
At that time there was still uncertainty about the etiology of CD although a pituitary origin had been suggested several times (first by Harvey Cushing). This was due to a number of reasons: many patients with adrenal tumors had similar symptoms, the first attempts to attack the pituitary gland in patients with CD (either with transcranial surgery or with radioactive seeds) had been unsuccessful and because patients with CD usually have a microadenoma, they often did not have an enlarged sella and/or pituitary gland. Hardy finally proved that CS could be caused by a pituitary adenoma when he successfully performed a selective adenomectomy in a patient with CD in 1963 [45]. This was in fact the first time TS was performed in a patient with CD. However, bilateral adrenalectomy remained the treatment of choice for CD until the first successful series on TS in CD had been published in the 1980s. Thereafter microscopic TS became the primary treatment option for CD.

In the 1990s a major change was introduced in the field of TS: the purely endoscopic technique of TS (that is, using the endoscope as the only visualizing tool) was introduced as an alternative to the microscopic technique thanks to the collaboration between neurological and otorhinolaryngological surgeons [46]. Although many groups were experimenting with the endoscopic technique of TS, Jho and Carrau, a neurosurgeon and otorhinolaryngologist of the University of Pittsburgh School of Medicine, are widely recognized as the pioneers of the purely endoscopic endonasal approach for the treatment of pituitary adenomas. They were the first to report upon the results of endoscopic TS in a series of 50 patients with pituitary adenomas in 1997 [47]. Thereafter Cappabianca and de Divitiis, from Naples, reported on their experience with the endoscopic technique [48] and they made major contributions to the technique by developing dedicated endoscopic instrumentation and suggesting technical improvements. From then onwards many neurosurgeons have adopted the procedure (Figure 10).

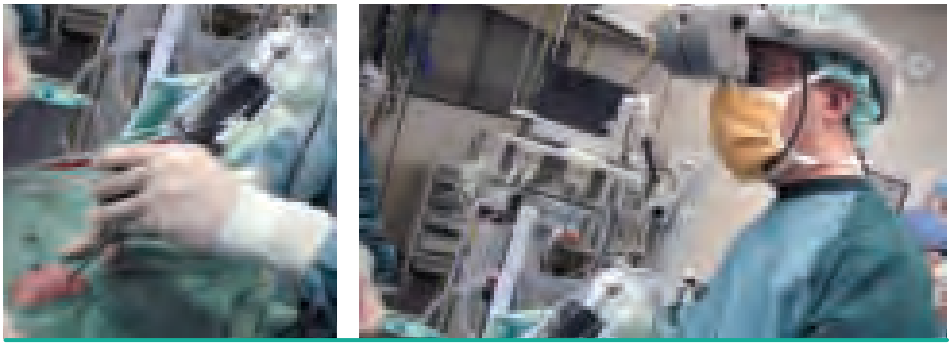

Figure 10) Endoscopic transsphenoidal pituitary surgery in the Radboud university medical center, Nijmegen, the Netherlands.

The endoscopic technique of TS has theoretical advantages and disadvantages compared to the microscopic technique of TS. Advantages are the panoramic view with increased illumination of the operating field and the possibility to use different angles making it possible to reach the suprasellar and parasellar regions. Disadvantages are the two-dimensional view and the easy clouding of the lens in case of bleeding [49, 50].

Since the introduction of the endoscopic technique of TS a vivid discussion has arisen about which technique should be the technique of choice for TS: the endoscopic or the traditional microscopic technique of TS. Important factors to take into account to answer this question are the abilities of the technique to achieve: 1) the primary objective of the surgery (in the case of pituitary adenomas: gross tumor resection in non-functional adenomas and remission of the hormonal syndrome in functioning adenomas), 2) patient safety, 3) patient comfort and, 4) cost-effectiveness. However, because both acromegaly and CD are rare diseases it is difficult to adequately answer these questions and it is crucial that results of both microscopic and endoscopic TS are reported systematically.

Our center introduced the endoscopic technique of TS in 1998 and entirely switched to this technique within a year. Thus our center was among the first centers in the world that adopted the endoscopic technique of TS and the first center in the Netherlands. Over the years we have gained a lot of experience with the endoscopic technique of TS but so far results had not been analyzed adequately.

The first aim of this thesis is to gain more insight in the role of endoscopic TS for the treatment of both acromegaly and CD. To do so we systematically analyzed the results of endoscopic TS in our hospital in patients with acromegaly and CD (Part 1 of this thesis).

In chapter 2 we describe the outcome of endoscopic TS (as the primary treatment modality for acromegaly) in 40 patients with acromegaly caused by a macroadenoma treated in our hospital between 1998 and 2007.
In chapter 3 we evaluate the results of endoscopic TS (as the primary treatment) in 86 patients with CD treated in our hospital between 1998 and 2011. Furthermore, we evaluated the recurrence rates after initial remission.
In chapter 4 we report on the results of repeated endoscopic TS in 14 patients with persistent or recurrent CD after an initial TS treated at our hospital between 1998 and 2007.

Other treatment modalities in acromegaly and Cushing's syndrome

Acromegaly

Medical therapy

Three forms of medical therapy are currently used to treat patients with acromegaly: 1) somatostatin receptor ligands (SRL, octreotide and lanreotide), 2) dopamine agonists (DA, cabergoline) and 3) GH receptor antagonists (GHRA, pegvisomant). Both SRL and DA target the pituitary adenoma itself and reduce GH secretion. GHRA are directed at blocking GH effects in the periphery [38].

Long-term studies have shown that SRL can lower GH values to ‹ 2.5 ng/ml and can normalize IGF-1 in up to 70% of all patients [37]. However, these studies have been performed in preselected patient groups. In unselected patients with acromegaly, acromegaly will be controlled with SRLs only in approximately 25% [38]. However, shrinkage of the adenoma (of › 20%) occurs in 75% of the patients with acromegaly treated with SRLs and these drugs have proven to be safe on the longterm. However the costs associated with the use of SRLs are high [51]: in the Netherlands lanreotide-autosolution 60 mg each 28 days costs about €12,000 annually and Sandostatin LAR 20 mg each 28 days costs about € 15,000 annually (Farmacotherapeutisch Kompas, 2015).

Cabergoline is the DA that is most effective for treatment of acromegaly, but monotherapy with this drug controls acromegaly in less than 10% of patients. The best response can be expected in patients with mildly elevated IGF-1 levels. However, it is a safe drug and far cheaper than SRLs and the GHRA pegvisomant, amounting only € 1000 a year.

The GHRA pegvisomant (which has been approved by the USA Food and Drug Administration (FDA) since 2003) is very effective in normalizing IGF-1 levels, provided that a sufficiently high dosage is given, and the medication is well tolerated. Normalisation of IGF-1

can be achieved in virtually all patients treated with GHRA. The first long-term safety reports are reassuring, showing a low incidence of adverse effects such as increase in pituitary tumor size, elevations of liver enzymes, and lipodystrophy at the injection site [52]. Pegvisomant is by far the most costly medication used for treatment of acromegaly [51]: in the Netherlands treatment with 20 mg/day has an annual cost of approximately € 55.000,-.

Because medical therapy cannot cure acromegaly and therefore requires life-long use of costly medication the current recommendation is to restrict primary medical therapy to patients in whom surgery is not feasible (e.g. because of comorbidities or refusal by the patient) or if surgical cure is not possible to achieve because of extensive growth of the tumor. For all other patients it is considered a second-line therapy. SRLs are the medical treatment of choice. If the response on SRL monotherapy is inadequate, pegvisomant and/or cabergoline can be added. If there is no response to a SRL, pegvisomant monotherapy is an option as well [38]. Pretreatment with SRL for 3-6 months before TS with the aim to reduce the size of the adenoma and improve the condition of the patient should be considered [53].

Radiation therapy (RT)

The general consensus is that RT, which was the primary therapy for acromegaly before the 1960s, is nowadays the third-line treatment, i.e. when TS and medical therapy have failed [37]. This is mainly because of limited effectiveness and safety issues.

Conventional fractionated RT can achieve remission of acromegaly in over 60% of patients, but its usefulness is limited by the delay between therapy and disease control, with a median time to complete remission between 6 and 10 years. More recently stereotactic RT has been introduced. In stereotactic RT a higher dosage of RT is given in usually a single dosage on a smaller area. This limits its value for larger adenomas that cannot be safely separated from surrounding normal radiosensitive tissues. Remission of acromegaly (usually after surgical debulking) can be achieved in approximately 50% of all patients with stereotactic RT and median time to remission is 3-10 years [54]. Control of tumor growth is excellent (>90%) with both stereotactic and conventional RT. However, hypopituitarism is observed in over 50% of patients 5–10 years after RT for acromegaly. There seems to be no difference between stereotactic and conventional RT in this respect. There is also a small risk of damage of the optic nerves, especially with stereotactic RT. After conventional RT there may be a small increased risk of developing secondary tumors or cerebrovascular events due to radiation vasculopathy, but results are conflicting [37, 54-56].

Cushing's disease/syndrome

When CS is caused by an adrenal adenoma the therapy of choice is unilateral adrenalectomy, which results in remission in virtually all cases.

In case of CD, the recommended second-line therapy, after TS has been unsuccessful (and if a repeated TS is not feasible or has also failed) is either bilateral adrenalectomy or RT. The choice should be made taking into account the wishes of the patient, the function of the pituitary gland after TS and whether a patient tolerates medical therapy, which will be necessary in case of RT.

Billateral adrenalectomy (ADX)

Traditionally, open procedures of adrenalectomy were associated with considerable morbidity and mortality. Nowadays the minimally invasive laparoscopic approach, which has reduced perioperative morbidity significantly, is the technique of choice. The major advantage of a bilateral ADX is that hypercortisolism is permanently cured in virtually all cases [57]. However, adrenalectomized patients require life-long substitution of glucocorticoids and mineralocorticoids, with the permanent risk of an adrenal crisis. A severe complication of bilateral ADX, which develops in approximately 20% of all patients, is Nelson's syndrome: an expanding invasive macroadenoma of the pituitary gland as a consequence of the lack of glucocorticoid feedback to control adenoma cells [58].

Radiotherapy

Radiotherapy in CD has the same advantages and disadvantages as in acromegaly (see above), although hypopituitarism may be more prevalent in CD in the long-term. Remission rates after conventional fractionated radiation therapy for CD range from 56% to 84% [59, 60]. The median time to remission ranges between 18 and 42 months [54]. The benefit of stereotactic RT seems to be a faster biochemical response to treatment with a median time to remission between 7.5 and 33 months [54]. However, the remission rates after stereotactic RT are still unclear. Recent literature of stereotactic RT series suggests that they lie between 35 and 80% [54].

Medical therapy

Various types of medication can be used for the treatment of CS: 1) Adrenal directed therapy. With steroidogenesis inhibitors (metyrapone, ketoconazole or mitotane). These drugs are all reasonably effective, and normalize urinary cortisol values in 70-80%, but they have major side effects and toxicity and availability is not always guaranteed [40]. 2) Pituitary targeted therapy including SRL (pasireotide) and dopamine agonists (cabergoline). Although these drugs are generally well tolerated, they only normalize cortisol excretion in 15-30% of patients [40, 61]. 3) The glucocorticoid receptor antagonist mifepri-

stone, which has recently been approved for CS by the FDA, induces significant clinical and metabolic improvement in patients with CS [62, 63]. However, as cortisol values rise during therapy it is unclear how one should assess adequate response and long-term safety still needs to be determined.

So unfortunately, in contrast to acromegaly, no drug that is currently available for the treatment of CS is both effective and well tolerated on the long-term. Furthermore, there is a lack of high-quality studies on medical therapy for CS [64]. Therefore currently, medical therapy in CS still has only an adjunctive role [61]. It can be prescribed to control severe hypercortisolism and improve the general condition of the patient prior to TS or when radiotherapy has not yet reached its full effect, and in the occasional patients that are not fit for or refuse other treatment modalities [61].

PART 2
Long-term Quality of Life after treatment of acromegaly and Cushing's syndrome

Over the past decade the interest in QoL of patients with pituitary adenomas has exponentially increased [65]. General health related questionnaires that had been developed for other patient categories, showed that QoL is decreased in all patients with (both functional and nonfunctional) pituitary adenomas [32, 65]. To adequately measure QoL in a specific patient group it is recommended that a general health questionnaire is used in combination with a disease-specific questionnaire [65]. To gain further insight in the specific problems in patients with different types of adenomas, disease-specific health questionaires have been developed like the ACROQoL for acromegaly [22, 66], the CushingQoL and the Tuebingen CD-25 for CS [33, 67].

Meanwhile a multitude of studies have investigated QoL after successful treatment of pituitary adenomas. All patient groups seem to have a decreased QoL compared to healthy matched control subjects [65, 68]. Patients that have successfully been treated for a non-functional pituitary adenoma perform best: the total patient group has a slightly impaired QoL [68, 69], but if comorbidities or hormonal deficiencies are adequately controlled it may be possible to achieve a normal or near normal QoL [70]. Even though QoL significantly improves after remission, patients in long-term remission of CD and acromegaly generally have a more impaired QoL than patients treated for other pituitary adenomas [65, 70, 71].

In order to understand why QoL remains more impaired in patients in long-term remission of CS and acromegaly compared to patients adequately treated for other pituitary adenomas, it is essential to determine which patient or disease-specific characteristics negatively influence QoL.

Therefore, the second aim of this thesis is to get more insight in patient — or disease-specific — characteristics that negatively influence QoL in patients in long-term remission of CS and acromegaly (Part 2 of this thesis).

Acromegaly

In patients in remission of acromegaly, the following factors have been found to negatively influence the QoL: the use of SRLs [72], previous treatment with radiotherapy [73, 74], persisting joint complaints or musculoskeletal pain [18], presence of clinical osteoarthritis [75] and presence of deficiency of growth hormone [76] but not of other pituitary hormones. Furthermore, as can be expected, most studies reported the negative effect on QoL of female gender and older age.
Because changes in appearance are highly prevalent in acromegaly and seem to persist, at least partially, after long-term remission, it is likely that this will also have a negative influence on QoL. However, this has not been adequately investigated, despite the fact that the appearance subscale was the most affected subscale in studies that used the AcroQoL to investigate QoL. Moreover, patients in remission of acromegaly had only slightly better results on this subscale than patients with active disease [21, 22, 77-79].

In **chapter 5** we investigate whether patients in long-term remission of acromegaly suffer from more psychological distress and psychological and social dysfunctioning related to self-consciousness of appearance than a gender-, age- and bosy mass index (BMI)-matched control group, and whether this negatively affects QoL. To do so, we use the Derriford appearance scale 59 (DAS59), which is a psychological questionnaire developed for research in plastic surgery and oncology. It also identifies the anatomical source of self-consciousness. This questionnaire has not been used previously in patients with acromegaly.

Cushing's syndrome

In patients in remission of CS, a number of studies reported that QoL remains impaired after treatment [31, 68, 80-87]. However most of these studies have major limitations and/or only investigated QoL in patients in remission of CD. The presence of hypopituitarism, female gender, older age and glucocorticoid deficiency seemed to negatively influence QoL . Furthermore, it has been suggested that a persisting effect of the previous period

of hypercortisolism might negatively influence QoL but this has never been adequately investigated.

In chapter 6 we report on the QoL of 123 patients in long-term remission of CS, compared to a sex- and age-matched healthy control group. We also investigate the influence of the aetiology of CS, the treatment strategy and coexisting hormonal deficiencies on QoL.

PART 3
Long-term physical sequelae after treatment of acromegaly and Cushing's syndrome

As stated before, both acromegaly and CS have a great impact on the physical health of patients. If the signs and symptoms of the disease are not totally reversible after remission, this will most likely have a negative impact on QoL.

The third aim of this thesis is to gain more insight in the long term effects of the previous period of growth hormone or cortisol hypersecretion on physical health of patients in long-term remission (Part 3 of this thesis).

Acromegaly

Because acromegaly is a rare disease, only little is known about the long-term physical consequences of the previous period of GH hypersecretion in patients in remission [88]. In the few studies that have been performed, most patients received medical therapy to control disease, so no conclusions can be made about patients that are really in remission of acromegaly. Furthermore, the studies that were performed had limitations, such as the lack of a control group or a very small sample size. Therefore, up to date, very little is known about the long-term physical effects of a longstanding period of GH excess in patients that are cured.

In general, the previous studies indicate that, by adequately controlling GH and IGF-1 levels, glucose-related metabolic abnormalities seem to improve and may even normalize[19] (although this may not account for the long-term [89]), lipid abnormalities seem to improve [88, 90], OSAS seems to be at least partially reversible [91, 92], cardiovascular disease (hallmarked by left ventricular dysfunction) improves [19, 93] and even arthropathy may be partially reversible [94]. However it is still unclear to what extent these abnormalities recover.

As stated before, craniofacial disproportions are highly prevalent in patients with active acromegaly and they affect QoL. However no study has investigated to what extent the craniofacial disproportions persist after long-term remission. In the past decade cephalometry using 3D imaging technology has rapidly evolved [95]. Because 3D cephalometry is much more accurate than traditional techniques to perform cephalometry, it offers new opportunities to accurately quantify craniofacial disproportions in patients with acromegaly.

In chapter 7 we evaluate the differences in craniofacial dimensions of soft and bony tissues between patients in long-term remission of acromegaly and control subjects matched for gender, age, BMI and ethnicity, using 3D cephalometry and 3D image fusion head models.

Cushing's syndrome

Over the past few years a number of groups started to investigate the long-term physical effects of the previous period of hypercortisolism in patients in remission of CS [34, 96]. Most studies have only focused on the long term effects of CS on the brain. They all found that structural abnormalities, like a smaller hippocampal volume and enlarged ventricles, persist even after long-term remission [96].

As previously stated, active CS is hallmarked by centripetal obesity and an increased cardiovascular risk. Only a limited amount of research has been performed to investigate if these changes persist after long-term remission of CS.

Centripetal obesity, with excess visceral adipose tissue (VAT), causes a state of systemic low-grade inflammation [97-99]. This process is triggered when macrophages infiltrate the VAT in response to microhypoxia and rupturing of adipocytes. As a result the secretion of adipocytokines by the VAT changes, leading to an adverse adipocytokine profile, which is associated with insulin resistance, endothelial dysfunction and eventually macrovascular cardiovascular disease. A small number of studies investigated if centripetal obesity and systemic low-grade inflammation persist after remission of CS [100-103]. However, these studies have methodological limitations (e.g. small numbers of patients, patients with untreated hormonal deficiencies included, patients included shortly after remission), and the results are conflicting.
In chapter 8 we investigate the adipose tissue distribution and adipocytokine profiles of patients in long-term remission of CS, and compared the results with a healthy gender-, age- and BMI-matched control group.

Only a small number of studies have previously investigated vascular health during remission of CS, using different (surrogate) markers of vascular function [101, 104-109] and these studies report contradicting results. Vascular health may theoretically be affected in patients in long-term remission of CS by two different mechanisms: **1)** through persisting centripetal obesity or comorbidities like impaired glucose tolerance, systemic hypertension and dyslipidaemia or **2)** via a persisting effect of the previous period of hypercortisolism itself on vascular health [6, 110].

In **chapter 9** we investigate whether micro- and macrovascular health is impaired in a well-selected group of patients in long-term remission of CS compared to healthy matched controls. To do so we use a broad spectrum of techniques: we measured serum biomarkers associated with endothelial dysfunction, performe gold standard measurements of endothelial function and investigate the presence of overt atherosclerosis.

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TREATMENT OF
ACROMEGALY
AND
CUSHING'S
DISEASE
by transsphenoidal surgery
PART 1

Results of endoscopic transsphenoidal pituitary surgery in forty patients with a growth hormone secreting macroadenoma

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Abstract

Objective: Transsphenoidal pituitary surgery (TS) is the primary treatment of choice for patients with acromegaly. Macroadenomas (>1 cm) are more difficult to resect than microadenomas (remission rate ± 50% compared to ± 90%). Besides the conventional microscopic TS, the more recently introduced endoscopic technique is nowadays frequently used. However, no large series reporting on its results have yet been published. We evaluated the outcome of endoscopic TS in 40 patients with a growth hormone (GH) secreting macroadenoma, treated in our hospital between 1998 and 2007.

Methods: Medical records were retrospectively reviewed. Remission was defined as disappearance of clinical symptoms of acromegaly, normal serum Insulin-like Growth Factor 1 levels (≤2 SD) and serum GH levels suppressed to < 2 mU/L after an oral glucose tolerance test within the first 4 months after TS.

Results: In four patients TS aimed at debulking of the tumor. In the remaining 36 patients, remission was achieved in 20 patients. In the first five years remission was achieved in 6 out of 18 patients (33%) compared to 14 out of 22 patients (63%) in the following 5 years (p=0.06). Thirteen patients had a mild perioperative complication. Before TS 15 patients received hormonal substitution therapy compared to 12 patients (33%) after TS.

Conclusion: Endoscopic TS is a good primary therapeutic option for patients with a GH secreting macroadenoma, resulting in a remission rate of up to 63% in experienced hands. This technique can potentially improve the outcome of TS in these patients.

Introduction

Untreated acromegaly causes significant morbidity, and is associated with a two- to threefold increase in mortality. When acromegaly is treated successfully and "safe" growth hormone (GH) and Insulin Growth Factor 1 (IGF-1) values are achieved, the mortality rate normalises [32]. Therefore, appropriate treatment of acromegaly is crucial. However, symptoms and signs of acromegaly develop insidiously, and there is often a delay in diagnosis for up to 10 years. Therefore, approximately 70% of GH secreting adenomas are ≥ 1cm (macroadenomas) at the time acromegaly is diagnosed [32].

According to experts, transsphenoidal pituitary surgery (TS) is the treatment of choice for acromegaly [28,31], potentially rapidly restoring normal physiology by a single intervention. Macroadenomas however are difficult to remove by TS, especially when invasive. This may explain the relatively low remission rate of about 50% reported after TS in macroadenomas, while remission rates up to 90% are achieved by TS in microadenomas (< 1 cm) [34]. Since more recently developed medical therapies achieve good results in controlling acromegaly, some authors have recommended medical therapy as a primary treatment option instead of TS for patients with a GH secreting macroadenoma that does not cause mass effects [13,25].

Nowadays, the endoscopic technique of TS is increasingly used by many neurosurgeons instead of the conventional microscopic technique. This technique, offering a panoramic wide angle view with increased illumination, was first developed in the 1990s. Different angles can be used, making it possible to effectively reach supra- and parasellar portions of the lesion and work around the corner [10,14]. Due to these advantages it has been suggested that the endoscopic technique may be preferable to the conventional technique, especially in patients with invasive macroadenomas [14,28,39]. However, due to the recent introduction of this technique, no large series reporting on the results of endoscopic TS in acromegaly have yet been published.

To gain insight in the role of endoscopic TS as a primary treatment option for patients with GH secreting macroadenomas, we evaluated the results of endoscopic TS in 40 consecutive patients with a GH secreting macroadenoma, treated in our hospital between 1998 and 2007.

Patients and methods

Patients

Between 1998 and 2007 40 patients with acromegaly and a macroadenoma on a preoperative Magnetic Resonance Imaging (MRI) scan underwent endoscopic TS in our centre. The medical records of these patients (19 males and 21 females, Table 1) were retrospectively reviewed. Age at time of TS was 47.4 ± 11.4 (mean ± SD) years and BMI was 29.0 ± 4.9 kg/m^2. We collected data on preoperative as well as early postoperative evaluation, complications that occurred during TS or in the early postoperative period and data on the follow-up of these patients.

Preoperative evaluation and perioperative treatment

The initial diagnosis of acromegaly was based on clinical grounds and biochemical tests, including assessment of serum GH levels (basal and after oral administration of glucose) and serum IGF-1 levels. Furthermore the thyrotropic, gonadotropic and pituitary-adrenal axes were assessed, as well as the prolactin blood level. Preoperative pituitary imaging by MRI was performed in all patients.

Long-acting somatostatin analogues (SA) were given preoperatively in 34 patients for a median period of 7 months (range 1-28), one patient received 10 mg/4 weeks, 20 patients received 20 mg/4 weeks and 13 patients received 30 mg/4 weeks.

One hour before surgery, administration of glucocorticoids (prednisolone, 25 mg i.v. every 8 h) was started. Two days after surgery glucocorticoid administration was changed from i.v. to oral and the dose was tapered rapidly.

Surgical technique

The endoscopic technique of TS was introduced in our hospital in 1994, and first used for acromegaly in 1998. From 1998 onward practically all TS (n=365) were performed endoscopically. The surgeries were exclusively performed by two neurosurgeons. The technique is very similar to the technique Jho et al. and Cappabianca et al. have described previously [7,8,21,22]. However, a binostril transsphenoidal approach to the sella turcica was used, during which the endoscope was handheld.

For the endoscopic transnasal TS 0º and 30° rigid endoscopes with a lens diameter of 4 mm with a separate shaft were used, which allow easy and comfortable holding, while offering a suction-irrigation-system for cleaning the lens (Karl Storz GmbH, Tuttlingen, Germany). The instruments used are principally the same as used with the microsurgical technique. Because an adenoma was visible on preoperative MRI a selective adenomectomy was performed in all patients.

Postoperative evaluation

A complication of TS was defined as any event occurring during or after TS which required treatment. As intraoperative cerebrospinal fluid (CSF) leakage is inherent to the surgical procedure and is closed during TS with a fat graft it was not regarded as a complication, whereas postoperative CSF leakage was considered a complication.

On the seventh day postoperatively, at least 48 hours after the last dose of glucocorticoids, early biochemical evaluation was carried out, by measuring the serum concentrations of IGF-1, fasting cortisol, adrenocorticotropic hormone (ACTH), thyrotropin (TSH), free thyroxine (FT4), gonadotropines (LH and FSH), testosterone, estradiol and prolactin. Patients were re-evaluated every 2 to 4 weeks during the first 3 months after surgery. Serum GH and IGF-1 were measured at each visit. Four months after surgery a new MRI of the pituitary was performed to check for tumour remnants. An oral glucose tolerance test (OGTT; 100 g of glucose [36]) was performed if IGF-1 was normalised or was marginally elevated. Thereafter patients who were in remission were evaluated at least once a year or earlier in case of clinical suspicion of a relapse.

Criteria for remission and relapse

Remission was defined as disappearance of clinical symptoms of active GH hypersecretion with in addition normal serum IGF-1 levels ($\leq$ mean + 2 standard deviations for age) and suppression of serum GH levels to $<$ 2 mU/L during OGTT within the first 4 months after surgery [19,20]. Relapse was defined as development of clinical signs of active GH hypersecretion with elevated serum IGF-1 levels ($>$ mean + 2 standard deviations for age) and serum GH levels $\geq$ 2 mU/L during OGTT [19,20].

Imaging

All preoperative and postoperative MRI-scans were evaluated by the same neurosurgeon to prevent bias. Maximal diameter of the adenoma was defined as the largest distance that could be measured in any direction of the adenoma. Invasion was defined as suspected growth of the adenoma beyond the sella into the cavernous sinus or the sphenoid sinus.

Analysis of factors influencing outcome and statistics

Data were analysed using SPSS 16.0. Characteristics of patients operated in the first and second 5 years were compared using unpaired T-test and Pearson's Chi-square test. The influence of various factors on the chance to achieve remission by TS was analysed by binary logistic regression. The factors analysed were: date of operation (as a surrogate measure for experience of the neurosurgeons), age, gender, the level of preoperative IGF-1 and GH, the diameter of the adenoma on preoperative MRI, evidence of

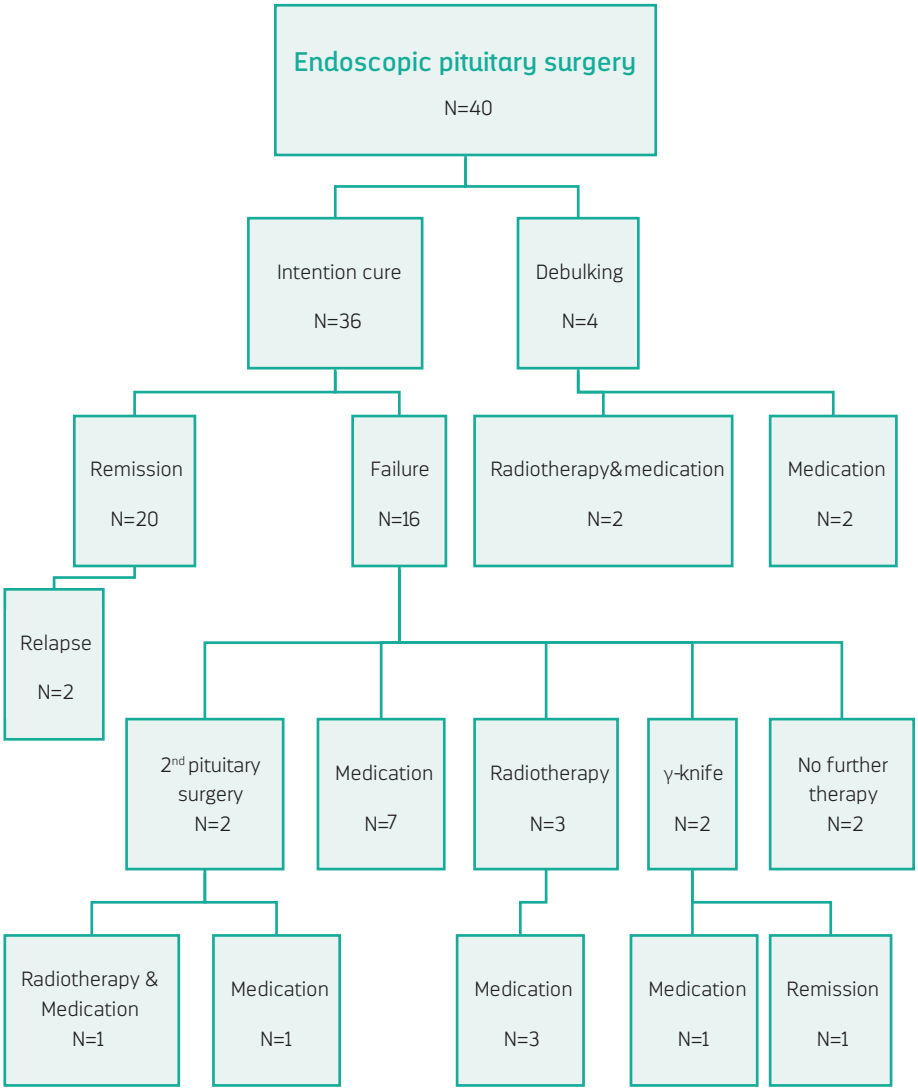

Figure 1) Results of endoscopic pituitary surgery in patients with a growth hormone (GH) secreting macroadenoma (1998-2007).

invasion on the preoperative MRI, occurrence of perioperative complications and the need for hormonal substitution therapy after TS. The influence of dichotomous variables (gender, substitution therapy before TS, evidence of invasion on the preoperative MRI, TS in the first or second five years, occurrence of perioperative complications and the need for hormonal substitution therapy after TS) on the chance of remission were also analysed using Pearson's Chi-square test. Statistical significance was defined as $p = <0.05$ (two-sided).

Results

Remission rates after TS

The results of endoscopic TS in the 40 patients with a GH secreting macroadenoma are shown in figure 1. The individual data per patient are presented in Table 1. Histological investigation of the removed tissue showed evidence of a GH producing adenoma in all cases. The overall remission rate in our series is 50%. However, four patients (patients 21, 30, 33 and 34, Table 1) had an invasive adenoma of more than 30 mm in diameter and suffered from local mass effects. The intention of the TS in these patients was to debulk the adenoma, as it was appreciated that cure could not be achieved by TS. In the remaining 36 patients, in whom the intent was cure, remission was achieved in 20 patients. In this group the remission percentage thus was 56%. Median follow-up was 56 months (range 6-126). Recently, 2 patients (patients 12 and 35) developed a mild relapse. Patient 12 is now treated with octreotide, while the relapse of patient 35 is very mild and no treatment had yet been initiated.

The date of TS significantly influenced the chance of remission after TS. If a patient was operated at a later date, the chance of achieving remission after TS was higher ($p=0.04$). If the results of TS during the first five year interval after the introduction of the endoscopic technique are compared with the second five year interval, remission was achieved in 6 out of 18 patients (33%) during the first 5 years, whereas in the next five years 14 out of 22 patients (63%) were in remission after TS ($p=0.06$). However, the four patients who only underwent debulking were all operated on in the last 5 years. If these patients are excluded, the remission rate achieved in the last 5 years is 77%, which is significantly better than the remission rate over the first 5 years ($p=0.01$). Table 2 shows that baseline characteristics of patients operated during the first 5 years do not significantly differ with baseline characteristics of patients operated during the last 5 years, except for preoperative IGF-1 levels which were significantly higher in de second group. This does not change if the four patients who underwent debulking are excluded.

Table 1) Results of endoscopic transsphenoidal pituitary surgery in patients with acromegaly (1998-2007). *(page 48-51)*

Patient number, gender, age (y)	Preoperative octreotide	MRI (mm)	Invasion on preoperative MRI	Year of TS
1.f, 52	Y	20	sc r	1998
2.m, 43	N	25	-	1999
3.f, 27	N	18	sc r	2000
4.m, 40	Y	10	-	2000
5.f, 24	Y	17	-	2000
6.f, 59	Y	20	ssphen,sc l	2001
7.f, 54	N	11	sc l	2001
8.m, 39	N	14	-	2001
9.m, 49	Y	43	ssphen, sc l + r	2001
10.f, 50	Y	12	-	2001
11. f, 49	Y	13	-	2001
12.m, 37	Y	20	-	2001
13.m, 44	Y	10	sc r	2001
14.f, 34	Y	16	-	2002
15.f, 55	Y	18	-	2002
16.f, 56	Y	11	sc r	2002
17.m, 56	Y	14	-	2002
18.m, 64	Y	12	-	2002
19.f, 66	Y	16	ssphen, sc r	2003
20.f, 45	Y	30	sc l	2003
21.m, 35	Y	39	ssphen	2003
22.f, 56	Y	15	sc r	2003
23.f, 41	Y	15	-	2003
24.m, 54	Y	13	-	2004

Postoperative IGF-1 nmol/L	SD	Postoperative oGTT	TS result	Additional therapy	Last IGF-1 nmol/L	SD	Follow-up (months)
50.3	›2	-	F	RT + cab	19.0	1	127
24.0	1	38	F	octr	32.8	›2	38
92.7	›2	9	F	GK	10.6	-2	109
23.1	1	‹2	R	-	12.9	-1	106
77.0	›2	-	F	octr + cab	41.0	›2	78
64.5	›2	-	F	TS, RT + peg	15.6	0	76
62.8	›2	-	F	RT+ octr	23.5	1	73
73.6	›2	-	F	RT+ octr, cab	28.1	2	68
60.5	›2	-	F	TS+ octr, cab	21.6	1	91
10.2	-2	‹2	R	-	15.4	0	74
38.4	›2	-	F	octr	14.0	0	91
28.3	1	‹2	R	octr	15.6	0	87*
83.6	›2	-	F	GK + octr, peg	27.9	2	86
10.5	-2	‹2	R	-	11.4	-2	70
30.9	›2	3	F	octr	9.8	-2	86
12.7	-1	‹2	R	-	13.6	0	79
16.8	0	‹2	R	-	15.2	0	77
18.0	1	2	F	octr	18.3	1	57
27.1	2	‹2	R	-	25.0	2	65
24.7	1	‹2	R	-	29.0	2	48
66.3	›2	-	F	octr	14.1	-1	69
15.3	0	‹2	R	-	16.5	0	47
28.0	1	‹2	R	-	22.7	1	66
19.8	1	‹2	R	-	16.6	0	44

Patient number, gender, age (y)	Preoperative octreotide	MRI (mm)	Invasion on preoperative MRI	Year of TS
25.f, 43	Y	11	-	2004
26.m, 48	N	12	-	2005
27.f, 29	Y	10	sc l	2005
28.f,51	Y	13	-	2005
29.m, 64	Y	14	sc r	2005
30.m, 68	Y	30	sc l + r, ssphen	2005
31.m, 46	Y	21	-	2005
32.m, 45	N	15	-	2005
33.m,35	Y	42	sc l + r	2005
34.f, 28	Y	45	sc l + r	2006
35.f, 67	Y	11	-	2006
36.m, 40	Y	21	sc r	2007
37.m, 62	Y	20	-	2007
38.f, 41	Y	24	sc re	2007
39.f, 46	Y	18	-	2007
40.m, 54	Y	27	sc r	2007

f: female; m: male; Preoperative octreotide Y: treated with octreotide before surgery; Preoperative octreotide N: not treated with octreotide before surgery; MRI: magnetic resonance imaging results given as maximal diameter of the visualized tumor in mm; sc r: cavernous sinus right; sc l: cavernous sinus left; ssphen: shenoid sinus; ssphen: sphenoid sinus; TS: transsphenoidal surgery; IGF-1 nmol/l: value of insulin-like growth factor-1; IGF-1 SD: standard deviation of insulin-like growth factor-1 compared to normal

There were no statistically significant differences between the patients who underwent successful or unsuccessful TS with respect to age, gender, occurrence of perioperative complications, preoperative IGF-1 levels or need for hormonal substitution therapy after TS. There was a trend that if the diameter of the adenoma was larger the chance to achieve remission was smaller (p= 0.06 in all patients), however if the four patients who underwent only debulking were excluded this trend was no longer present (p= 0.56). Eight of 19 patients (42%) with evidence of invasion on preoperative MRI, and

Postoperative IGF-1 nmol/L	SD	Postoperative oGTT	TS result	Additional therapy	Last IGF-1 nmol/L	SD	Follow-up (months)
29.5	2	‹2	R	-	22.8	1	43
35.8	›2	-	F	octr	23.6	1	31
25.6	1	‹2	R	-	23.5	1	37
11.7	-1	‹2	R	-	15.6	0	49
30.0	›2	-	F	cab	21.4	1	36
31.5	› 2	-	F	octr	16.8	1	30
43.1	› 2	‹2	F	-	36.2	›2	32
46.0	›2	‹2	F	-	31.3	›2	38
163.8	› 2	595	F	RT + octr,cab	56.9	›2	33
98.7	›2	-	F	RT+ octr	33.3	›2	15
21.5	1	‹2	R	-	27.4	›2	31*
22.9	0	‹2	R	-	21.3	1	14
18.4	1	‹2	R	-	18.4	1	6
31.7	2	‹2	R	-	12.0	-1	15
16.2	0	‹2	R	-	21.3	1	8
26.6	2	‹2	R	-	26.6	2	6

*values in people of the same age and sex; oGTT: Minimal value of growth hormone achieved during the postoperative oral glucose tolerance test; GTT -: no oral glucose tolerance test performed after surgery; TS result R: remission; TS result F: failure; RT: conventional radiotherapy; octr: octreotide; cab: cabergoline; GK: gamma knife radiosurgery; peg: pegvisomant *****: relapse at last follow-up*

12 of 21 patients (57%) without invasion achieved remission after TS (p=0.34). If the four patients in whom the intention of the TS was to debulk the adenoma were not taken into account the remission rate in patients with suspected invasion was 53%, indicating that in this study invasion did not significantly influence the chance to achieve remission (p=0.82).

Additional treatment and benefits of TS in patients with persistent acromegaly after TS.

Although remission was not achieved via TS in 20 patients, the maximal diameter of the adenoma was reduced from median 18 mm (range 10-45) on the preoperative MRI to median 7 mm (range 0-35) on the MRI performed four months after surgery. The adenoma was reduced in size in all cases, and in 6 cases no residual adenoma was visible on the postoperative MRI. In three patients (patients 11, 15 and 18) normal IGF-1 levels could be achieved with a dose of octreotide which was the same or even lower than the dose prescribed before the operation and which had been insufficient to suppress IGF-1 to normal levels before TS.

Figure 1 shows how the 20 patients with persisting acromegaly after TS were treated. A second TS was attempted in two patients but failed to result in cure. Of the eight patients receiving additional conventional radiotherapy none is presently in remission and all still receive medical treatment to control the acromegaly. Radiosurgery (-knife) was performed in 2 patients and resulted in remission in one of them. Of the remaining patients not cured by the TS, all patients except patient 32 and 33 were exclusively treated with medication. Patient 32 and 33 did not receive any further treatment. In patient 32 IGF-1 is only slightly elevated with no symptoms of active acromegaly, GH is suppressed to ‹ 2 mU/L after OGTT and mean GH values are below 6.5 mU/L. Patient 33 refused to be tested or treated further after TS, because the symptoms of acromegaly had disappeared.

Complications of TS and influence of TS on deficiencies of pituitary hormones.

Only mild complications occurred in our series. Fourteen patients developed a very mild transient diabetes insipidus (DI), for a maximum of 2 to 3 days. This was not regarded as a complication. Only patient (patient 33) had a more severe transient DI. Five patients, of whom two had had mild transient DI early after the operation, were treated with fluid restriction when they developed a mild hyponatremia due to inappropriate ADH secretion. Four patients had mild epistaxis, controlled with nasal tampons. Three patients had cerebrospinal fluid (CSF) leakage postoperatively. They were treated successfully with an external lumbar drain.

Fifteen patients (38%) already received substitution therapy for deficiency of one or more hormones before TS. After TS 33% of all patients receive long-term hormonal substitution. Twenty percent of the patients receive substitution with levothyroxine, 12.5% receive androgens, 12.5% receive glucocorticoids, 2.5% receive GH therapy and 2.5% receive desmopressin (Table 3).

Table 2) Comparison of baseline characteristics of the patients operated during the first 5 years and the patients operated during the second 5 years.

	First 5 years (n = 18)	Second 5 years (n = 22)	Significance
Gender (% male)	8 (44%)	11 (50%)	p = 0.76
Age (years)	46.2 (± 11.0)	48.4 (± 11.9)	p = 0.56
BMI (kg/m2)	29.8 (± 4.4)	26.6 (± 5.3)	p = 0.32
Preoperative medication	13 (72%)	20 (91%)	p = 0.12
Adenoma diameter (cm)	16.9 (± 7.8)	20.9 (10.4)	p = 0.18
Invasion on preoperative MRI	7 (39%)	12 (54%)	p = 0.32
Preoperative IGF-1 value (nmol/L)	93.3 (± 23.5)	116.6 (±36.9)	p = 0.03*

Data are expressed as means and standard deviations in case of continuous variables and as exact numbers and percentages in case of nominal or ordinal variables. BMI: Body mass index; IGF-1 : insulin-like growth factor-1; MRI: magnetic resonance imaging

Table 3) Influence of transsphenoidal surgery on substitution therapy in 40 patients with acromegaly caused by a macroadenoma.

Substitution therapy	Levothyroxine	Androgen	Glucocorticoid	GH	Desmopressin	Total
Discontinued after TS	5	5	1			11
Started after TS	4	2	2	1	1	10
Continued after TS	4	3	3			10

TS: transsphenoidal surgery, GH: growth hormone.

Discussion

In this study we report on the results of endoscopic TS in 40 consecutive patients with acromegaly and a pituitary macroadenoma on preoperative MRI, operated on in our hospital between 1998 and 2007. Although some results of endoscopic TS in small numbers of patients with acromegaly have been mentioned in large series of patients with different pituitary tumours [6,11,26,42], no series focusing on results of endoscopic TS in patients with acromegaly have yet been published. All previous published series on results of TS in patients with GH-secreting macroadenomas used the conventional technique of TS.

Remission rates after conventional TS reported in these larger series of patients with GH-secreting macroadenomas (including giant macroadenoma) vary widely, from 15 to 71% (Table 4) [1,2,4,15,18,23,29,34,40,41,43,45]. The overall remission rate of 50% in this study is in concordance with these results. However, not all series have used the same criteria to describe remission. The studies which used the criteria for remission formulated by Giustina et al. in 2000 [19,20], as we did in our series, reported remission rates in patients with macroadenomas of maximally 50%. Therefore the remission rate of 50% reported in our series is comparable to the best previously published remission rates achieved in patients operated on by the conventional microscopic method of TS.

However, the remission rate of 63% (or 77% if the patients who underwent debulking are excluded) we achieved in the last 5 years, compared to a remission rate of 33% in the first 5 years is very promising for the future. The characteristics of patients operated in the first 5 years and second 5 years were comparable (Table 2).The only significant difference was that the patients operated upon in the second 5 years had a significantly higher IGF-1 level. Therefore we believe that the higher remission rate achieved in the last 5 years is not biased by patients that were easier to operate on. So, it is more likely that the large difference between the remission rate achieved in the first 5 years and the second 5 years after introduction of the endoscopic technique of TS can be explained by the increasing experience of the two neurosurgeons who performed all endoscopic TS in our hospital. Strong evidence exists indicating that success rates of microscopic TS critically depend on the skills and experience of the neurosurgeon [3,15,18]. Our data indicate that this is no different for endoscopic TS. This argues in favour of concentrating endoscopic TS for acromegaly in a limited number of experienced centres.

Previously published series on conventional microscopic TS in patients with acromegaly and a macroadenoma found that the chance of remission after TS could be predicted

by the suspected invasiveness of the macroadenoma on the preoperative MRI scan [2,4,18]. However, in this study, although we observed a non-significant trend towards a lower chance of successful TS if tumour invasion was suspected, remission was still achieved in 42% of patients with suspected invasion. This may be explained by the fact that the endoscopic technique enables the use of different angles to operate, making it possible to effectively reach suprasellar and parasellar portions of the lesion [10,14]. If this is the case, the endoscopic technique might be preferable in case of invasive macroadenomas.

Table 4) Review of the criteria to define remission of acromegaly and remission percentages in macroadenomas reported in the most recently published series (1997-2005).

Author	N	Criteria of remission	Remission %
v Lindert (1997)	40	GH<2 ng/ml after OGTT, IGF1 N	55
Abosch (1998)	254	basal GH<5 ng/ml	71
Swearingen (1998)	129	GH<2 ng/ml after OGTT or IGF1 N or basal GH<2.5ng/ml	48
Gittoes (1999)	45	GH<2 mU/l after OGTT or basal GH<5mU/l	51
Laws (2000)	51	GH<1 ng/ml after OGTT or IGF1 N or basal GH<2.5ng/ml	51
Kaltsas (2001)	50	basal GH<2.5 ng/ml, IGF1 N	26
Abe (2001)	126	basal GH<2.5 ng/ml, IGF1 N	68
Shimon (2001)	44	GH<2 ng/ml basal or after OGTT, IGF1 N	64
Beauregard (2003)	77	GH<1 ng/ml after OGTT or IGF1 N or basal GH<2.5ng/ml	49
Trepp (2005)	64	GH<1 ng/ml after OGTT or IGF1 N or basal GH<2.5ng/ml	39
Erturk (2005)	19	GH<2 ng/ml basal or after OGTT	15
Nomikos (2005)	364	GH<1 ng/ml after OGTT or IGF1 N or GH<2.5ng/ml	50

N: number of patients included; GH: Growth hormone; OGTT: oral glucose tolerance test; IGF1: insulin-like growth factor type 1.

Due to the good results that have been achieved by medical therapy in patients with acromegaly and the relatively low remission rates after TS for patients with a GH secreting macroadenoma, some authors have recommended medical therapy as a primary treatment option instead of TS for patients with a GH secreting macroadenoma not causing mass effects [13,25,39]. Nowadays long-acting somastatin analogues (SA) have the potential to normalize IGF-1 levels in two thirds of patients, additionally controlling tumour size [17]. The more recently developed GH receptor antagonist pegvisomant can normalise IGF-1 in up to 97% of patients [44]. Furthermore, studies on combination therapies with SA and pegvisomant or SA and dopamine agonists have shown that combination therapy may be successful when monotherapy has failed [16,33,38]. Although medical treatment can result in long-term remission, it cannot cure acromegaly. Moreover, pegvisomant, which is effective by preventing GH action in the target tissues (organs), lacks a direct effect on the tumour to control long-term tumour growth. This might limit its use as primary therapy for patients with macroadenomas, until more long-term data on safety are available. Last but not least, lifelong use of expensive medication is required with the risk of serious side effects.

Studies have shown that surgical debulking can improve control of acromegaly by SA [12,24,35]. So even if a patient cannot be cured by TS, TS should still be considered, especially if acromegaly cannot be controlled by SA before TS. In this study TS reduced the size of the adenoma in all patients who were not cured by TS and improved the response to SA treatment in at least 5 of these patients. Unfortunately, a preoperative IGF-1 value during SA therapy was not available in all patients, so possibly more patients benefited from the TS to control their acromegaly.

Preoperative treatment with SA has been associated with improved results of TS, especially in macroadenomas [1,5,9,30]. This could possibly be explained by adenoma shrinkage or change in consistency of the adenoma [9]. However, most published studies have limitations. They are retrospective, have poor remission rates or small numbers of patients. Furthermore, other studies have not confirmed this positive effect [27,37]. A negative effect of preoperative treatment with SA on the outcome of TS results, however, has never been found. Therefore and because pretreatment with SA improves metabolic control, we prescribed preoperative therapy with octreotide in all but 6 patients. Of these 6 patients, none achieved remission after TS. However, because of the small number of patients who did not receive preoperative treatment and the retrospective character of this study it was not possible to evaluate whether preoperative treatment had an effect on the results of TS.

Thirteen patients in our study had a perioperative complication. All complications were mild and no serious complications occurred. This is in concordance with the incidence of complications associated with TS via the microscopic technique [1,2,4,15,18,23,29,34,40,41,43,45]. However, the endoscopic technique is probably more comfortable for the patients as the nose septum is almost left intact and usually no nasal packing is required after surgery. Besides the four patients with mild epistaxis, no rhinologic/local complications occurred, which seems to be less than those reported with the conventional technique. However, most patients that are operated upon via the microscopic technique do not need nasal packaging, but receive it because of a longstanding surgical habit.
In this series the number of hormonal deficiencies caused by TS was equal to the number of deficiencies cured by TS. All patients had a macroadenoma, which frequently causes a hormonal deficiency by itself before surgery. If the adenoma is selectively removed, normal pituitary function can potentially be restored [45]. Therefore in macroadenomas, the fear of creating new hormonal deficiencies should probably not be a reason to restrain from TS.

Conclusion

Endoscopic TS is a treatment which should be considered as a primary therapeutic option for patients with a GH secreting macroadenoma. In this series of patients operated on by experienced surgeons, it resulted in a remission rate of at least 50%, with only mild complications. The relatively high remission rate of 63% (or 77% excluding the patients who underwent primary debulking) we achieved in the last 5 years indicates that operation results can improve further if experience is gained. Because the endoscopic technique enables the surgeon to use different angles this technique can potentially improve the outcome of TS in macroadenomas, especially in patients with invasive macroadenomas. However, a randomized clinical trial, comparing endoscopic and conventional TS in patients with a GH secreting macroadenoma, is needed to determine the exact pros and cons of both techniques.

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Endoscopic transsphenoidal pituitary surgery:

a good and safe primary treatment option for Cushing's disease, even in case of macroadenomas or invasive adenomas

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Abstract

Context: Although the endoscopic technique of transsphenoidal pituitary surgery (TS) has been widely adopted, reports on its results in Cushing's disease (CD) are still scarce and no studies have investigated long-term recurrence rates. This is the largest endoscopic series published until now.

Objective: To gain insight in the role of endoscopic TS as a primary treatment option for CD, especially in patients with MRI-negative CD and (invasive) macroadenomas.

Design: retrospective cohort study.

Patients and methods: The medical records of 86 patients with CD who underwent endoscopic TS were examined. Data on preoperative and postoperative evaluation, perioperative complications and follow-up were collected. Remission was defined as disappearance of clinical symptoms with a fasting plasma cortisol level ≤ 50 nmol/l either basal or after 1 mg dexamethasone.

Results: The remission rate in different adenoma subclasses varied significantly: 60% in MRI-negative CD (n=20), 83% in microadenomas (n=35), 94% in non-invasive macroadenomas (n=16) and 40% in macroadenomas that invaded the cavernous sinus (n=15). The rate of recurrence was 16% after 71 ± 39 months follow-up (mean ± SD, range 10-165).

Conclusions: Endoscopic TS is a safe and effective treatment for all patients with CD. Recurrence rates after endoscopic TS are comparable to those published for microscopic TS. Our data suggest that in patients with non-invasive and invasive macroadenomas, the endoscopic technique of TS should be the technique of choice, as remission rates seem to be higher than remission rates reported for microscopic TS, although no comparative study has been performed.

Introduction

Persisting Cushing's disease (CD) after unsuccessful treatment is associated with a four- to five fold increased standardized mortality ratio and substantial morbidity [1-3]. Successful treatment of CD is therefore crucial. Since Hardy introduced the operating microscope to selectively remove adrenocorticotropic hormone (ACTH) secreting microadenomas via the transsphenoidal route in 1963, transsphenoidal surgery (TS) has become the treatment of choice for CD [4]. Over the years a large number of reports on the results of microscopic TS in patients with CD have been published. These reports show that, in experienced hands, excellent remission rates of over 80% can be achieved if a microadenoma is visualized on preoperative imaging [5-16]. However, if no adenoma is visualized on preoperative imaging (Magnetic Resonance Imaging (MRI) negative CD), or if a macroadenoma is the cause of CD, remission rates are substantially lower [5-10, 13-16]. Furthermore CD relapses in up to 25% of the patients. Therefore it has been suggested that TS is the optimal primary treatment for only a subset of patients with CD [17].

In the 1990s the purely endoscopic technique of TS has been introduced as an alternative to the conventional microscopic technique. The endoscopic technique offers a panoramic view with increased illumination of the operating field. Furthermore different angles can be used making it possible to reach the supra- and parasellar regions. Due to these advantages the endoscopic technique has been widely adopted [18-22]. Compared to historical microscopic series the endoscopic technique seems to result in improved outcome rates in macroadenomas [18-22]. However, reports on the results of endoscopic TS in CD are still scarce and in the studies that have been published (including our own report on the results in the first 35 patients) all patients have a short follow-up period so no statements about recurrences can be made [23-25]. Furthermore it is still unclear if the endoscopic technique has advantages or disadvantages in patients with very small microadenomas (<5 mm), which occur frequently in patients with CD [26, 27]. Therefore some neurosurgeons are reluctant to use this technique in patients with CD [27].

To gain more insight in the role of endoscopic TS as a primary treatment option for patients with CD, especially in patients with MRI-negative CD or (invasive) macroadenomas, we evaluated the results of endoscopic TS in the first 86 patients treated in our centre. Furthermore we evaluated the rates of recurrence after initial remission.

Patients and methods

Patients

From 1998 onwards all TS in our hospital were performed endoscopically. All patients who underwent endoscopic TS as primary treatment for CD between January 1998 and December 2011 (n=86) were identified for this retrospective cohort study. The medical records of these 86 consecutive patients were examined. Data on preoperative and early postoperative evaluation, perioperative complications and follow-up were collected according to the guidelines of our local medical ethical committee. Seventy-two percent of all patients were female. At the time of TS the age of the patients was 42.3 ± 14.9 years (mean ± SD) and body mass index (BMI) was 30.5 ± 7.2 kg/m^2. The initial results of the first 35 TS have been published previously [23].

Diagnostic evaluation

At presentation all patients had symptoms of active hypercortisolism. The clinical diagnosis of hypercortisolism was confirmed by standard biochemical investigations (elevated 24-h urinary free cortisol (UFC) excretion, loss of diurnal variation of plasma and/or salivary cortisol levels and failure to suppress plasma cortisol after 1 mg dexamethasone overnight) [28]. ACTH dependency was confirmed by normal or elevated ACTH values. The diagnosis of pituitary-dependent CD was established as follows: pituitary imaging by MRI scanning with intravenous contrast (gadolinium) was performed in all patients. If no adenoma was seen on the MRI scan (MRI-negative CD, n=20) or if the adenoma was < 6 mm (n=21) inferior petrosal sinus sampling (IPSS) was performed (n=22) or (in case IPSS could not be performed because of technical reasons, comorbidity or refusal by the patient) a corticotrophin-releasing hormone (CRH) test (100 µg human CRH i.v.) and a high dose dexamethasone suppression test (DST) (7 mg dexamethasone in 7 h i.v.) were performed (n=19).

Perioperative treatment

Eighty-seven percent of all patients received cortisol lowering medication (ketoconazole in 48 patients, metyrapone in 22 patients and a combination of these drugs in 8 patients) before TS for 3.6 ± 1.8 months. One hour before surgery, administration of glucocorticoids (prednisolone, 25 mg i.v. every 8 h) was started and after surgery the dose was tapered rapidly.

Surgical procedures

The endoscopic technique of TS was introduced in our hospital in 1994 and was first used for CD in 1998 (n=2). Thereafter an increasing number of patients with CD were operated endoscopically, reaching an average of 8 patients per year from 2003 onwards. The technique of endoscopic TS has been described before [22, 23, 29]. In short: a binostril, transsphenoidal, endoscopic approach to the sella turcica during which the endoscope is handheld was used. Until 2010 endoscopic TS was exclusively performed by two neurosurgeons and thereafter by three neurosurgeons.

A selective adenomectomy was performed if an adenoma could be localized during TS (n=76). In 5 patients no adenoma could be localized peroperatively and a hemihypophysectomy was performed (based on the results of IPSS). In one patient the whole pituitary gland seemed abnormal and a resection of the total anterior pituitary gland was performed. In 4 patients only debulking of the adenoma was performed, because it was clear that it was impossible to remove the total adenoma based on preoperative imaging. However, these patients required an operation because of local mass effects of the adenoma.

All material removed during TS was examined histopathologically and immunohistochemical staining for pituitary hormones was performed to confirm the diagnosis of ACTH-secreting pituitary adenoma.

A complication of TS was defined as any event occurring during or in the month after TS that required treatment. As intraoperative cerebrospinal fluid (CSF) leakage, which occurred in eleven patients and was closed with a fat graft peroperatively, is inherent to the surgical procedure this was not regarded as a complication.

Postoperative evaluation

On the fourth day postoperatively biochemical evaluation was carried out (after glucocorticoid substitution had been stopped for at least 24 hours), by measurement of fasting (08:00 h) plasma cortisol, ACTH, thyrotropin, free thyroxine, gonadotropins, testosterone or estradiol and insulin-like growth factor type 1.

If basal plasma cortisol was lower than 200 nmol/l substitution therapy with hydrocortisone, 30 mg a day, was prescribed. Patients were reevaluated every 2–4 weeks during the first 3 months after TS and thereafter at two- to three months intervals during the first year. The fasting plasma cortisol concentration was measured at each visit. In addition, a 1 mg overnight DST was carried out 1 and 3 months postoperatively and there-

after in patients who were in remission, once a year. If a patient received glucocorticoid substitution therapy postoperatively, the dose was reduced and stopped, if possible, between 3 and 12 months after TS. Thereafter the integrity of the hypothalamic–pituitary–adrenal axis was assessed by an insulin tolerance test (ITT). The thyrotrophic, gonadotrophic and somatotrophic axes were checked regularly. If growth hormone deficiency was suspected a growth hormone (GH) stimulation test was performed (preferably an ITT, but in case of contraindications a GHRH-Arginine test).

Additional procedures for this study

All preoperative MRI scans were reviewed by one neurosurgeon (H.B.). The diameter of the adenoma was measured in 3 orthogonal planes. The volumes of the adenomas were estimated using the following formula: $4/3\pi$ (a/2 • b/2 • c/2) (30). Furthermore the adenomas were divided into four groups, based on the modified Hardy criteria [31]: 1) MRI-negative CD (23.3% of all patients), 2) microadenomas without cavernous sinus invasion (< 1 cm, 40.7% of all patients), 3) macroadenomas with or without suprasellar extension but no cavernous sinus invasion (noninvasive macroadenomas, ≥ 1cm, 18.6% of all patients) and 4) macroadenomas with invasion in the cavernous sinus (invasive macroadenomas, 17.4% of all patients). Invasion in the cavernous sinus was subclassified according to the KNOSP classification [32].

In all patients who were assumed to be in remission, a new 1 mg DST was performed to exclude a mild recurrence. Data on postoperative substitution therapy were collected at the last follow-up visit for the patients who were in remission. For patients with persistent or recurrent disease data on postoperative substitution therapy were collected at the last visit before additional therapy was initiated.

Criteria of remission and recurrence

Remission was defined as disappearance of clinical symptoms of hypercortisolism with basal plasma cortisol level ≤ 50 nmol/l after glucocorticoid withdrawal for 24–48 h and/or suppression of plasma cortisol level ≤ 50 nmol/l after a 1 mg overnight DST within the first 3 months after surgery [28, 33].

Recurrence after initial remission was defined as inadequate suppression of plasma cortisol levels after a 1 mg overnight DST (> 50 nmol/l) in combination with elevated midnight salivary cortisol levels and/or elevated 24-h UFC levels.

Statistics

Data are presented as mean ± standard deviation and range for continuous variables, and as frequency for categorical variables. Categorical variables were analyzed with Chi-square tests or Fisher's exact tests. Continuous variables were analyzed with unpaired Mann-Whitney U tests. A stepwise forward logistic regression analysis was performed to determine possible independent predictors of remission using the following variables: gender, age, BMI, mean preoperative cortisol levels, treatment with cortisol lowering agents, adenoma volume on preoperative MRI, adenoma classification and operation date (as a surrogate marker for the experience of the neurosurgeons). Kaplan–Meier analysis was used to estimate the probability of recurrence-free survival for the patients who were initially in remission after TS (both for the total group as separately for the different adenoma classes). Statistical significance was defined as $P \leq 0.05$ (two sided). Data were analyzed using SPSS 20.0.

Results

Remission and recurrence rates after endoscopic TS

The results of endoscopic TS in the 86 patients with CD are shown in Figure 1. The remission rates between the adenoma classes were significantly different (Figure 2, $p<0.01$). Remission was achieved in 12 of 20 patients with MRI-negative CD (60%), 29 of 35 patients with a microadenoma (83%), 15 of 16 patients with a noninvasive macroadenoma (94%) and 6 of 15 patients with an invasive macroadenoma (40%). In all patients with invasive macroadenomas the invasion of the cavernous sinus was classified as a KNOSP grade of ≥ 2. Remission was achieved in 2 of the 4 patients with KNOSP grade 2 invasion, 3 of the 8 patients with KNOSP grade 3 invasion and one of the 3 patients with KNOSP grade 4 invasion. Two patients with KNOSP grade 3 invasion developed a relapse after initial remission.

The proportion of the different adenoma classes varied significantly over the years, with a higher percentage of patients with MRI-negative CD in the first 5 years and a higher percentage of invasive macroadenomas in the last 5 years ($p<0.01$). As investigated with a stepwise logistic regression analysis, adenoma classification was the only preoperative variable that significantly influenced the chance of remission ($p<0.01$).

Evidence of an ACTH-producing pituitary adenoma was found during immunohistochemical investigation in 79% if the patients were in remission of CD after TS compared to 54% of the patients with persistent CD ($p=0.02$).

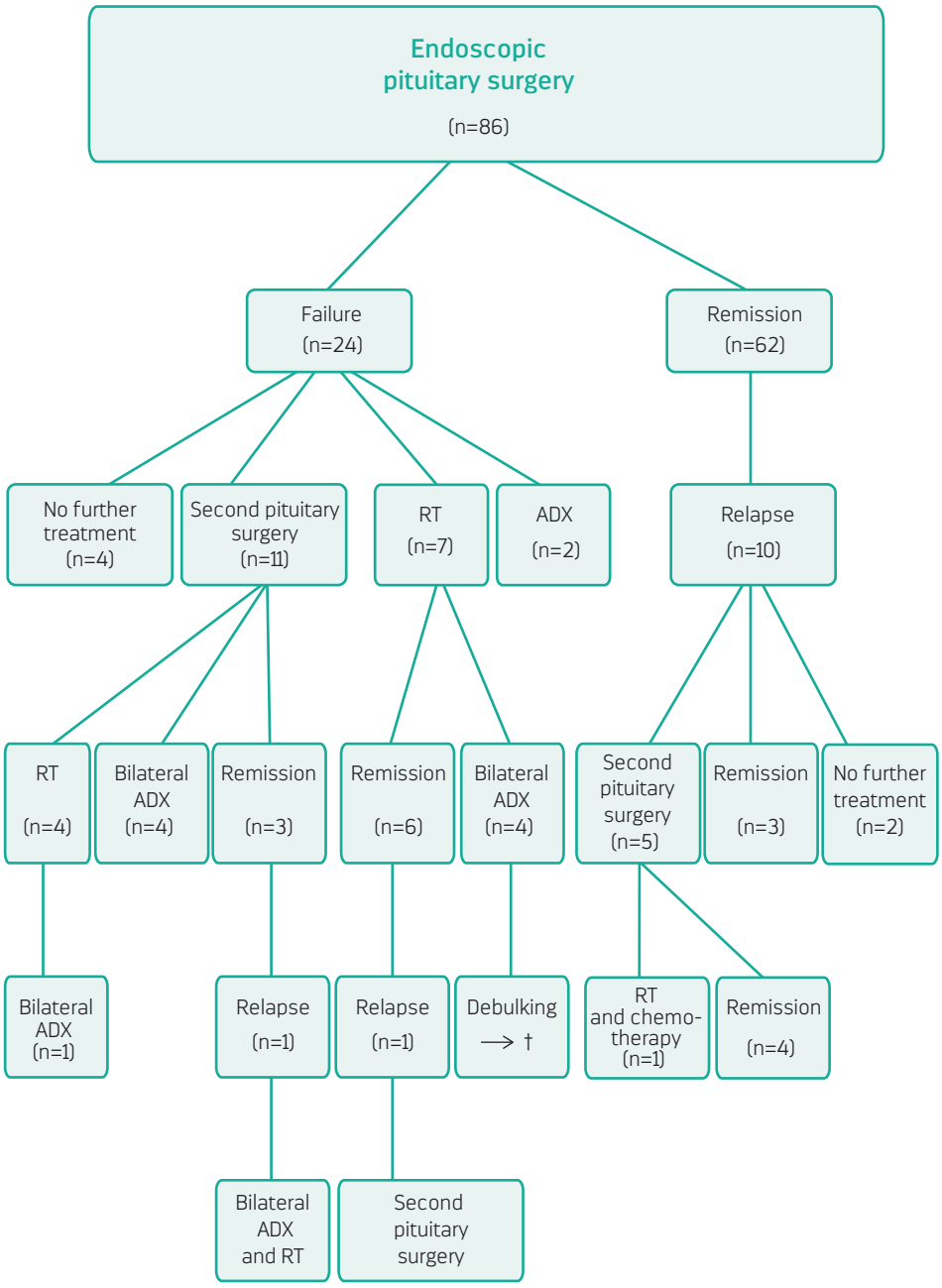

Figure 1) Results of endoscopic pituitary surgery in 86 patients with Cushing's disease (1998-2011). *ADX: adrenalectomy; RT: radiation therapy; †: deceased.*

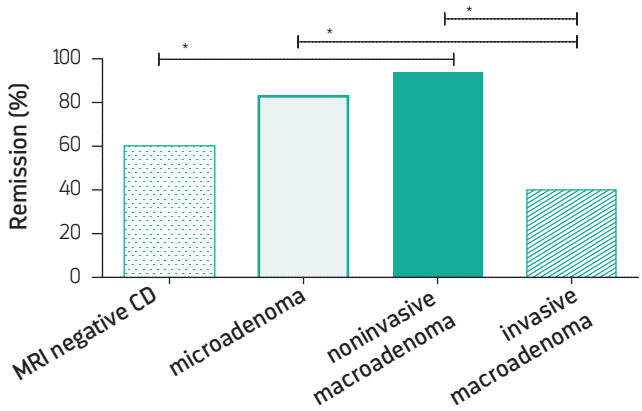

Figure 2) Overview of the remission rates of Cushing's syndrome after endoscopic pituitary surgery for the different adenoma classes.

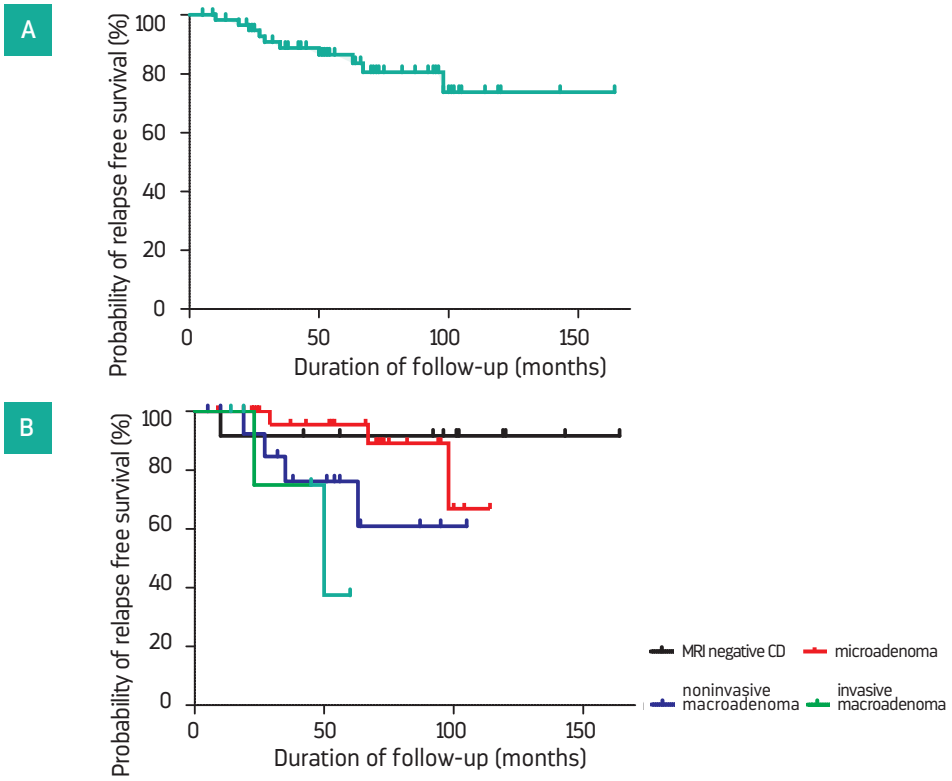

Figure 3) Kaplan Meier survival analysis demonstrating recurrence-free survival of Cushing's disease after endoscopic pituitary surgery for A) the total patient group and B) the different adenoma classes.

Table 1) Influence of transsphenoidal surgery on hormonal substitution therapy in 86 patients with Cushing's disease.

Substitution therapy	Levothyroxine	Androgen	Glucocorticoid	GH	Total
Discontinued after TS	5	3	0	0	8
Continued after TS	6	2	0	0	8
Started after TS	13	1	12	5	31

TS: transsphenoidal surgery, GH: growth hormone.

The follow-up period of the total patient group was 71 ± 39 months (5-164). After 42 ± 27 months (10-98) CD recurred in 10 of the 62 patients who were initially in remission (16%). The probability of recurrence-free survival, as estimated with a Kaplan-Meier analysis, was 98% one year after successful primary TS, 84% after 5 years and 73% after 8 or more years (Figure 3a). The chance of recurrence significantly increased with a larger adenoma classification: probability of recurrence-free survival after successful primary TS was 91.7% after a mean of 100 months in the MRI-negative CD group, 66.8% after 45 months in the microadenoma group, 60.9% after 43 months in the noninvasive macroadenoma group and 37.5% after 35 months in the invasive macroadenoma group (p=0.04, Figure 3b).

Complications of endoscopic TS and influence on pituitary hormone secretion

In this series all complications of endoscopic TS were relatively mild and did not cause any permanent damage. The most severe complication, which occurred in one patient, was postoperative bleeding originating from the sphenopalatine artery. This directly required reoperation. One patient with persistent CD after TS developed a pulmonary embolism two weeks after surgery. Ten patients (11.6% of all patients) had mild epistaxis controlled with nasal tampons. Four patients (4.6%) had postoperative CSF leakage, which was successfully treated with an external lumbar drain (ELD). Four patients (4.6%) developed transient diabetes insipidus (polyuria for more than two days and less than 6 months requiring desmopressin substitution), which resolved spontaneously in the first few weeks after surgery. Ten patients (11.6%) developed transient hyponatremia of < 130 mmol/L postoperatively, caused by inappropriate ADH secretion and/or (relative) glucocorticoid deficiency. Three patients (3.4%) had an infection postoperatively and were treated with antibiotic therapy: 2 patients because of a local urinary tract infection and one patient because of a local infection at the entry point of the ELD.

Table 2) Remission and recurrence rates of Cushing's disease in micro- and macroadenomas after primary transsphenoidal pituitary surgery: a selection† of recently published single centre series. *(page 71-72)*

Author	N	Remission criteria	Remission percentage	Recurrence %
Microscopic series				
Swearingen (1999) [16]	154	• Basal cortisol <138 nmol/L and • UFC < 55 nmol/L	Overall 87% *Microadenomas (n=137) 90%* *Macroadenomas (n=17) 65%*	7% follow-up mean 8.7 y
Rees (2002) [13]	54	• Basal cortisol < 50 nmol/L	Overall 70% *MRI-negative CD (n=16) 69%* *Microadenomas (n=23) 100%* *Macroadenomas (n=10) 40%*	5% follow-up mean 7 y
Shimon (2002) [15]	77	• Normal UFC and • Cortisol < 138 nmol/L after 48h DST	Overall 78% *MRI-negative CD (n=10) 80%* *Microadenomas (n=42) 79%* *Macroadenomas (n=3) 33%*	5% follow-up mean 4.2 y
Yap (2002) [34]	97	• Basal cortisol < 50 nmol/L	Overall 68,5% *MRI-negative CD n=34* *Microadenomas n=45* *Macroadenomas n=10*	11.5% follow-up mean 7.6 y
Chen (2003) [6]	174	• Cortisol <83nmol/L after a 1mg DST	Overall 79% *Microadenomas (n=133) 92%* *Macroadenomas (29) 17%*	6.5% follow-up mean 5 y
Hammer (2004) [9]	200*	• Basal cortisol < 140nmol/L or • Cortisol <140 nmol/L after a 1mg DST	Overall 85% *Microadenomas (n=140) 86%* *Macroadenomas (n=52) 83%* *Invasive adenomas (n=8) 63%*	9% follow-up median 11.1 y
Esposito (2006) [8]	39*	• Basal cortisol < 140 nmol/L	Overall 79% *MRI-negative CD (n=8) 50%* *Microadenomas (n=23) 91%* *Macroadenomas (n=8) 75%*	6% follow-up mean 2.8 y
Rollin (2007) [14]	108	• Glucocorticoid dependence and • Cortisol <82,8 nmol/L after a 1mg DST	Overall 85% *MRI-negative CD (n=21) 71%* *Microadenomas (n=59) 95%* *Macroadenomas (n=23) 74%*	7% follow-up mean 6 y

Author	N	Remission criteria	Remission percentage	Recurrence %
Fomekong (2009) [35]	40	• Normal UFC or • Glucocorticoid dependence	Overall 65% *MRI-negative CD (n=3) 0%* *Microadenomas (n=25) 64%* *Macroadenomas (n=12) 83%*	11.5% follow-up mean 7 y
Alwani (2010)[5]	79	• Normal UFC and • Cortisol <50 nmol/L after a 1mg DST	Overall 65% *MRI-negative CD (n=14) 57%* *Microadenomas (n=44) 77%* *Macroadenomas (n=21) 43%*	21% follow-up median 7 y
Ciric (2012)[7]	136	• Basal cortisol < 138nmol/L	Overall 85% *Microadenomas (n=123) 90%* *Macroadenomas (n=13) 31%*	9.7% follow-up mean 5.7 y
Honegger (2012)[10]	83	• Normal UFC and • Cortisol <55 nmol/L after a 1mg DST	Overall 84% MRI-negative CD (n=20) 77% *Microadenomas (n=46) 93%* *Macroadenomas (n=5) 100%* *Invasive macroad. (n=7) 43%*	7% follow-up mean 3.2 y
Endoscopic series				
Dehdashti (2007)[25]	25	• Basal cortisol < 100 nmol/L and • Suppression of cortisol after 1mg DST and • Normal UFC	Overall 80% MRI-negative CD *(n=5) 60%* *Microadenomas (n=13) 100%* *Macroadenomas (n=5) 80%* *Invasive macroad. (n=2) 0%*	0% follow-up median 1.4 y
Starke (2012)[24]	62	• Basal cortisol < 138 nmol/L or • Decreased UFC	Overall 95% MRI-negative CD *(n=16) 100%* *Microadenomas (n=30) 97%* *Macroadenomas (n=15) 87%*	10% follow-up mean 2.3 y
This series	86	• Basal cortisol < 50 nmol/L or • Cortisol, 50 nmol/L after 1mg DST	Overall remission 72% MRI-negative CD *(n=20) 60%* *Microadenomas (n=35) 83%* *Macroadenomas (n=16) 94%* *Invasive macroad. (n=15) 40%*	16% follow-up mean 5.6 y

†: *Only single centre studies with clear criteria for remission and relapse and in which remission rates were mentioned or could be calculated for different adenoma subclasses were included in this literature overview. N: number of patients;* *: *for a few patients the operation was the second operation; DST: dexamethasone suppression test; UFC: 24 hour urinary free cortisol; y: years; MRI: magnetic resonance imaging scan; CD: Cushing's disease.*

Thirteen patients (15%) already received substitution therapy for deficiencies of one or more pituitary hormones before surgery. After TS, six patients who received hormonal substitution before TS did not need any hormonal substitution anymore and 22 patients started with substitution therapy. So at last follow-up (or at the follow-up visit before additional treatment was initiated) 35% of all patients received one or more hormonal substitution therapies. Twenty-two percent of all patients received substitution with levothyroxine, 14% received long-term glucocorticoid substitution (for a minimum duration of 2 years), 6% received GH and 3.5% received androgens (Table 1).

Additional treatment for persistent and recurrent CD

Figure 1 shows how the patients with persistent and recurrent CD were treated. Four of the 24 patients with persistent CD did not receive any additional treatment. The persisting CD is subclinical in three of these patients and the fourth patient refused further treatment. Eleven patients with persistent CD were treated with repeat endoscopic TS which was only successful in 3 patients. Seven patients with persistent CD were treated with radiation therapy (RT), which was successful in 6 patients. In the seventh the pituitary tumor turned out to be a pituitary carcinoma. The patient died of metastatic disease. Two patients with persistent CD were treated with a bilateral ADX.

Five of the ten patients with recurrent CD were treated with a second endoscopic TS which was successful in 4 patients. In the fifth patient the intention of the repeat TS was to debulk the adenoma which had grown very rapidly in a short period of time and also turned out to be a pituitary carcinoma. Three patients with recurrent CD (30%) were treated with radiation therapy and two patients have not yet received additional treatment.

Discussion

This study reports on the results of endoscopic TS in 86 patients with CD treated at the Radboud University Nijmegen Medical Centre between 1998 and 2011. To our knowledge this is the largest series on the results of endoscopic TS in CD published till now, with the longest follow-up time. In addition, in contrast to most previously published series on results of both endoscopic and microscopic TS, we included a relatively large number of patients with macroadenomas, including invasive macroadenomas.

The most important finding of our study is the high remission rate of 94% achieved in patients with noninvasive macroadenomas. This compares favourably to previously published remission rates after microscopic TS in patients with ACTH secreting macroad-

enomas which are on average 60%, despite the fact that most studies used less strict criteria for remission than we used (Table 2) [5, 7-10, 13-16, 34-36]. In line with our results, Starke et al. recently reported a high remission rate of 87% in patients with ACTH secreting macroadenomas (n=15, of which 6 were invasive) achieved with endoscopic TS. Furthermore, the remission rates after endoscopic TS also seem to be higher in patients with a GH secreting or a non-functioning macroadenoma [18-22]. We therefore believe that the endoscopic technique of TS should be the treatment of choice in patients with an ACTH secreting macroadenoma.

It is of interest that in the previously published series on microscopic TS only a few patients with ACTH secreting macroadenomas that invaded the cavernous sinus were included, probably because it was believed to be virtually impossible to achieve remission in these patients. Remission was nevertheless achieved in 40% of the patients with an invasive macroadenoma in the current series, of whom one patient even had a total encasement of the intracavernous carotid artery (KNOSP grade 4). This relatively good result can be explained by the fact that compared to the microscopic technique of TS, the endoscopic technique enables the use of different operating angles which makes it possible to effectively reach suprasellar and parasellar portions of the lesion, including the cavernous sinus [37]. In our opinion invasion of the adenoma in the cavernous sinus should therefore not be a reason to refrain from surgery, especially because at present TS remains the only treatment that potentially restores normal physiology and all other treatment options for CD have major disadvantages.

In patients with an intrasellar ACTH secreting microadenoma we achieved a remission rate of 83% which is in concordance with remission rates previously reported in both conventional microscopic and endoscopic series (Table 2). Thus in patients with a microadenoma the technique of TS is probably equivocal, as long as the TS is performed by an experienced neurosurgeon. Furthermore, the number and type of complications, the complication rates and the rates of postoperative hormonal deficiencies that are reported are similar with both techniques [18, 19]. The preference of the neurosurgeon and/or the patient should therefore determine the technique of TS that is used in patients with a microadenoma.

In MRI-negative CD the remission rate we achieved was 60%, which is significantly lower than the remission rate achieved in microadenomas that could be visualized on preoperative MRI. This is in concordance with most conventional microscopic series, that also report lower remission rates in MRI-negative CD (Table 2). Interestingly Starke et al achieved remission in 100% of the patients with MRI-negative CD with the endoscopic

technique [21]. More data are needed in order to determine the real value of endoscopic TS in patients with MRI-negative CD.

This is the first study that reports on long-term recurrence rates after endoscopic TS in CD. We found that, after a mean of 5.6 years of follow-up, 16% of the patients who were initially in remission after endoscopic TS developed a recurrence. All patients were actively investigated for recurrence and a strict definition of recurrence was used in order to obtain a good insight in actual recurrence rates. There are only few previously published microscopic series that carefully analyzed patients for recurrence [3, 5, 38, 39] and these series also report relatively high recurrence rates varying between 13 and 21%. Therefore recurrence rates of CD after endoscopic TS seem to be comparable to recurrence rates after microscopic TS. Because most recurrences occur in the first 5 years after TS and more recurrences occur in larger/invasive adenomas (Figure 3), the cause of a recurrence is most likely a small adenoma remnant left behind during TS.

We previously reported that endoscopic TS seems to be a good therapeutic option for recurrent or persistent CD, after analyzing the outcome in 14 patients [29]. At the time we did not separately analyze the results of persistent and recurrent CD, but remission was achieved in 7 out of 8 patients with recurrent CD and 3 out of 6 patients with persistent CD. This study confirms that repeated endoscopic TS seems to a good therapeutic option for patients with recurrent CD with remission achieved in 4 out of 5 patients. However, repeated endoscopic TS seems to be of less value for patients with persistent CD as remission was only achieved in 3 out of 11 patients.

A limitation of this study is that, although the total patient group is relatively large, the number of patients in the different adenoma subclasses is relatively small. Another limitation of this study is its retrospective nature. Ideally a comparison between the microscopic and endoscopic technique of TS in CD would be made via a randomized controlled trial, with one or two neurosurgeons performing both operations. However, because CD is a rare disease, such a trial is very difficult to organize. Furthermore only a few neurosurgeons have extensive experience with both techniques of TS. Thus the only way that the results of the microscopic and endoscopic technique of TS in CD can currently be compared in a large number of patients is via a meta-analysis. However, at present remission rates between different studies are difficult to compare as remission criteria vary immensely between different studies (Table 2). It is therefore of the greatest importance that a good consensus statement is established about the definition of remission and recurrence of CD after TS for future research purposes.

In conclusion, endoscopic TS is a safe and effective treatment for all patients with CD. Recurrence rates after endoscopic TS are comparable to those published for microscopic TS. Our data suggest that in patients with non-invasive and invasive macroadenomas the endoscopic technique of TS should be the technique of choice as remission rates seem to be higher than remission rates reported for microscopic TS, although no formal comparative study has been performed. In MRI-negative CD or microadenomas the preference of the neurosurgeon and/or the patient should determine the technique of TS that is used, because remission and complication rates that are reported in microscopic TS and endoscopic TS are comparable.

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Repeated transsphenoidal pituitary surgery via the endoscopic technique:

a good therapeutic option for recurrent or persistent Cushing's disease

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Abstract

Background: No data on results of repeated transsphenoidal surgery via the endoscopic technique for patients with persistent or recurrent Cushing's disease are available.

Design and patients: We retrospectively evaluated the remission rates and complications of repeated transsphenoidal surgery via the endoscopic technique in 14 patients with persistent (N=6) or recurrent (N=8) Cushing's disease treated in our centre between 1999 and 2007.

Main Outcomes: Remission was defined as disappearance of symptoms of hypercortisolism with basal plasma cortisol level ≤ 50 nmol/L 24-48 hours after glucocorticoid withdrawal and/or suppression of plasma cortisol level ≤ 50 nmol/L after 1 mg dexamethasone overnight within the first 3 months after transsphenoidal surgery.

Results: With repeated endoscopic transsphenoidal surgery a remission rate of 10/14 (71%) was achieved. No patient had a relapse during a median follow-up of 24 months. Cerebrospinal fluid leakage was the most frequent complication (6 patients) and 11 patients required hormonal substitution after surgery. The success of repeated transsphenoidal surgery could not be predicted by visualisation of an adenoma on MRI before first or second surgery, histopathological confirmation of an ACTH secreting adenoma after first or second surgery, treatment with cortisol lowering agents before first or second surgery, the operation technique used during the first surgery, persistent versus recurrent disease after the first surgery, age, gender and interval between the two surgeries.

Conclusion: Repeated transsphenoidal surgery via the endoscopic technique is a good treatment option for selected patients with recurrent or persistent Cushing's disease following primary pituitary surgery.

Introduction

Cushing's disease (CD) is a potentially life threatening condition that requires aggressive treatment [1,2]. At present transsphenoidal pituitary surgery (TS) is the primary treatment of choice. Remission rates vary from 50 to 90%. However, CD persists in the remaining patients, and 5-25% of the patients who are initially in remission develop a recurrence [3-8]. Treatment options for persistent or recurrent CD include radiation therapy, bilateral adrenalectomy, medical therapy and repeated TS. Thus far, no consensus exists on which therapy is preferable [5,9,10].

Pituitary radiotherapy has been used to treat persistent or recurrent CD for several decades. Remission rates after conventional fractionated radiation therapy range from 56 to 84% [5,9]. However, its usefulness is limited by the delay between therapy and disease control and the 50% to 100% incidence of hypopituitarism several years after treatment. Additional complications include radiation necrosis, cerebral vasculopathy, damage to surrounding structures and the development of radiation-induced neoplasms. Stereotactic radiosurgery, which was introduced more recently, may lead to faster normalisation of hormone levels with a lower complication risk than conventional radiotherapy, but long-term follow-up is needed to determine its real value [5,9,11,12].

Bilateral adrenalectomy has a very high success rate of reversing hypercortisolism, ranging from 88% to 100%. Traditionally, open procedures of adrenalectomy were associated with considerable morbidity and mortality [13,14]. Nowadays the minimally invasive laparoscopic approach is preferred, which has reduced perioperative morbidity significantly [9,15]. However, adrenalectomised patients require lifelong glucocorticoid and mineralocorticoid replacement. The major concern is the development of Nelson's syndrome in 15 to 46% of the patients [5,16,17].

Medical therapy in CD mostly serves an adjunctive role after unsuccessful TS. At present only steroidogenesis inhibitors have been proven effective. Furthermore, discontinuation of medical treatment will invariably result in recurrence of CD and all available medicaments have significant side effects [18].

Repeated TS may be a good treatment option for persistent or recurrent CD. It has the potential to instantly cure CD, while adrenal and pituitary function may remain intact. However, few investigators have addressed the results of repeated TS and the reported risk of hypopituitarism and other complications is higher than after the first TS [19-26]. Furthermore all previous studies operated patients via the conventional microscopic

technique of TS. Cappabianca et. al. have suggested that the endoscopic technique of TS might be considered the procedure of choice in recurrent or residual pituitary adenomas, as the wider and direct visual control of the surgical field provides an advantage if the anatomy has been distorted by previous surgery [27]. No results on repeated TS via the endoscopic technique in CD have been published until now [27,28].

In order to gain insight into the role of repeated TS as a treatment option for persistent or recurrent CD, we evaluated the remission rates and complications of repeated TS in 14 patients, treated in our hospital between 1999 and 2007. All patients had surgery via the endoscopic technique.

Patients and methods

Patients

Between 1999 and 2007, 68 endoscopic TS were carried out for CD in our hospital. Fourteen of these operations were repeated TS, for persistent CD in 6 patients and recurrent CD in 8 patients. The medical records of these patients were retrospectively reviewed. There were 5 males and 9 females and age at time of the second surgery was 37.2 ± 10.3 years (Table 1).

Between 1999 and 2007 a total of 24 patients were treated for persistent or recurrent CD. Besides the fourteen patients that underwent an endoscopic repeated TS, 5 patients underwent conventional pituitary radiotherapy, 4 patients underwent stereotactic radiosurgery and 1 patient underwent a bilateral adrenalectomy. A multidisciplinary team of neurosurgeons, endocrinologists and a radiotherapist decided which therapy for persistent or recurrent CD was the optimal therapy for each individual patient, taking into account the invasiveness of the adenoma, findings at previous surgery, the physical condition of the patient and the patient's preference.

Diagnostic evaluation before the first and second TS

The initial diagnosis of hypercortisolism was based on clinical symptoms and biochemical tests, including 24-hour urinary free cortisol measurements, assessment of the plasma cortisol and ACTH circadian rhythm and an overnight low dose dexamethasone suppression test (1 mg at 23.00) [29]. A CRHtest (100 µg human CRH i.v.) [30]and a high-dose dexamethasone suppression test [31] were performed in most patients. In addition pituitary imaging by contrast enhanced magnetic resonance imaging (MRI) was performed in all patients, except in patient one, in whom contrast enhanced computed tomography

(CT) scanning was used to search for a pituitary adenoma. Eight patients underwent bilateral inferior petrosal sinus sampling with i.v. stimulation with 100 µg human CRH, which confirmed the pituitary origin of hypercortisolism in all 8 patients [32].

To document the persistence of hypercortisolism after TS or relapse of hypercortisolism in patients who were in remission for some time after TS, 24-hour urinary free cortisol was measured, an overnight 1 mg dexamethasone suppression test was performed, and midnight plasma cortisol was assessed. A new MRI scan was performed before the second TS in all patients.

Surgical procedures

All repeated pituitary operations in this series were performed via an endoscopic binostril endonasal transsphenoidal technique. This technique was introduced in our hospital in 1994, and first used for CD in 1997. From 1998 onward practically all TS were performed endoscopically. The surgeries were exclusively performed by two neurosurgeons. The technique is very similar to the technique Jho et al. and Cappabianca et al. have described previously [33-36]. However, a binostril transsphenoidal endoscopic approach to the sella turcica during which the endoscope is handheld was used.

For endoscopic transnasal TS, 0° and 30° rigid endoscopes with a lens diameter of 4 mm with a separate shaft were used, which allow easy and comfortable holding, while offering a suction-irrigation-system for cleaning the lens (Karl Storz GmbH, Tuttlingen, Germany). The instruments used are principally the same as with the microsurgical technique. The technique of repeated surgery is in no way different from primary surgery. In two recent cases electromagnetic neuronavigation was used for orientation and localization of a very small tumor.

In patients with recurrent CD a selective adenomectomy was performed if MRI identified the tumor. In case of negative MRI investigations a hemihypophysectomy was performed based on intraoperative findings. In patients with persistent CD after first TS, a total hypophysectomy was performed during repeated TS. Although this term suggests complete removal of all contents of the sella, it is never the intention to remove all pituitary tissue. Total hypophysectomy indicates the surgical exploration of the complete contents of the sella and removal of all anterior pituitary tissue that might harbor adenoma tissue. As soon as this goal seems to be achieved, further surgery is discontinued and thus pituitary tissue may be left in place.

Perioperative treatment

Cortisol lowering agents (metyrapone and/or ketoconazole) were given for a period of 4 months preoperatively in 9 patients before the first TS and in 7 patients before the second TS.

Administration of glucocorticoids i.v. (prednisolone, 25 mg every 8 h) was started one hour before surgery. After two days glucocorticoids were given orally and the dose was tapered rapidly. There was an interval of at least 48 hours between the last dose of glucocorticoids and the first postoperative measurement of fasting plasma cortisol on the seventh day postoperatively.

Postoperative evaluation after first and second TS

On the seventh day postoperatively, early biochemical evaluation was carried out, consisting of measurement of fasting (0800 h) plasma cortisol. If it was lower than 200 nmol/L, substitution therapy with hydrocortisone 30 mg a day, or cortisone acetate, 37.5 mg daily, was prescribed. Patients were re-evaluated every 2 to 4 weeks during the first 3 months after surgery and then at 2-3 months intervals during the first year. Thereafter they were evaluated at least once a year.

The fasting plasma cortisol concentration was measured at each visit. In addition an overnight 1 mg dexamethasone suppression test was carried out 1 and 3 months post-operatively, and thereafter in patients who were in remission once yearly or earlier in case of clinical suspicion of relapse. If a patient received glucocorticoid substitution postoperatively, the dose was reduced gradually and stopped, if possible between 3 and 12 months after surgery. Thereafter the integrity of the hypothalamic-pituitary-adrenal axis was assessed by an insulin-hypoglycaemia test [37]. The thyrotropic, gonadotropic and somatotropic axes were checked regularly.

Criteria for remission and relapse

Remission was defined as disappearance of clinical symptoms of hypercortisolism with basal plasma cortisol level ≤50 nmol/L after glucocorticoid withdrawal for 24-48 hours and/or suppression of plasma cortisol level ≤ 50 nmol/L after overnight 1 mg dexamethasone within the first 3 months after surgery.[6,7,10] Relapse was defined as development of clinical symptoms of hypercortisolism and inadequate suppression of plasma cortisol level after an overnight 1 mg dexamethasone suppression test in patients who were in remission after the first surgery according to the previously mentioned criteria.

Statistics

Statistical analyses were performed using Pearson's Chi-square tests (P) and Wilcoxon's two sample tests (P*). Statistical significance was defined as $P<0.05$ (two tailed).

Results

Remission rates after repeated TS (Figure 1)

The individual clinical data of the 14 patients are shown in Tables 1 and 2. The mean interval between the first and the second TS was 59 months (median 39, range 2-185). After repeated TS remission was achieved in 10 patients. Nevertheless, patients 1 and 9 received additional therapy. Patient 1 was concerned that hypercortisolism would recur and asked for additional radiotherapy. Patient 9 was the only patient with an invasive macroadenoma that could not entirely be removed during the second surgery. Therefore the patient received additional gamma knife surgery, although he was clinically and biochemically in remission.

None of the patients who were in remission after the second TS had a relapse during follow-up of mean 34 months (median 24, range 4 to 97). At last follow-up plasma cortisol was not suppressed to ≤ 50 nmol/L after 1 mg dexamethasone overnight in patients 1,2,7 and 10 (table 2), but they had no clinical symptoms of hypercortisolism and 24-hour urinary free cortisol excretion was not elevated. Four patients had persistent CD after the second TS. Patient 3 underwent a bilateral adrenalectomy and patients 5, 6 and 8 received conventional radiotherapy.

There were no statistically significant differences between the patients who underwent successful or unsuccessful repeated TS with respect to the following parameters: visualisation of an adenoma on MRI before first or second TS, histopathological confirmation of an ACTH secreting adenoma after first or second TS, treatment with cortisol lowering agents before first or second TS, the operation technique used during the first TS, persistent versus recurrent disease after the first TS, age, gender and interval between the two TS.

Table 1) Clinical characteristics per patient: first and second transsphenoidal pituitary surgery (1999-2007). *(page 88-89)*

			1st surgery		
Patient number, Gender, Age (years)	MRI	Technique	Histology	Result	1st - 2nd surgery (months)
1. f, 36	‹10*	TE	+	R	131
2. f, 45	-	Endo TS	-	F	2
3. f, 36	-	Endo TS	-	F	3
4. m, 30	-	Endo TS	-	R	11
5. m, 41	‹10	TS	+	R	98
6. f, 30	‹10	Endo TS	-	F	5
7. m, 46	‹10	TS	+	F	185
8. f, 22	‹10	Endo TS	-	F	38
9. m, 27	‹10	Endo TS	+	R	53
10. f, 40	35	Endo TS	-	R	41
11. m, 56	‹10	TS	+	R	124
12. f, 27	-	Endo TS	-	F	2
13. f, 54	-	Endo TS	-	R	32
14. f, 31	‹10	TS	-	R	104

*Age: age at second surgery; f: female; m: male; MRI: magnetic resonance imaging results given as maximal diameter of the visualised adenoma in mm; MRI *: Computed tomography scan was made instead of an MRI scan; MRI - : no adenoma identified; MRI in: invasion in cavernous sinus or other parasellar structures; TE: transethmoidal pituitary surgery; TS: microscopic transsphenoidal pituitary surgery; Endo TS: endoscopic transsphenoidal pituitary surgery; Histology +: evidence of ACTH-producing adenoma on histo-*

2nd surgery							
CLA	MRI	Year	Technique	Histology	Cortisol (nmol/L)		Result
					Basal	After Dex	
-	‹10	1999	Endo TS	+	0.50	0.02	R
M	-	2001	Endo TS	-	0.05	0.02	R
-	-	2002	Endo TS	-	0.55	0.21	F
M	‹10	2002	Endo TS	-	0.02	0.05	R
K	‹10, in	2005	Endo TS	NT	0.38	-	F
K	‹10	2005	Endo TS	+	0.57	0.25	F
K	‹10	2005	Endo TS	+	0.05	0.07	R
K	‹10	2006	Endo TS	-	0.59	0.50	F
-	22, in	2006	Endo TS	+	0.15	0.04	R
-	‹10	2006	Endo TS	+	0.02	0.03	R
-	‹10	2006	Endo TS	+	0.21	0.02	R
K	‹10	2006	Endo TS	-	0.01	-	R
-	-	2006	Endo TS	-	0.02	-	R
-	‹10	2007	Endo TS	+	0.01	-	R

logical examination; Histology -: no evidence of ACTH-producing adenoma on histological examination; Histology NT: no tissue obtained during surgery; CLA: preoperative therapy with cortisol lowering agents; M: metyrapone; K: ketoconazole; Cortisol basal: fasting plasma cortisol level at 0800h on seventh day after surgery; Dex: plasma cortisol level at 0800h after 1 mg dexamethasone overnight; R: remission; F: failure.

Table 2) Substitution therapy and follow-up after second pituitary surgery (1999-2007).

Patient number	Substitution after 1st TS*	Substitution after 2nd TS**	Result 2nd surgery	Additional Therapy	Cortisol at last follow-up (nmol/L)		Follow-up (months)
					Basal	After dex	
1	-	-	R	CR (45 Gy)	0.46	0.08	94
2.	-	T, GH	R	-	0.57	0.06	83
3.	-	T	F	ADX	-	-	68
4.	-	A, GH, G	R	-	0.15	0.03	61
5.	-	-	F	CR (50 Gy), K	-	-	30
6.	T	T	F	CR (50 Gy)	-	-	28
7.	-	T, G	R	-	0.13	0.09	30
8.	-	T	F	CR (45 Gy), K	-	-	15
9.	-	A	R	RS	0.10	<0.02	17
10.	-	T	R	-	0.18	0.08	15
11.	T	T, GH	R	-	0.26	<0.02	15
12.	-	-	R	-	0.40	<0.02	13
13.	-	G	R	-	0.06	<0.02	13
14.	G,T,GH,E	G,T,GH,E	R	-	0.04	<0.02	3

*: *at last evaluation before relapse was diagnosed or in case of persistent Cushing's disease immediately before second TS;* **: *At last follow-up; A: androgens; D: desmopressin; E: estrogens; G: glucocorticoids; GH: growth hormone; T: Levothyroxine; CR: conventional radiotherapy; RS: radiosurgery; ADX: bilateral adrenalectomy; K: ketoconazole; Cortisol at last follow-up: fasting plasma cortisol level at 0800h and plasma cortisol level at 0800h after 1 mg dexamethasone overnight, measured in January 2008 in patients who were clinically in remission after 2nd surgery.*

Table 3) Overview of previously published results of repeated TS in patients with persistent or recurrent CD.

	Population	Criteria remission	Remission (%)	Relapse (%)
Friedman et al. (1989)	33 persistent + recurrent	Morning C < 6 µg/dl and UFC < 90 µg/day	73	13
Ram et al. (1994)	17 persistent*	Morning C < 5 µg/dl and UFC < 90 µg/dag	71	25
Knappe et al. (1996)	10 persistent * / 17 recurrent	Not defined	71/ 56	24/22
Benveniste et al. (2005)	44 persistent + recurrent	Not defined	57	25
Locatteli et al. (2005)	12 persistent *	Clinical symptoms of hypocortisolism and morning C ≤ 2µg/dl	67	0
Hofman et al.(2006)	16 recurrent	C < 2 µg/dl after 2 mg dexamethasone and morning C 10 –21 µg/dl	37	0

*TS: transsphenoidal pituitary surgery; CD: Cushing's disease; * : immediate reoperation; C: plasma cortisol; UFC: urinary free cortisol excretion.*

Complications of repeated TS

Six of the 14 patients had mild transient diabetes insipidus. Six patients had cerebrospinal fluid (CSF) leakage, 4 intraoperatively (closed with a fat graft) and 2 postoperatively. All six patients with CSF leakage were treated successfully with an external lumbar drain. Two patients required a reoperation to close the leak. More serious complications occurred in 2 patients. Patient 5 had blood loss from the right cavernous sinus during second TS. Damage of the internal carotid artery was excluded via an angiogram. Surgery was terminated before any tissue was removed and a tampon was placed that was removed via reexploration one week later. A few hours after the reexploration the patient developed a hemiparesis, due to occlusion of the left internal carotid artery just above the bifurcation. This was very unlikely a direct complication of the TS because the site of occlusion of the internal carotid artery was lower than the operation area. Although the patient improved significantly during the revalidation process, permanent disabilities remain. Patient 11 had postoperative bleeding from the sphenopalatine artery. Reexploration was required with successful coagulation of the artery. The patient recovered within a few days without other complications.

Only 3 patients required hormonal substitution after the first TS. After the second TS 11 patients required long-term hormonal substitution (Table 2). Of all patients, 8 required levothyroxine, 4 glucocorticoids, 4 growth hormone and 2 androgens and 1 estrogens after repeated TS.

Discussion

The present study is the first study to report on the outcome of repeated TS via the endoscopic technique in patients with persistent or recurrent CD. In 1994 the endoscopic technique for TS was introduced in our hospital. Because of the minimal invasiveness, the excellent view of the surgical field and wider working angle, practically all TS were done endoscopically from 1998 onward. Before the introduction of the endoscopic technique we regarded repeated TS as a technically difficult procedure. Therefore, most patients with persistent and recurrent CD treated in our centre before 1999 underwent bilateral adrenalectomy or radiotherapy, and only 4 patients underwent repeated TS. Only 1 of these 4 patients was cured. After the introduction of the endoscopic technique repeated TS became more feasible. The wider view of the endoscopic technique provides an advantage, during repeated TS, as the anatomy is altered due to the previous operation [27]. A substantially higher remission rate of 71% was achieved in the fourteen patients operated on endoscopically and there was no relapse during follow-up of median 24 months.

The remission rate of 71% achieved in this study compares favourably with the remission rates previously reported after repeated TS via the conventional microscopic technique, which vary between 37% and 73%, with a 0-25% relapse rate [19-24] (Table 4). When comparing the reported remission rates, it is important to note that the studies were carried out in different patient groups. Three studies included patients with both persistent and recurrent CD [19-21], whereas other studies reported on patients with either persistent [22-23] or recurrent [24] CD. Criteria for remission also differed. In three studies immediate repeated TS was performed in case of persistent CD [21-23]. However, this strategy ignores the fact that some patients who do not have a low early postoperative basal plasma cortisol level do achieve remission, so by using this strategy some patients will unnecessarily undergo immediate repeated TS [8]. Overall, the remission rates reported after second TS in persistent or recurrent CD seem to be slightly lower than the remission rates reported after first TS, which vary from 50-90%, but are mostly 70-85% [3-9]. Nevertheless, the majority of the patients with persistent or recurrent CD can still instantly be cured by repeated TS, and the remission rate we achieved via the

endoscopic technique seems to be at least as good as the remission rate achieved by repeated TS via the conventional microscopic technique.

In this study 57% of the patients had a perioperative complication of the repeated TS. Although most complications in our series were mild and transient more serious complications occurred in two patients: one patient had a cerebrovascular accident postoperatively and another patient had postoperative bleeding from the sphenopalatine artery. The latter complication is not a rare complication of microscopic TS, occurring in 3.4 % of all cases. Vascular complications of endoscopic TS and microscopic TS are identical [38]. We previously reported a complication rate of 29% in our study on results of primary endoscopic TS in patients with CD[3], which is similar to the complication rates reported in studies of primary microscopic TS in patients with CD. The studies on repeated TS in patients with persistent or recurrent CD report complication rates varying between 0 and 91% [19-24]. Although complication rates are influenced substantially by differences in definitions, the complication rate of repeated TS seems to be higher than the complication rate of primary TS.

Especially CSF leakage has been reported to occur more frequently during repeated TS than during the first TS, probably because of postoperative changes such as scar tissue, but also as a result of a more aggressive surgical procedure in a usually small sella with a concave diaphragm [22,28,39]. CSF leakage occurred in 43% of the patients in this study, intraoperatively in 4 patients and postoperatively in 2 patients. Previously, we reported a CSF leakage rate of 8.5% in primary endoscopic TS in patients with CD operated on by the same neurosurgeons that operated on the patients in this study [3]. When comparing rates of CSF leakage it is important to note that most studies only report on the rates of postoperative CSF leakage. The rate of postoperative CSF leakage is lower (1-4% during first TS) than the rate of intraoperative CSF leakage (9-25% during first TS) [39-40]. Because intraoperative CSF leakage, which is usually not regarded as a complication, also occurs frequently during the first TS and the risk of meningitis is minimal if patients are given a lumbar drain, [41] we believe that the higher chance of CSFleakage during repeated TS should not be a reason to refrain from repeated TS.

The chance of hypopituitarism after repeated TS is higher than after the first TS. Reported percentages of hormonal deficiencies in patients with CD after the first TS vary from 2-48%, but are mostly around 20%. Ram et al. [23] and Friedman et al. [20] reported rates of hormonal deficiencies after repeated TS in 41% and 50% of the patients. Locatelli et al. found an extremely high rate of hormonal deficiencies (100%), but all patients underwent a total hypophysectomy [22]. Hofmann et al. [24] and Knappe et al. [21] report on new

hormonal deficiencies in 0% and 8%. In the present series the percentage of patients with hormonal deficiencies was 78% after the second TS, with 6 patients having only one hormonal deficiency. The rate of hormonal deficiencies was 48% in our earlier study on results of first endoscopic TS in patients with CD [3]. The higher rates of hypopituitarism after repeated TS compared to after the first TS can be expected because additional pituitary tissue is removed. Even so, the risk of hypopituitarism after second TS seems to be lower than reported rates of hypopituitarism several years after radiotherapy [9,11].

The success rate of repeated TS for persistent or recurrent CD depends on the correct selection of patients. In our opinion TS is a good treatment option if there is a reasonable chance that it will be successful. CD can be controlled instantly with the possibility to normalise cortisol circadian rhythm and keep the pituitary adrenal axis intact. Radiotherapy, however, is a good option if an adenoma is invasive.

Success rates of TS also critically depend on the skills and the experience of the neurosurgeons [42]. Repeated TS is an even more difficult procedure than a first TS, and repeated TS is infrequently performed. Thus it is advised to concentrate repeated TS for CD in a limited number of specialised centres.

In conclusion, repeated TS is a good therapeutic option for selected patients with persistent or recurrent CD, with the potential to achieve remission in the majority of patients. The remission rate of 71% we achieved via the endoscopic technique of repeated TS compares favourably with the remission rate of repeated TS via the conventional microscopic technique previously described in the literature. The excellent view of the surgical field during endoscopic TS provides an advantage in case of altered anatomy due to previous surgery.

However, only a randomised trial can definitively assess whether the endoscopic or the microscopic technique of TS is the preferred technique for repeated surgery in patients with persistent or recurrent CD.

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Long-term Quality of Life after

TREATMENT OF ACROMEGALY AND CUSHING'S SYNDROME

PART 2


5

Persistent self-consciousness about facial appearance, measured with the Derriford appearance scale 59, in patients after long-term biochemical remission of acromegaly

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Abstract

Context: Acromegaly is associated with impaired quality of life (QoL) and causes anatomical disproportions, which may contribute to the decreased QoL after successful treatment. The Derriford appearance scale 59 (DAS59) is a questionnaire measuring psychological distress and disruptions to everyday life associated with self-consciousness of appearance.

Objective: To investigate the psychological distress and dysfunction related to selfconsciousness about appearance and its effect on - QoL in patients in long-term remission of acromegaly.

Patients, design and methods: Patients (>18 years old) treated for acromegaly at the Department of Endocrinology of the Radboud University Medical Center Nijmegen were invited to participate. A gender-, age- and body mass index (BMI)- matched control group was provided by the patients themselves. Participants were asked to complete the modified DAS59-, research and development 36- (RAND-36), acromegaly quality of life questionnaire- (AcroQoL) and a sociodemographic questionnaire. Differences between patient and control groups and correlations between questionnaire scores and clinical characteristics collected from medical records were analyzed.

Main outcome measures: Questionnaire scores.

Results: Of the 120 respondents, 73 agreed to participate [all cured or under biochemical control, median remission time 10.5 years (range 2.3-43.6 years)]. Of these, 34 (46.6%) reported self-consciousness about their appearance. Twenty-nine of these patients (85.3%) pointed out their face to be a prominent source of self-consciousness. Fifty-seven matched control subjects were included as well. Significant correlations were found between the scores of the DAS59 and the AcroQoL, RAND-36 and VAS in patients.

Conclusions: Even after long-term remission of acromegaly, a large number of patients are self-conscious about their appearance, leading to psychological distress and disruptions to everyday life and decreased QoL. Facial features were the most important source of self-consciousness. This stresses the importance of addressing self-consciousness of appearance and the need for additional support in this regard during follow-up in these patients.

Introduction

Craniofacial disproportions due to soft tissue swelling and new bone formation are highly prevalent in patients with active acromegaly [1]. Although clinically there is a slight improvement in facial appearance after biochemical control of growth hormone (GH) hypersecretion, we have recently shown that significant differences in craniofacial characteristics persist even after long-term remission [2]. It has been suggested that these changes in appearance can lead to self-consciousness about appearance, leading towards self-esteem disintegration, social withdrawal, body image distortion and an impaired quality of life (QoL) [3].

Previous studies, which used the disease-specific AcroQoL questionnaire, have shown that patients with both active and controlled acromegaly have an impaired QoL. Remarkably, the appearance subscale of the AcroQoL was the most affected subscale in these studies [4-7]. Moreover, the patients in remission of acromegaly had only slightly better scores on the appearance subscale than patients with active disease [4,6,8]. However the appearance subscale of the AcroQoL is limited and does not investigate which aspect of appearance specifically causes the self-consciousness related distress.

The Derriford appearance scale 59 (DAS59) is a psychological questionnaire, developed for research in plastic surgery and oncology. It measures self-consciousness about one's appearance and identifies the anatomical source of this self-consciousness. It is validated for both clinical and research settings. It has an excellent validity and reliability, and has been independently recommended as a measure of choice for the assessment of feelings about appearance [9,10]. However, the DAS59 had never been used in patients with acromegaly.

The primary aim of the present study was to investigate, by using the DAS59 questionnaire, whether patients in long-term remission of acromegaly suffer from more psychological distress and psychological and social dysfunction related to self-consciousness of appearance than a gender-, age- and BMI- matched control group, and whether this affects QoL. In addition we aimed to identify the anatomical sources of self-consciousness in these patients. We hypothesised that (irreversible) changes in craniofacial characteristics caused by the previous period of GH hypersecretion are related to the distress related to appearance in patients in long-term remission of acromegaly.

Subjects and methods

Patients

All patients (18-80 years old) treated at the Department of Medicine, Division of Endocrinology of the Radboud University Medical Center Nijmegen, who had been in remission of acromegaly for more than 2 years, were invited to participate in this study by letter.

Exclusion criteria were active malignancy, cardiovascular disease (unstable coronary artery disease, heart failure NYHA III-IV), (recent) pregnancy (within 1 year prior to the study), depression, psychosis and personality disorder.
Because the assessment of one's own appearance differs in relation to gender, age and BMI, participants were asked to find their own control subject with the same gender and roughly the same age and BMI [9,11,12].

The study was approved by our institutional ethics committee and conformed to the declaration of Helsinki. All participants gave written informed consent for participation in the study.

Questionnaires

DAS59

The DAS-59 is a self-reporting questionnaire that generates a series of valid and reliable measures of the specific psychological distress and disruption to everyday life that are associated with self-consciousness of appearance. It is intended for use in an adult population (› 16 years) [9]. An introductory section identifies whether a subject is self-conscious about their appearance and the aspect of appearance that is of the greatest concern to the respondent. This is referred to as the respondent's 'feature' in the rest of the questionnaire. The DAS59 contains 59 statements and questions with response categories in a Likert format to measure frequency of symptomatology ('almost never'... 'almost always') and levels of associated distress ('not at all distressed'...'extremely distressed'). Fifty-seven items assess relevant psychological distress and dysfunction, and two items assess physical distress and physical dysfunction. The following subscales were examined as described by the questionnaire manual [13]: general self-consciousness (GSC), social self-consciousness (SSC), facial self-consciousness (FSC), Sexual and bodily self-consciousness (SBSC) and negative self-concept (NSC). A higher score on the DAS59 is associated with a greater degree of self image-related distress and dysfunction. The format of the introductory section and a 'not applicable' response category for most items make the scale acceptable for respondents who are not concerned about appearance at all.

In this study a linguistic and cultural translation of the DAS59 from English to Dutch was performed following internationally accepted guidelines [14,15] and used with permission of the original authors [9]. The translation into Dutch was successful. The backward-translation was compared to the original English version and did not show conceptual discrepancies. During piloting the items were assessed as conceptually and linguistically meaningful and appropriate, and in line with the original (English) items.

The DAS59 questionnaire was modified for the purpose of this study by including a self-rated Visual Analogue Scale (VAS) for the participants to score the level of satisfaction with their facial appearance in general. The VAS is a 10 cm 'ruler' scaled 0 (not at all satisfied) to 10 (very satisfied) [16].

RAND – 36: For the assessment of general QoL the Dutch translation of the RAND-36 was used [18]. It comprises 36 items regarding general wellbeing during the past 30 days. The items are formulated as statements or questions with Likert scale or yes/no response options to assess 9 health concepts: 1) physical functioning; 2) social functioning; 3) role limitations because of physical health problems; 4) role limitations because of emotional problems; 5) mental health; 6) vitality; 7) bodily pain, 8) general health perceptions, 9) health change in the last year. Scores are calculated for each health aspect on a 0-100 scale, in which higher scores represent a better QoL.

AcroQoL: This is a disease-specific questionnaire developed to assess health-related QoL in patients with acromegaly [17]. It comprises 22 items. Responses are given as the frequency of occurrence (ranging from always to never) or degree of agreement (ranging from completely agree to completely disagree) on a five-point scale. The questionnaire includes two different scales; a physical performance scale and a psychological well-being scale. The psychological scale is further subdivided into 2 subscales regarding appearance and personal relationships, containing seven items each. The highest achievable score is 110 (100%), which is indicative of an excellent QoL, while the lowest score is 22 (0%) [5].

Sociodemographical and clinical variables: Sociodemographical and clinical characteristics of patients were collected by means of a sociodemographical questionnaire and medical chart review in the patient group.

Definitions

Remission was subdivided into 2 groups and defined as: 1. cure: successful surgical and/or radiotherapeutical treatment of the GH-producing adenoma followed by a normalization of the IGF-1 concentration (≤ mean + 2 SD for age) and suppression of serum GH levels <1.0µg/l during OGTT (after surgical treatment)[18] and 2. disease control: normal

IGF1 concentrations (≤ mean +2 S.D.S for age) during the use of somatostatin analogues, dopamine agonists and/or GH receptor antagonists.

Hormonal deficiency was defined as deficiency of one or more pituitary hormones. Hypothyroidism was defined as the use of thyroid hormone substitution therapy. Hypogonadism was defined as use of testosterone substitution in men and use of oestrogen in women. GH deficiency was defined as the need for GH substitution therapy as defined by a maximal GH response <15.3mU/l during an insulin tolerance test (ITT) or a maximal GH response of <12.3 mU/l during an arginine/GHRH test [19]. Glucocorticoid deficiency was defined as the need for glucocorticoid substitution therapy as defined by a plasma morning cortisol <100nmol/l, after withdrawal of glucocorticoids for 24 hours, or a maximal cortisol response <550nmol/l during an ITT [20].
Regarding current disease status, cure or biochemical disease control had been confirmed by a recent (<1 year) normal IGF-1 level in all participating patients.

Statistical analysis

All data are presented as means and standard deviations (SD) or median and range. Data were tested for normality of distribution by means of the Kolmogorov-Smirnov test. T tests or Mann-Whitney U tests depending on normality of distribution or χ^2 tests (for categorical variables) were performed to compare the variables age, gender and BMI of the patient and control group. T tests or Mann-Whitney U tests were performed to compare the scores on the subscales of the DAS59, RAND, AcroQoL and VAS of the patient and control group. To investigate the presence of correlations between sociodemographic characteristics, clinical characteristics and outcomes of the questionnaires, Spearman's rank correlation testing or point-biserial correlation was used as appropriate. Statistical significance was set at the 5% level ($p<0.05$). All statistical analyses were performed using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

The clinical characteristics of the patients and control subjects are depicted in Table 1. One hundred and thirty-one patients were invited to participate. Of these, 120 subjects responded (response rate 91.6%). Forty-seven of the patients who responded decided not to participate because of a lack of time (n=6), a lack of interest (n=14), physical or emotional problems (n=19) or because they did not have a problem with their appearance (n=5). Three of the patients who responded did not report their reason for refusal

to participate. No statistically significant differences were found between the participants and the subjects who refused to participate in this study with respect to clinical parameters, with the exception of age, with the non-participants being slightly older than the participants. A total of 73 patients (45.2% female, mean age 59.4 ±10.5 years and BMI 28.1 ± 4.7 kg/m^2) agreed to participate. Mean age at diagnosis was 41.6 (11.2) years. Regarding current disease status, cure or biochemical disease control had been confirmed by a recent (<1 year) normal IGF-1 level in all participating patients. Median remission time was 10.5 (2.3-43.6) years. The treatment processes of all patients are depicted in Figure 1. Sixty-three (86.3%) patients had undergone primary pituitary surgery and 3 (4.1%) patients had undergone primary radiotherapy. At the time of the study acromegaly was controlled by medical therapy in 43.8% of the patients: 20 patients (27.4%) used somatostatin analogues (SA) monotherapy, 4 (5.5%) dopamine agonists (DA) monotherapy, 2 (2.7%) GH receptor blockers (GHRB) monotherapy and 6 were using combination therapy. The other 41 (56.2%) patients were in remission of acromegaly without medical therapy. In 41 (56.2%) patients no substitution of pituitary hormones was necessary after surgery or radiotherapy, 32 (43.8%) had some kind of hormonal deficiency, of whom 15 (20.5%) had a hypopituitarism.

Fifty-seven control subjects were included in this study (47.4% female, age 58.6 ± 11.3 years, BMI 26.4 ±3.5 kg/m^2). There was no significant difference between the patient and the control group regarding age (p=0.510), gender (p=0.860) and BMI (p=0.050), which confirmed the adequate matching.

Derriford appearance scale scores

Table 2 shows the sources of self-consciousness and scores of the two groups on the DAS59. Thirty-four (46,6%) of the patients in long-term remission of acromegaly were self-conscious about their appearance compared to 13 (22.8%) of the control subjects (p<0.01). Twenty-nine (85.3%) of the patients indicated that their face was the most prominent source of self-consciousness compared to 3 (23.1%) controls (p<0.01). Compared to the control group the patient group had statistically significanty higher scores on all subscales (p < 0.01). No statistically significant correlations were found between the DAS59 scores of the patient group and clinical characteristics like age, gender and BMI (Table 1). However a statistically significant correlation was found between the DAS59 scores of the control group and age (r=-0.327, p=<0.05). Furthermore, in the patient group no correlation was found between the duration of remission and the DAS59 scores (r= -0.169, p=0.153) and between disease status (cure versus biochemical disease control) and the DAS59 scores (r=0.021, p=0.860). A slight but significant correlation was found between pituitary function and the DAS59 score (r=0.279, p<0.05) with patients with a preserved pituitary function scoring lower amounts of self image-related distress.

Table 1) Patient characteristics and correlation with the DAS59 score.

	Patients treated for acromegaly (N=73)	Control subjects (N=57)	p-value	Correlation to DAS59 score	
				Spearman's-rho	p-value
Gender (n): male/ female	40/ 33	30/27	0.860	0.139	0.240
Age: mean (SD) (years)	59.4 (10.5)	58.6 (11.3)	0.510	-0.085	0.504
BMI (kg/m²): mean (SD)	28.1 (4.7)	26.4 (3.5)	0.050	0.087	0.466
Age at diagnosis: mean (SD) (years)	41.6 (11.16)			-0.095	0.425
Duration since cure: median (range) (years)	12.6 (41)			-0.169	0.153
2-5 years remission n (%)	8 (11.0)				
5-10 years remission n (%)	27 (37.0)				
10-15 years remission n (%)	20 (27.4)				
› 15 years remission n (%)	18 (24.7)				
Treatment modality n (n) (%):					
Surgery	64 (87.7)			0.071	0.549
Medical	39 (53.4)			-0.115	0.189
SA	26 (35.6)				
DA	8 (11.0)				
P	5 (6.8)				
Irradiation	14 (19.2)			-0.111	0.351
Disease status: n (%)				0.021	0.860
Cure	41 (56.1)				
Biochemical remission	32 (43.8)				
Hormonal deficiencies: n (%)					
Preserved pituitary function	41 (56.1)			0.279	‹ 0.05*
Hypothyroidism	26 (35.6)			-0.249	‹ 0.05*
Hypocortisolism	19 (26.0)			-0.106	0.372
Growth hormone deficiency	17 (23.3)			-0.082	0.493
Hypogonadism	19 (26.0)			-0.150	0.206
Diabetes insipidus	17 (23.3)			-0.068	0.565
Panhypopituirism	15 (20.5)			-0.077	0.516
Comorbidities					
Hypertension	29 (39.7)			-0.042	0.725
Diabetes mellitus	14 (19.2)			0.007	0.956
Joint related complaints	12 (16.4)			-0.109	0.360

SA somatostatin analogues, DA dopamine agonists, GHRB growth hormone receptor antagonists

**P ‹0.05 by Spearman's rho*

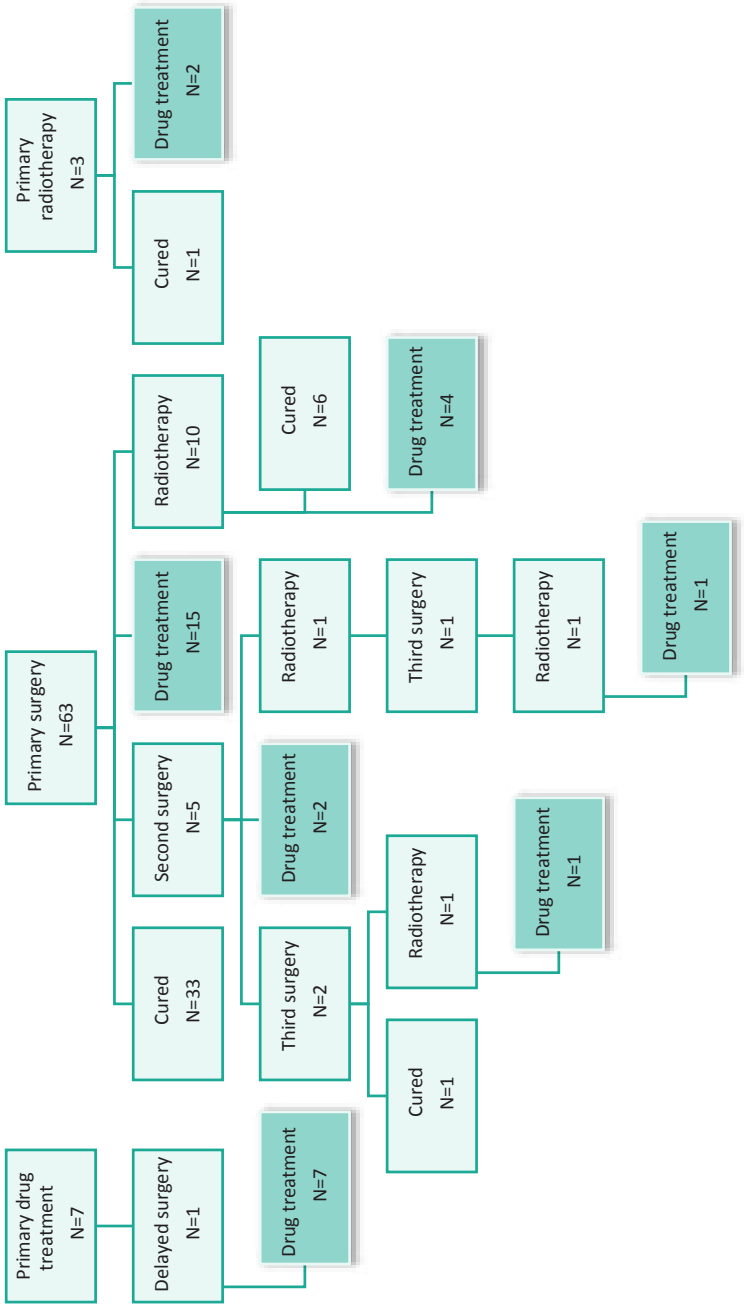

Figure 1) Treatment process of patients.

Patients biochemically controlled using drug treatment are depicted in dark green.

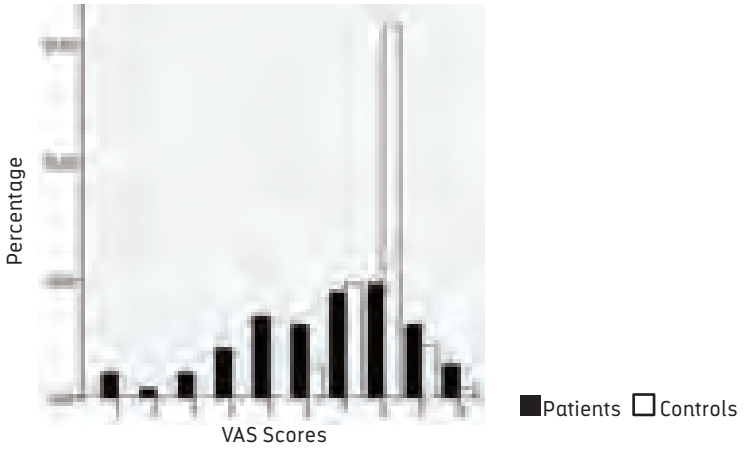

Figure 2) Visual Analogue Scale (VAS) scores.

Another correlation was found between hypothyroidism and the full scale DAS59 score (r=-0.249, p<0.05) (Table 1) with patients with hypothyroidism scoring higher. More specifically, a significant correlation was found between hypothyroidism and the GSC-subscale (r=-0.262, p<0.05). In the patient group, no correlations were found between the DAS59 score and having a partner (r=0.040, p=0.739), education level (r=-0.038, p=0.749) or employment status (r= -0.037, p= 0.756). In the control group, these correlations were also not found to be statistically significant; having a partner (r=0.003, p=0.368), education level (r=-0.006, p=0.963), employment status (r=-0.122. p=0.368).The scores of the AcroQoL and the RAND-36 are shown in Table 3. The DAS59 score correlates inversely with all subscales of the AcroQoL (p<0.01). The DAS59 scores were also inversely correlated with most of the subscales of the RAND-36.

Figure 2 shows the VAS scores. Scores of the patients were significantly lower than the scores of the control-subjects (p <0.01). The total satisfaction score ranged from 0 to 10 in the patient group (mean score = 6.2 ± 2.4). Twenty-five patients (34.2%) scored the satisfaction with their facial appearance below 6. Scores in the control subject group ranged from 6-10 (mean score 7.8 ± 0.8). In the patient group the scores on the VAS scale also showed an inverse correlation with the DAS59 score (r=-0.567, p<0.01).

RAND 36 scores

Mean scores of the patients in long-term remission of acromegaly were significantly lower than the scores of the control group (Table 3). Subscale scores of patients and controls and comparisons between the groups are depicted in Table 3.

Table 2) Derriford appearance scale 59.

	Patient (N=73)	Controls (N=57)	P value
Prevalence of self-consciousness, n (%):	34 (46.6)	13 (22.8)	‹ 0.01*
Sources of self-consciousness, n (% of self-conscious subjects):			
Face/facial features	29 (85.3)	3 (23.1)	‹ 0.01*
Large/coarse feet	1 (2.9)	0 (0.0)	
Curved back	1 (2.9)	0 (0.0)	
Unsightly skin	2 (5.9)	4 (30.8)	
Overweight	3 (8.8)	1 (7.7)	
Breasts	0 (0.0)	1 (7.7)	
Scalp hair	0 (0.0)	1 (7.7)	
General aging	0 (0.0)	1 (7.7)	
Abdomen	0 (0.0)	2 (15.4)	

	Mean score (SD) Patients (N=73)	Mean score (SD) Controls (N=57)	p-value
Full-scale (FS)	66.6 (33.7)	38.3 (22.7)	‹ 0.01*
General self-consciousness of appearance (GSC)	21.3 (16.3)	8.8 (8.5)	‹ 0.01*
Social self-consciousness of appearance (SSC)	15.2 (12.3)	8.2 (8.9)	‹ 0.01*
Sexual and bodily self-consciousness of appearance (SBSC)	7.8 (6.8)	4.2 (5.0)	‹ 0.01*
Negative self-concept (NSC)	14.4 (3.9)	12.2 (2.6)	‹ 0.01*
Facial self-consciousness of appearance (FSC)	2.8 (2.3)	1.7 (2.6)	‹ 0.01*
Not loading on a specific factor			
53. how irritable do you feel?	2.2 (1.0)	1.7 (0.7)	‹ 0.01*
59. how hostile do you feel?	1.7 (1.0)	1.3 (0.8)	‹ 0.01*

P ‹ 0.01, by Mann-Whitney U test

AcroQoL scores

In the patient group, the mean (SD) AcroQoL score was 64.0% (16.6%). The most affected scale was the subscale "appearance" mean (SD) 57.1% (19.5%). The AcroQoL appearance subscale score was significantly correlated to the DAS59 full scale score ($r=-0.597$, $p<0.01$). Other mean subscale scores are further depicted in Table 3.

Sociodemographic characteristics

Of the patients in remission 55 (75.3%) were married or engaged into a relationship versus 47 (82.5%) of the controls ($p=0.528$), 18 (24.7%) of the patients in remission had no relationship, were divorced or a widow/widower versus 10 (17.5%) of the controls. Two of the patients in remission (2.7%) had less than eight years of education versus 1 (1.8%) of the controls, 6 (8.2%) of the patients in remission versus 6 (10.5%) of the controls had 8-12 years of education, 45 (61.6%) of the patients in remission versus 21 (36.8%) of the controls had 12-16 years of education, 10 (13.7%) of the patients in remission versus 19 (33.3%) of the controls had 16-21 years of education and 10 (13.7%) of the patients in remission versus 9 (15.8%) of the controls had a university degree. Twenty-two of the patients in remission (30.1%) had full- or part-time employment versus 30 (52.6%) of the controls ($p<0.05$), 29 (39.7%) of the patients in remission versus 18 (31.6%) of the controls were retired, and 22 (30.1%) of the patients in remission versus 9 (15.8%) of the controls were unemployed.

Discussion

In this study we evaluated self-consciousness about appearance and its relation with QoL in 73 patients in long-term remission of acromegaly, and determined which physical aspects have the most influence on the self-consciousness about appearance.

The main finding of the present study is that, compared to a matched control group, a significantly larger proportion of the patients are still self-conscious about their appearance (46.6% vs. 22.8%), even after more than 12 years of remission. This is regardless of most clinical and sociodemographic characteristics. The significant correlations between the DAS59 scores and the AcroQoL and RAND-36 scores indicate that the concerns about appearance are clearly related to an impaired quality of life and general well-being. In addition, this is the first study that investigates the association between specific "features" and self-consciousness about appearance in patients in long-term remission of acromegaly. An impressing 85.3% of the patients who reported self-consciousness of appearance in our cohort indicated that their face is the main source of

Table 3) The Derriford Appearance Scale (DAS59) scores and patient group correlations to the scores of the RAND-36 and the scores of the AcroQoL.

Questionnaire	Mean score (SD)			Correlation to DAS59-score	
	Patients treated for acromegaly (N=73)	Controls (N=57)	P-value	Spearman's rho	P-value
RAND-36					
Physical functioning	72.8 (23.1)	91.2 (13.3)	< 0.01*	-0.114	0.335
Social functioning	75.7 (23.1)	93.0 (11.1)	< 0.01*	-0.236	< 0.05**
Role limitations due to physical problems	53.8 (44.8)	94.7 (14.7)	< 0.01*	-0.234	< 0.05**
Role limitations due to emotional problems	78.1 (38.2)	94.7 (18.7)	< 0.01*	-0.262	< 0.05**
Vitality	57.5 (20.2)	77.1 (12.5)	< 0.01*	-0.225	0.055
Mental health	74.9 (17.5)	84.4 (10.4)	< 0.01*	-0.322	< 0.01*
Bodily Pain	71.5 (21.7)	89.0 (14.9)	< 0.01*	-0.159	0.179
General Health Perception	54.8 (20.8)	73.9 (16.6)	< 0.01*	-0.189	0.109
Change in health	50.0 (17.2)	56.1 (17.2)	0.064	-0.086	0.470
AcroQoL					
Total	64.0 (16.6)			-0.570	< 0.01*
Physical performance	61.3 (20.0)			-0.365	< 0.01*
Psychological well-being	65.5 (17.4)			-0.638	< 0.01*
Appearance	57.1 (19.5)			-0.597	< 0.01*
Personal relations	73.9 (18.2)			-0.570	< 0.01*

*P < 0.01, **P <0.05 by Mann-Whitney U test or Spearman's rho

discontent. In the general population the distribution of 'features' that cause self-consciousness of appearance is much more evenly distributed throughout the body with only 25-27% focus on the face [11]. This is in concordance with the results of our control group. Moreover, 34 % of the patients scored their facial appearance below 6 on the 0-10 VAS scale compared to 0% in the control group (Figure 2). Our study therefore clearly demonstrates that patients in long-term remission of acromegaly still have significant problems with their facial appearance.

The findings in this study are in line with the findings of previous studies that found that the most affected dimension of the AcroQoL was appearance [4,5]. Matta *et al.* [6] and Paisley *et al.* [7] showed an improvement in the appearance subdomain of the AcroQoL after treatment-induced improvement in IGF-1 levels. However they did not compare these "after treatment" outcomes with the normal population, because the AcroQoL is a disease-generated questionnaire specifically designed for patients. Furthermore, previous studies were not able to point out the specific anatomical features that caused the concern and discontent about appearance, while the DAS-59 clearly identifies the face as the primary source of self-consciousness.

Studies about the prevalence of concern about appearance in the general population showed that concern about physical appearance is twice as common among women as among men. The prevalence of concern about appearance is highest during the late teens and early twenties, while it normally decreases with age [8,11]. In our series there was no correlation between the DAS59 score and gender or age in the patient group, but a statistically significant correlation between the DAS59 scores and age was found in the control group ($r=-0.327$, $p=<0.05$). This indicates that, in contrast to the general population, the individuals in the patient group remained self-conscious about their appearance despite the fact that they aged and men and women are equally affected.

A positive perception of appearance is related to successful psychological functioning and well-being [11]. Therefore, the self-consciousness about appearance and impaired QoL in patients in long-term remission of acromegaly are even more concerning, as these patients are already in remission and these disturbances seem to persist. The concerns about appearance in patients in remission of acromegaly are probably caused by persisting anatomical alterations such as craniofacial disproportions remain present, even after long-term remission [2]. A delay in initial diagnosis, often by several years, probably is the major cause of the irreparable changes and damage [6,21]. In our study persisting comorbidities may have also contributed to decreased QoL as previously reported [22], as hypertension was present in 39.7% of our patients, diabetes mellitus

in 19.2% and joint related complaints in 16.4% of our patients. However no correlations were found between comorbidities and DAS59 scores.

When the results of the DAS59 mean subscale score on facial self-consciousness of appearance (FSC) of our patient group are compared to the results of previously described clinical subgroups, it is notable that the score of our patients (2.8) is comparable or worse than the scores in males considered suitable for rhinoplasty (2.6), and other cosmetic surgery (2.5) and females considered suitable for a facelift (2.5) and other cosmetic surgery (2.5) [9]. Surprisingly plastic/craniofacial surgery is not widely performed in (former) acromegaly patients, especially not in Europe. Only a limited number of case reports about corrections of the acromegalic face have been published in literature. However individualized procedures, for example reduction of the vertical dimension of the body of the mandible, rhinoplasty, posterior displacement of the prominent frontal bone, recession and remodeling of cheeks (zygoma) or correction of mandibular prognathism can greatly improve appearance and potentially reduce self-consciousness and associated distress about appearance [23-26]. Another method to support patients that still have concerns about their appearance after remission of acromegaly could be psychological support or psychotherapeutical interventions to prevent evolution of a maladjusted coping style [27-29].

An additional finding in this study is that patients in remission are more likely to be unemployed. This could be a result of the previous illness, but it is interesting to consider the possibility that their appearance could play a role in their unemployment. Literature shows that faces are key to our perception of others and people widely believe that the character of a person can be recognized from his or her face. This phenomenon is commonly labeled the *halo effect* [30]. In addition the slightly higher education level in the control group could also in part explain the differences we observed. Furthermore, an interesting correlation was found between hypothyroidism and the DAS59 full scale score. Especially the GSC subscale of the DAS59 turned out to be correlated with hypothyroidism, which could be explained by the fact that hypothyroidism, even when adequately treated, is known to be associated with depressive symptoms [31].

Some limitations of this study should be acknowledged. A cross-sectional design was used, which did not account for variability of QoL and perception of appearance over time. However good test-retest reliability for both the AcroQoL and DAS59 has previously been demonstrated [4,9]. The refusal of 5 patients to participate because they did not have any problems with their appearance might have introduced some bias. However even when these patients would have participated the proportion of patients that

reported to be self-conscious about a 'feature' would still be 43.6%. The difference in BMI between both groups was at the limit of significance (p=0.05). However, no correlation was found between BMI and full scale DAS59 score. Therefore, we believe that this cannot explain the difference in DAS59 scores found between patients and controls.

In conclusion, even after long-term remission of acromegaly, a large number of patients are self-conscious about their appearance, leading to psychological distress and disruptions to everyday life and a decreased QoL. The facial features were the most important source of self-consciousness. The findings in this study highlight the importance of the fact that physicians should not ignore self-consciousness about appearance in patients treated for acromegaly, but have to address these concerns during follow-up. Patients should be encouraged to discuss their concerns about their appearance and should be offered individualized advice about corrective interventions to improve not only their appearance but also functionality. There is a lot of potential for improvement in the support of people with acromegaly regarding their difficulties concerning their appearance and therefore their QoL as shown by a recent study that detected a significant effect of exercise on body self-perception in patients with acromegaly [32]. To optimize management, not only biochemical and radiological parameters but also dimensions like appearance that reflect QoL should be evaluated. In practice the DAS59 is easy to use by physicians and can be an excellent instrument during follow-up. For patients, the DAS59 provides the opportunity to more easily address problems with their appearance and to discuss possible treatment options like psychological support or plastic/craniofacial surgery.

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5) Self-consciousness about appearance after remission of acromegaly

Impaired quality of life in patients in long-term remission of Cushing's syndrome of both adrenal and pituitary origin:

a remaining effect of longstanding hypercortisolism?

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Abstract

Objective: The determinants that cause impaired quality of life (QoL) in patients in long-term remission of Cushing's syndrome (CS) are unknown. The aim of this study was to get more insight in the patient and disease characteristics related to impaired QoL in these patients.

Design: Cross-sectional study.

Methods: The QoL of 123 patients in remission of CS (age 52.2 ± 12.0 years, 106 women, duration of remission 13.3 ± 10.4 years, 80% pituitary CS), assessed with seven validated questionnaires, was compared to the QoL of an age- and sex-matched control group (n=105). To investigate the influence of the aetiology of CS on QOL, patients in remission of pituitary and adrenal CS were compared. Furthermore, the influence of hormonal deficiencies, treatment strategy, duration of remission, gender and age on QoL was investigated.

Results: QoL in the total patient group and each patient subgroup was significantly worse on practically all dimensions of questionnaires compared to the control group ($p < 0.05$), except for patients in remission of pituitary CS without hormonal deficiencies who had an impaired QoL on 50% of the QoL dimensions. Subgroup analysis revealed no difference in QoL between different patient groups, especially no difference between patients in remission of adrenal and pituitary CS. Female gender and a shorter duration of remission had a negative influence on QoL in the patient group.

Conclusions: QoL remains impaired in patients in long-term remission of CS regardless of aetiology, presence of hormonal deficiencies and treatment strategies. More research is needed to establish the causes.

Introduction

Endogenous Cushing's syndrome (CS) is associated with significant morbidity and mortality [1]. Although the Cushingoid phenotype improves after curative treatment, there is accumulating evidence that not all changes in body composition, cardiovascular risk profile and cognitive function are reversible [2, 3, 4]. Furthermore, a number of studies have reported that quality of life (QoL) remains impaired in patients in long-term remission of CS [5-15]. However, most of these studies have major limitations (e.g., assessment of QoL was not the main purpose of the study, small numbers of patients, no control group, unvalidated questionnaires, missing data on clinical characteristics and biochemical follow-up). Furthermore, most studies have only investigated QoL in patients in remission of pituitary CS [8, 9, 11, 12, 14], while studies that did include patients in remission of adrenal CS, did not compare QoL between different patients groups [5, 13, 15]. This has made it difficult to establish patient or disease characteristics related to impaired QoL in patients in long-term remission of CS. In this respect hypopituitarism, glucocorticoid deficiency or a persisting effect of the previous period of hypercortisolism have been suggested as possible determinants.

The aim of this study was to investigate QoL in a large group of patients in long-term remission of CS using 7 validated questionnaires concerning different dimensions of QoL and to compare the QoL of the patients to a sex- and age-matched healthy control group. Furthermore, to investigate the influence of the aetiology of CS on QOL, we compared the QoL of patients in remission of pituitary CS to that of patients in remission of adrenal CS. We also investigated the influence of treatment strategy and coexisting hormonal deficiencies on QoL in patients in remission of pituitary CS.

Subjects and methods

Protocol

After approval of the study by the Medical Research Ethics Committee region Arnhem-Nijmegen, all patients treated for CS from 1967 until 2007 in our hospital were identified. Medical records of all patients were retrospectively reviewed to assess data on demographics, diagnosis of CS, the aetiology of CS, the type and number of treatments that patients received and follow-up data on remission, relapses, hormonal deficiencies, and mortality. Patients that were assumed to be in remission for at least two years, according to the medical records, were eligible for inclusion. Patients with ectopic CS, active malignancy or systemic treatment for malignancy in the past were excluded.

Furthermore, all patients that were unable to answer the questionnaires (because they could not comprehend the questionnaires or the Dutch language) were excluded. All other eligible patients were asked to participate in this study. They were sent two sets of questionnaires by mail. Non-responders received a reminder letter after four weeks. If they still did not respond we attempted to contact them by telephone.

To obtain a sex- and age-matched control group, patients were asked to find their own control subject with the same age and gender among their family or friends. Written informed consent was obtained from all patients and controls before their questionnaires were enrolled in the study.
All patients underwent a 1 mg dexamethasone suppression test after they completed the questionnaires to confirm that they were still in remission. In patients who had been treated with radiation therapy of the pituitary gland a 24-hour urinary free cortisol was measured.

The results of the questionnaires of the patient group were compared to the results of the control group. Furthermore we compared the results of different patient subgroups. To investigate if the aetiology of CS influences QoL we compared patients in remission of adrenal CS to patients in remission of pituitary CS. To investigate if the presence of hormonal deficiencies influences QoL we compared patients in remission of pituitary CS with hormonal deficiencies to patients in remission of pituitary CS without hormonal deficiencies. We hypothesized that the hormonal deficiency that would impact QoL the most would be glucocorticoid deficiency. Therefore we compared patients in remission of pituitary CS with hormonal deficiencies including glucocorticoid deficiency to those with hormonal deficiencies without glucocorticoid deficiency. To investigate if treatment strategy influences QoL we compared patients in remission of pituitary CS treated with bilateral ADX to those treated without bilateral ADX. Furthermore, the results of each patient subgroup were compared to the results of the control group.

Study population

Two hundred and thirty-six patients met the inclusion criteria. However, 38 patients were known to be deceased and 9 patients were lost to follow-up. The remaining 189 patients were invited to participate in this study. One hundred and forty-nine patients (79%) responded, of whom 127 (85% of the patients that responded, 67% of the patients that were invited) were willing to participate. Four patients had recurrent CS and had to be excluded, leaving 123 patients (age 52.2 ± 12.0 years, 106 women) that were included. The duration of remission at the time of answering the questionnaires was 13.3 ± 10.4 years (range 2-39).

There was no significant difference in response rate and willingness to participate between patients that were in remission of pituitary CS or adrenal CS: 114 of 146 patients (78%) in remission of pituitary CS responded, of whom 99 (68%) were willing to participate, compared to 31 of 43 patients (72%) in remission of adrenal CS, of whom 24 (56%) were willing to participate.

One hundred and five controls (age 51.3 ± 12.8 years, 71 women) completed the questionnaires. Sex and age did not differ significantly between the patient and control group (Table 1).

Table 1) Socio-economic characteristics and comorbidity of patients and controls.

	patients (n=123)	controls (n=105)	P - value
age (years)	52.2 (± 12.0)	51. 3 (± 12.8)	0.56
gender (female)	78%	71%	0.25
self-reported comorbidity	79%	57%	‹0.01*
hypertension	28%	20%	0.18
chronic back complaints	18%	12%	0.25
osteoporosis / arthrosis	13%	10%	0.40
psychological problems	11%	5%	0.07
pulmonary disease	11%	12%	0.67
recurrent sinusitis	10%	6%	0.26
diabetes mellitus (type 1 or 2)	9%	4%	0.12
cardiovascular disease	6%	7%	0.76
number of self-reported diseases	1.8 (±1.6)	1.1 (±1.3)	‹0.01*
active smoker	20%	12%	0.15
smoked in the past	33%	40%	0.29
paid work	51%	70%	‹0.01*
self-reported inability to work	25%	0%	‹0.01*
education level	1.8 (±0.8)	2.1 (±0.8)	0.02*
relationship	75%	89%	0.01*
number of children	1.5 (± 1.5)	1.6 (± 1.3)	0.53

*Education level: 1=low, 2=middle, 3=high. *: significant difference between patients and control subjects.*

Definitions

Remission was defined as suppression of plasma cortisol to ≤ 50 nmol/L after overnight 1 mg dexamethasone, and absence of clinical signs and symptoms of active hypercortisolism (16) or, if a patient had received pituitary radiotherapy, a 24-hour urinary free cortisol value of <240 nmol/24h for men or <150 nmol/24h for women (reference range at our institution) and absence of clinical signs and symptoms of active hypercortisolism.
Hormonal deficiency was defined as deficiency of one or more hormones. Hypothyroidism was defined as the use of thyroid hormone substitution therapy. Hypogonadism was defined as use of testosterone substitution in men and use of estrogen in women (no difference was made between suppletion or contraceptive therapy). GH deficiency was defined as a maximal GH response < 15.3 mU/L during an insulin tolerance test (ITT), or as a maximal GH response of < 12.3 mU/L during an Arginine/GHRH test (17). Glucocorticoid deficiency was defined as the use of glucocorticoids. In virtually all cases glucocorticoid deficiency was confirmed by a plasma morning cortisol <100 nmol/L, or a maximal cortisol response < 550 nmol/L during an ITT (18). Hypopituitarism was defined as deficiency of one or more of the hormones that are secreted by the pituitary gland.

Quality of life questionnaires

The following set of QoL questionnaires was used in this study:

RAND-36. The Dutch translation of the RAND-36 was used, which is very similar to the Short Form-36 questionnaire [19]. It comprises 36 items regarding general wellbeing during the past 30 days. The items are formulated as statements or questions to assess 9 health concepts: 1) physical functioning; 2) social functioning; 3) role limitations because of physical health problems; 4) role limitations because of emotional problems; 5) mental health; 6) vitality; 7) bodily pain, 8) general health perceptions, 9) health change in the last year. Scores are calculated for each health aspect on a 0-100 scale, in which higher scores represent a better QoL [20].

Hospital Anxiety and Depression Scale (HADS). The HADS consists of 14 items concerning anxiety and depression, measured on a four-point scale [21, 22]. Scores range from 0-21 for the anxiety and depression subscales and from 0-42 for the total score. Higher scores indicate more anxiety or more depressive symptoms,

Checklist Individual Strength Questionnaire (CIS). The CIS is designed to measure several aspects of fatigue [23]. It consists of four dimensions: 1) the subjective experience of fatigue; 2) reduction in motivation; 3) reduction in activity and 4) reduction in concentration. The questionnaire consists of 20 statements, scored on a 7-point

Likert scale. The statements refer to aspects of fatigue experienced during the last two weeks. Scores range from 20 to 140 points. Higher scores indicate a higher degree of fatigue.

Cognitive Failures Questionnaire (CFQ). The CFQ detects everyday mistakes and problems regarding perception, memory, motor function and orientation [24]. The first part of the questionnaire consists of 25 items and the second part indicates the influence of these items on daily life. Scores range from 0 to 100, in which a higher score indicates more severe problems.

Appearance Self-Esteem (ASE). The ASE is a section of the *Self-report State Self-Esteem Scale* and comprises satisfaction with ones appearance [25]. Separate use of the ASE has been reported before [26]. The questionnaire consists of six statements, scored on a 5-point Likert scale. Scores range from 6 to 30. A higher score indicates more self-esteem concerning a person's own appearance.

Cushing's Quality of Life (CushingQoL). This unidimensional questionnaire, with a time frame referring to the preceding four weeks, covers several Cushing-related problems. It has been designed to analyse QoL in patients with active CS [27]. We used the questionnaire to analyse if patients in long-term remission of CS still have more CS-related symptoms than healthy controls. The answers are scored on a five-point Likert scale. The total score is converted to a 0-100 scale in which 0 indicates the worst and 100 the best possible QoL.

Nottingham Health Profile (NHP): The NHP contains 38 yes/no items that focus on general wellbeing and current health perception [28, 29]. The items are subdivided into six subscales that assess different health aspects: 1) energy; 2) pain; 3) emotional reaction; 4) sleep; 5) physical ability and 6) social isolation. Subscale scores are calculated as a weighted mean of the associated items and are expressed as a value between 0 and 100. A higher score is associated with a worse QoL.

Employment status and self-reported comorbidity. All participants received a questionnaire regarding their employment status. The most important aspects of this questionnaire were current employment, number of working hours per week, current sick leave and inability to work. Furthermore, participants received a checklist mentioning 16 common diseases (e.g. diabetes mellitus, cardiovascular disease, hypertension, and psychiatric problems). They were asked if they currently had any of these problems. They could also describe other health problems they experienced at the moment.

Statistics

Data were analysed using SPSS for Windows version 16.0 (SPSS Inc., Chicago, Ill). Data were expressed as mean ± SD, unless otherwise mentioned. To compare biometric and socio-economic characteristics of patients and controls we used unpaired t tests and χ^2 tests. To compare the results of the questionnaires of patients and controls, and those of the different patient subgroups, we chose to use the Kruskal-Wallis equality-of-populations rank test because the data of several questionnaires were not normally distributed. Furthermore, using multiple regression analysis, the influence of duration of remission (between 2 and 5 years, 5 and 10 years and more than 10 years), age and sex on the results of the questionnaires was investigated. Statistical significance was defined as $P < 0.05$.

Results

Patient characteristics

Eighty percent of the patients (n=99) were in remission of pituitary CS. Transsphenoidal surgery (TS) was the primary therapy for 85% (n=84) of these patients (Figure 1). Twelve percent (n=12) underwent a bilateral ADX and 3% (n=3) underwent radiotherapy of the pituitary gland as primary treatment. Forty patients received one or more additional treatments because of persistent or recurrent hypercortisolism (n=28), and/or because they developed Nelson's syndrome after bilateral ADX (n=12).

Twenty percent of all patients (n=24) were in remission of adrenal CS and were previously treated by unilateral ADX.

Partial or total hypopituitarism was present in 51% of all patients (n= 63) and all of these patients had been treated for pituitary CS. Hormonal deficiencies due to any cause, including primary adrenal insufficiency after bilateral ADX and primary hypothyroidism, were present in 65% of all patients (n=81) of whom 4 had been treated for adrenal CS.

In contrast to all other hormonal deficiencies, presence of GH deficiency had only been investigated in 74% (n=73) of the patients that were in remission of pituitary CS (insulin tolerance test or GHRH-arginine test). GH deficiency was present in 58% (n=42) of these patients. However, 21 of these 42 patients were not receiving treatment with GH at the time of the study, despite the proven GH deficiency, for various reasons (the initiation of treatment was pending at the time of the study, the treatment was discontinued or refused by the patients, there was a (relative) contraindication for GH substitution therapy, or the deficiency was not severe enough for treatment to be covered by the

health insurance companies). Four of these patients did not have other hormonal deficiencies. Furthermore, 11 of the 26 patients in whom GH deficiency had not been investigated, did not have other hormonal deficiencies. For our analyses we classified these patients as patients without hormonal deficiencies.

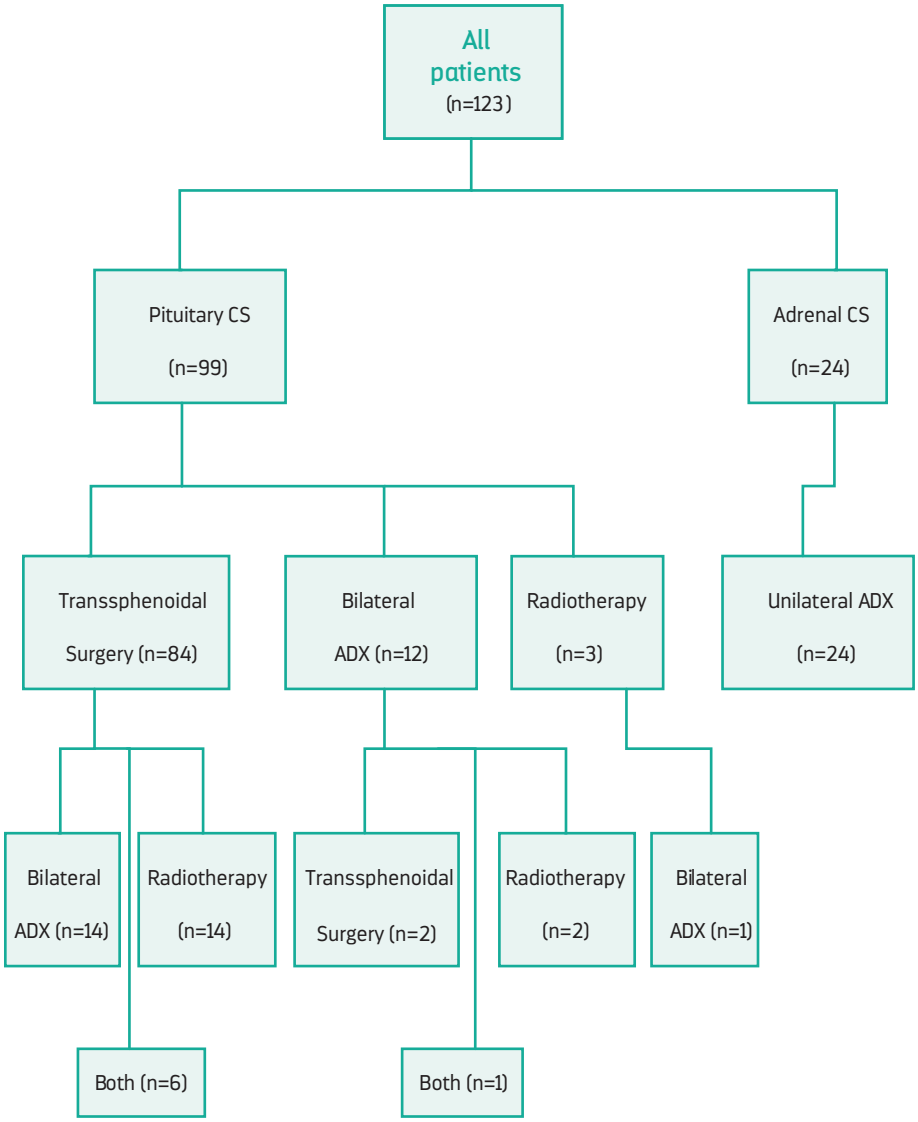

Figure 1) Overview of the treatments that the 123 patients in remission of Cushing's syndrome had received.

At the time they participated in the study, 63% of all patients (n=77) received replacement therapy with at least one hormone (replacement of cortisol in 55 patients (45%), thyroid hormone in 49 patients (40%, of whom 9 patients had primary hypothyroidism), growth hormone in 21 patients (17%), testosterone in 11 of the 17 males (68% of all males), estrogen in 5 females (5% of all females), desmopressin in 6 patients (6%), and fludrocortisone in the 33 patients that had been treated by bilateral ADX). Substitution therapy was individually titrated to achieve at least the lower limit of our institutional reference values if a hormonal deficiency was present (except for GH therapy in the 21 patients with GH deficiency described earlier).

Comparison of QoL between the total patient group and the healthy control group

Each questionnaire showed that QoL in the total patient group was significantly reduced compared to the healthy control group. The only items that were not significantly different between the patient and control group were 'health change' and 'emotional role limitation' of the RAND-36. Furthermore, compared to the control subjects, significantly fewer patients had a relationship, their education level was lower, they had less paid work and more patients were unable to work (Table 1). Compared to the control subjects significantly more patients reported comorbidity and the average number of comorbidities was significantly higher (Table 1). However, the prevalence of different diseases (e.g. hypertension, cardiovascular disease, diabetes) in the patient and control group did not significantly differ, nor did smoking habits (Figure 2).

Comparison of QoL between different patient subgroups:

Adrenal CS (n=24) versus pituitary CS (n=99) (Figure 2*):* QoL did not significantly differ between these groups on any item of any questionnaire. Both patients with adrenal CS and pituitary CS scored significantly worse on all items compared to the control group with the exception of the items 'health change' and 'emotional role limitation' of the RAND-36.

Pituitary CS with hormonal deficiencies (n=77) versus pituitary CS without hormonal deficiencies (n=22): Patients treated for pituitary CS without permanent hormonal deficiencies scored significantly better than patients with hormonal deficiencies on the items fatigue and motivation of the CIS questionnaire, the CushingQOL questionnaire, and the item energy of the NHP questionnaire. Furthermore, although the patients in remission of pituitary CS without hormonal deficiencies do have a significantly impaired QoL compared to the control group on approximately 50% of the items of QoL questionnaires (physical functioning, role limitations because of physical health prob-

lems, vitality, and general health perceptions of the RAND-36, depression of the HADS, experience of fatigue and concentration of the CIS, the CushingQOL questionnaire and emotional reaction and physical ability of the NHP), differences were not significant on the other items.

Pituitary CS treated with bilateral ADX (n=33) versus no bilateral ADX (n=66) and Pituitary CS with hormonal deficiencies with glucocorticoid substitution (n=58) versus without glucocorticoid substitution (n=19): QoL did not differ between these groups on all items of all questionnaires.

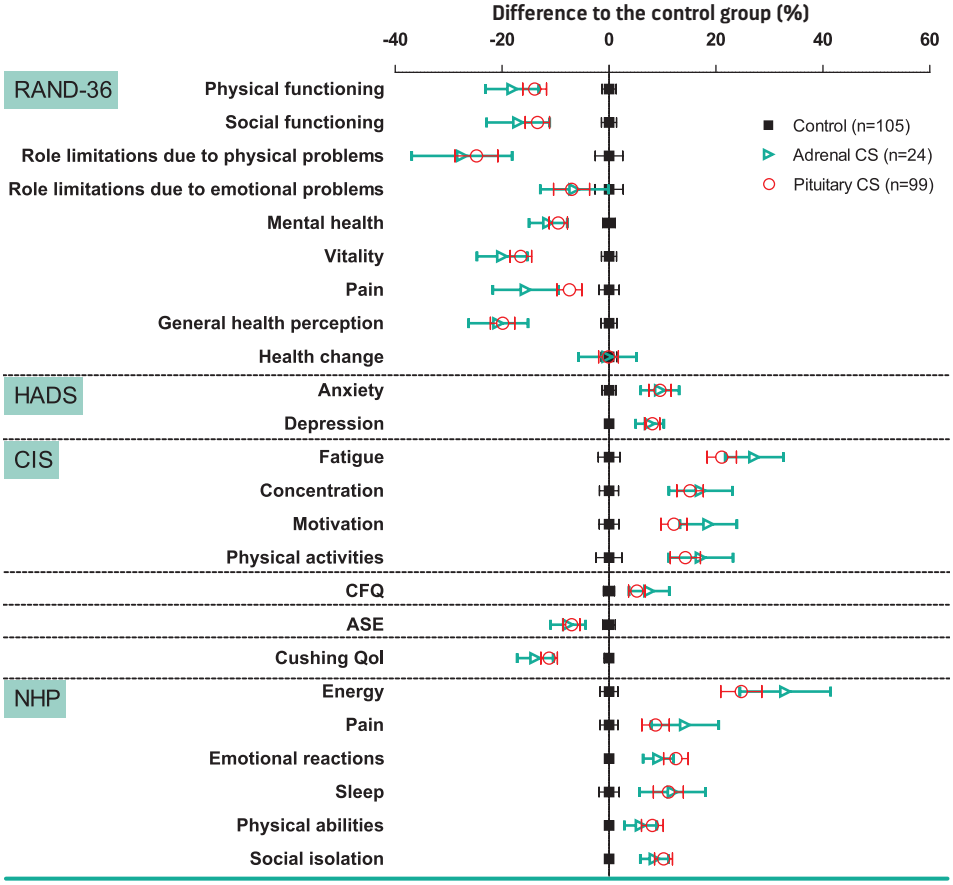

Figure 2) The difference in mean score on each dimension of the quality of life questionnaires between patients in remission of pituitary and adrenal Cushing's syndrome compared to the control group, with the standard error of the mean. The maximum score has been recalculated to be 100 for all questionnaires.

Influence of duration of remission, age and gender on QoL

Multiple regression analysis showed that in the patient group duration of remission (analysed as remission for 2-5 years, 5-10 years or 10 years and longer) significantly affected QOL, with a lower QoL if duration of remission was shorter, on the dimension 'depression' of the HADS, the dimension 'concentration' of the CIS, on the CFQ and on the CushingQOL questionnaire. Furthermore, as could be expected, duration of remission significantly affected the dimension 'health change' of the RAND-36, with more improvement in health in the past year, if duration of remission was shorter. Older age negatively influenced QoL only on the dimension 'depression' of the HADS questionnaire. Female gender negatively influenced QoL on the dimensions 'physical functioning', 'mental health', 'vitality', 'pain', and 'general health perception' of the RAND-36, the dimension 'fatigue' of the CIS questionnaire, the CFQ, ASE and Cushing QoL questionnaire, and the dimension 'energy', 'pain', 'sleep' and 'social isolation' of the NHP.

In the control group, older age had a much stronger negative influence on the reported QoL, significantly affecting QoL on most items. On the other hand, female gender only significantly affected QoL in the CushingQOL questionnaire and the dimension 'mental health' of the RAND-36.

Discussion

In this study we evaluated QoL in 123 patients in long-term remission of CS, to get more insight in the determinants related to impaired QoL. The results of this study show that, compared to a gender- and age-matched control group, QoL remains impaired in all subgroups of patients in long-term remission of CS, regardless of the aetiology of CS, presence of hormonal deficiencies and the treatment received. Importantly, QoL was impaired to the same extent in patients treated for adrenal CS and in patients treated for pituitary CS. Furthermore, duration of remission only affected QoL on a number of dimensions, with a better QoL if duration of remission was longer. Women in remission of CS scored worse than men on approximately 60% of items of QoL questionnaires, while gender was of no significant influence on QoL in the control group. Age, however, did not have a strong influence on QoL in the patient group while it did have a strong influence in the control group.

Although a few previous studies on QoL in patients in remission of CS have included patients that were in remission of adrenal CS, until now there were no studies that had specifically made a comparison between QoL in patients in long-term remission of pituitary CS and in long-term remission of adrenal CS [5, 7, 13, 15, 27]. Lindholm et al. [7] made a short

comment that QoL seemed quite satisfactory in the patients treated for an adrenal adenoma. However QoL was not the main outcome of their study and data about the clinical characteristics of their patients are sparse. Furthermore, they defined relative health impairment as a score below the 25th percentile of the Danish norm. No direct comparison was made with an age- and sex-matched control group. Webb et al (27) compared 18 patients with adrenal and 107 patients with pituitary CS and did not find a difference in QoL between the two groups, assessed by two questionnaires (SF-36 and CushingQOL). However a substantial part of their patients (31%) were hypercortisolemic at the moment of investigation or had only recently been treated. Interestingly, we found that after long-term remission, QoL is at least as impaired in patients in remission of adrenal CS as in patients in remission, of pituitary CS. To our knowledge, this is the first study to show this, which is surprising as we hypothesized that patients in remission of adrenal CS would have a better QoL than patients in remission of pituitary CS because they are expected not to have any hormonal deficiencies and normal endocrine physiology is restored.

The presence of hormonal deficiencies in patients treated for CS has previously been associated with impaired QoL. Van Aken et al. previously found that hypopituitarism was the main independent predictor of impaired QoL in patients in remission of pituitary CS [9]. We confirmed this finding in our study, and found that not only hypopituitarism, but also hormonal deficiencies caused by bilateral ADX were associated with worse QoL. Our patients in remission of pituitary CS without hormonal deficiencies did have a significantly better QoL than patients with hormonal deficiencies on a number of QoL items, and they were the only patient group that did not have significantly worse QoL than the control group on approximately 50% of QoL dimensions, but QoL remained impaired in the other 50% of dimensions.

Current glucocorticoid replacement regimens are not physiological and there is a risk of overreplacement. QoL has been found to be impaired in patients receiving glucocorticoid substitution (30). Therefore we hypothesized that glucocorticoid substitution might be an important cause of impaired QoL in patients with hormonal deficiencies. To investigate this hypothesis we first compared the QoL of patients receiving glucocorticoid substitution with the QoL of patients with hormonal deficiencies but without glucocorticoid substitution, and second the QoL of patients that had been treated with bilateral ADX to the QoL of patients with hormonal deficiencies that had not been treated with bilateral ADX. Because there was no difference in QoL between these patient groups it is unlikely that the impaired QoL in patients in long-term remission of CS with hormonal deficiencies is strongly influenced by the glucocorticoid replacement regime.

Because QoL is impaired in all patient groups in long-term remission of CS, the cause of impaired QoL could be the previous period of longstanding exposure to high cortisol levels. There is accumulating evidence that longstanding hypercortisolism has irreversible effects on cardiovascular risk and that patients in remission of CS have persistent centripetal obesity which may lead to a state of low-grade inflammation [2, 3, 31, 32]. Furthermore, CS is associated with morphological changes of the brain (including brain atrophy, reduction in total and cortical grey matter, and reduced hippocampal volume), which at least partially persist after long-term remission [33, 34]. This may explain the chronic cognitive impairments described in patients in long-term remission of CS [4, 35] which may negatively influence QoL. Other explanations for the impaired QoL could be the previous experience of suffering a severe illness, ineffective coping strategies or ineffective illness perceptions [35, 36]. Future research will have to determine the exact causes of impaired QoL in patients in long-term remission of CS.

Multiple linear regression analysis showed that, in our patient group, female gender negatively influenced QoL. This effect was not present in the control group. Although previous studies have also described that female gender negatively influences QoL in patients in remission of CS, it is still unclear what causes this phenomenon [9, 27]. A possible explanation of the negative influence of female gender on QoL could be differences in the long term effects of the previous period of hypercortisolism in men and women. This could be caused by a different clinical presentation, which has previously been described in pituitary CS [37]. Another explanation could be differences in coping strategies and illness perceptions between men and women. Although gender differences in coping strategies and illness perceptions have been reported in numerous other diseases [38, 39], this has not yet been investigated in patients with CS.

Although shorter duration of remission did have a negative impact on QoL on a number of dimensions, most dimensions of QoL was not affected by duration of remission on most dimensions of QOL, suggesting that the majority of effects of CS on QoL have reached a stable situation after 2 years of remission. Van Aken et al. even found that duration of remission did not affect QoL at all [9]. However, in contrast to van Aken, who analysed duration of remission as a continuum, we analysed duration of remission in different categories (from 2-5 years, 5-10 years and >10 years), because we hypothesized that the largest effect of remission on QoL would take place in the first decade after treatment. Interestingly, in our study, the (dimensions of the) QoL questionnaires that were affected by duration of remission with a lower QoL if duration of remission was shorter, were the dimension 'depression' of the HADS, 'concentration' of the CIS, the CFQ and on the CushingQOL questionnaire. The combination of these QoL dimensions

suggests problems in coping with the recent experience of CS, which improves as time goes by. Another possible explanation could be that the effect of hypercortisolism on the brain, causing psychological problems and cognitive impairments, takes longer to reach a stable situation after remission than other effects of hypercortisolism.

Strengths of this study are the relatively large number of patients included and the long average duration of remission (13.3 ± 10.4 years). A limitation of this study is the fact that GH deficiency was not ruled out in 26 patients in remission of pituitary CS. Because 11 of these patients had no other hormonal deficiencies and normal IGF-1 values, we analysed these patients as having no hormonal deficiencies. If some of these patients do have GH deficiency this may have negatively influenced QoL in the patient group without hormonal deficiencies. Furthermore 21 patients with proven GH deficiency were not receiving treatment for a number of different reasons. This could have negatively influenced the QoL of the total patient group and the patient group with hormonal deficiencies. However, in patients with adult-onset GH deficiency, GH therapy has only been found to have a positive effect on QoL if disease-specific questionnaires are used. In studies that use generic QoL questionnaires, as we used in this study, the positive effect of GH therapy on QoL in GH deficient patients is much more controversial and most studies that did find a positive effect of QoL only found this in a few dimensions of the generic QoL questionnaires [40]. Therefore the presence of untreated GH deficiency can certainly not account for the obvious impairment of QoL of the total patient group. Another possible limitation of this study is that not all patients found a control subject and not all control subjects were gender matched. However, we controlled for gender in our analysis by using a multiple regression analysis. Furthermore, not all control subjects were healthy, reporting comorbidity in 57% (Table 1). However, if this influenced QoL in the control group it would have been a negative influence on QOL, reducing the difference in QoL between the patient and control group.

In conclusion, QoL remains impaired in patients in long-term remission of CS, regardless of aetiology, presence of hormonal deficiencies and treatment strategies. The cause of impaired QoL remains unclear and is probably multifactorial. More research is needed to establish the causes of impaired QOL, and hopefully this will provide targets for interventions that can improve QoL in patients in long-term remission of CS in the future.

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Long-term physical sequelae after

TREATMENT OF ACROMEGALY AND CUSHING'S SYNDROME

PART 3

Three-dimensional facial analysis in acromegaly:

a novel tool to quantify craniofacial characteristics after long-term remission

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Abstract

Purpose: The exact quantification of craniofacial characteristics in patients with acromegaly is important because it provides insight in the pathophysiology of the disease and offers a tool to evaluate the effects of treatment on tissue-specific endpoints. However, until recently this was not feasible due to limitations of available cephalometric methods. The new technique of three-dimensional (3D) cephalometry enables the accurate quantification of facial anatomical characteristics of both soft tissue and bone. This is the first study that uses 3D cephalometry to analyze craniofacial disproportions in patients in long-term remission of acromegaly.

Methods: Sixteen patients in remission of acromegaly for over 24 months (50% male, mean age 56.0 ± 10.7 years, mean BMI 29.3 ± 5.5 kg/m^2) were compared to 16 matched control subjects. A 3D cone beam computed tomography scan and 3D stereophotograph of each individual were acquired and analyzed using 3D cephalometry.

Results: In addition to an accurate quantification of the classical craniofacial characteristics, 3D cephalometry, shows that many typical soft tissue deformities persist, even after long-term remission. Furthermore, we found that, compared to controls, the patients in remission of acromegaly have a wider face at the level of the zygoma and a longer maxilla (p<0.05).

Conclusions: 3D cephalometry is an attractive novel imaging modality to accurately investigate craniofacial disproportions of both soft tissue and bony parts of the face in patients with acromegaly, which makes it a promising technique for future research purposes and clinical practice.

Introduction

Acromegaly is a rare clinical syndrome caused by prolonged exposure to excessive amounts of growth hormone (GH) [1]. The GH excess causes proliferation of many tissues, including connective tissue, cartilage, bone and skin [2]. Because the signs and symptoms of acromegaly develop insidiously, there is often a delay in diagnosis for up to ten years. Therefore, most patients have a pathognomonic phenotype with marked craniofacial disproportions when acromegaly is finally diagnosed [1].

Since acromegaly was first described in 1886 the craniofacial disproportions have been investigated. In the early days, a multitude of case reports already described the characteristic frontal bossing, prominence of the supraorbital ridges and mandibular prognathism in combination with hypertrophy of the tongue, lower lip and nose, which can be seen in virtually all patients with active acromegaly [3-6].

The changes in appearance in active acromegaly are associated with a significantly impaired quality of life [7], both due to cosmetic aspects as to maxillofacial complaints [8]. However, nowadays acromegaly can almost always be cured or controlled. After successful treatment some features of acromegaly, such as sleep apnea and cartilage thickening in joints, show at least partial reversibility [9,10]. The exact quantification of craniofacial characteristics in patients with acromegaly is important because it offers a tool to evaluate the effects of treatment on tissue-specific endpoints and provides insight in the pathophysiology of the disease. However, at present it is unknown if the craniofacial disproportions are reversible after successful treatment, as the current clinical assessment of craniofacial changes does not enable exact quantification. As a consequence, it is unclear how patients should be informed about cosmetic changes after successful treatment, and if and how maxillofacial pathologies should be followed-up or treated.

Since the 1960's standardized radiographic cephalometry by using two-dimensional (2D) cephalograms has been widely adopted for the evaluation of craniofacial morphology and growth [11]. A number of studies have analyzed the extent of craniofacial disproportions in acromegaly using this technique [8, 12-16]. However, 2D cephalometry has many limitations. The data collected from 2D cephalograms can only describe the hard tissue and soft tissue profile with regard to the midfacial plane, omitting the morphologic features in all other facial regions. Thus, the use of 2D imaging to describe complex three-dimensional (3D) craniofacial structures will inevitably lead to loss of data. Furthermore the measurements obtained are subject to magnification and land-

mark identification errors. This limits the value of all previous cephalometric studies on craniofacial characteristics in patients with acromegaly. Furthermore, all previous studies have other limitations, such as mixed study populations of active and cured acromegaly and unspecified duration of follow-up in cured patients.

In the past decade cephalometry using 3D imaging technology has rapidly evolved [17]. Cone beam computed tomography (CBCT) has become a well-established alternative to 2D cephalograms, providing 3D image data of bony tissue structures in the facial region. In order to capture the 3D soft tissue facial profile, 3D stereophotogrammetry was developed [18-19]. By image fusion, the hard tissue data of CBCT and soft tissue data of 3D stereophotogrammetry can be combined into a 3D virtual head model, making it possible to quantify all dimensions of the face (bone and soft tissues) [20]. Because 3D cephalometry is much more accurate than 2D cephalometry, and relatively easy to perform with the use of computers, it has already been implemented in the daily practice in oral and maxillofacial surgery, but it has never been used to quantify the craniofacial disproportions in patients with acromegaly.

The aim of the present study was to evaluate the differences in craniofacial dimensions of soft and bony tissues between patients in long-term remission of acromegaly and control subjects matched for gender, age, body mass index (BMI) and ethnicity, using 3D cephalometry and 3D image fusion head models, which were used for the first time for this purpose.

Subjects and Methods

Subjects

All adult patients of the Department of Medicine, Division of Endocrinology, of the Radboud University Nijmegen Medical Center, who were in remission of acromegaly for at least 2 years after successful transsphenoidal pituitary surgery, were eligible for this cross sectional case-control study. The initial diagnosis of acromegaly was based on clinical grounds and biochemical tests, including assessment of serum GH levels (basal and during an oral glucose tolerance test (OGTT)) and serum insulin-like growth factor type-1 (IGF-1) levels. Remission was defined as disappearance of clinical symptoms of active GH hypersecretion with normal serum IGF-1 levels (≤ mean + 2 standard deviations for age) and suppression of serum GH levels to < 2 mU/L during OGTT [21]. All patients who had undergone maxillofacial surgery in the past were excluded from this study. Furthermore, all patients that received GH substitution therapy because of GH deficiency after

remission of acromegaly were excluded. A total of 5 patients used another form of hormonal replacement therapy: 4 patients used levothyroxine suppletion and 1 patient used testosterone suppletion. They had received this suppletion for at least 2 years. All patients had been screened for hormonal deficiencies several times after transsphenoidal pituitary surgery.

Sixteen patients met all inclusion and no exclusion criteria, and agreed to participate. Fifty percent of the patients were male, age at time of study was 56.0 ± 10.7 years (mean ± SD) and BMI was 29.3 ± 5.5 kg/m². The patients had been in remission of acromegaly for an average of 8.5 ± 8.6 years (range 2-36). Each patient was matched to a healthy gender-, age-, BMI- and ethnicity-matched healthy control subject. The 16 control subjects were recruited via an advertisement in a local newspaper. They had no history of maxillofacial surgery or trauma. In the control group 50% of the subjects were male, age was 56.6 ± 11.1 years and BMI was 29.5 ± 4.7 kg/m², which is not statistically different from the patient group. As all control subjects were healthy, they did not use any hormonal replacement therapy.

The study was approved by the Ethics Committee of the Radboud University Nijmegen Medical Center and conformed to the declaration of Helsinki. All participants gave written informed consent prior to their participation in the study.

Study protocol and image acquisition

On the day of the study, serum IGF-1 was determined in all patients to exclude recurrent acromegaly. A CBCT scan and 3D stereophotograph were acquired from all patients and control subjects. The CBCT scan was acquired using the i-CAT™ 3D Imaging System (Imaging Sciences International, Hatfield, PA, USA). CBCT scanning was performed in "Extended Field" mode (field of view: 16 cm diameter/22 cm height; scan time: 2 x 20 seconds; voxel size: 0,4 mm) at 120 kV and 3-8 mAs pulse mode resulting in a radiation dose of 136 μSv for a single scan. All subjects were scanned vertically in a natural, seated position with the occlusal plane parallel to the horizontal positioning line of the scanner. They were asked to bite in maximum intercuspidation, relax their lips and keep their eyes open.

Three-dimensional stereophotographs were acquired using a stereophotogrammetrical camera set-up (3dMDCranial™ System, 3dMD LLC, Atlanta, USA), which generates a 3D stereophotograph from six 2D photographs taken simultaneously (four grey-scale photographs and two full color photographs). All subjects were photographed seated, with their head in a natural position, habitual occlusion, opened eyes and relaxed facial musculature.

3D cephalometric analysis

Data from the CBCT scan and 3D stereophotography were registered using a surface based registration algorithm [20] and rendered to a 3D virtual head model using Maxilim® software (Medicim NV, Mechelen, Belgium). In order to align all virtual head models in the same orientation, a 3D cephalometric reference frame was set up according to the procedure described by Swennen et al. [22]. Thirty-three soft tissue and 21 hard tissue landmarks were identified (Table 1). A modified Swennen hard tissue and soft tissue cephalometric analysis was carried out [17]. This resulted in a total of 39 hard tissue and 111 soft tissue measurements, consisting of distances, angles and proportions.

Distance color mapping

Four average faces were computed according to the method described by Zhurov et al [23] (an average male acromegalic face, an average female acromegalic face, an average male control face and an average female control face). Comparing these average faces, a color histogram was rendered, illustrating the differences between the acromegaly group and control group, for both males and females.

Statistical analyses

Data were analyzed using SAS 9.2. (SAS Institute, Cary, NC). Data are presented as median unless otherwise stated. The outcomes of the soft tissue and hard tissue cephalometric measurements of the patient and control groups were compared using the Wilcoxon signed rank test. Confidence intervals for the median difference were calculated according to the method of Conover. Statistical significance was defined as $P < 0.05$.

In order to investigate whether the increase in total mandibular length (Co-Pog) is correlated to the observed increase in horizontal (Go-Pog) and/or vertical (Co-Go) dimension of the mandible, differences in Co-Go, Go-Pog and Co-Pog between the patients and controls were calculated, resulting in ΔCo-Go, ΔGo-Pog and ΔCo-Pog. ΔCo-Pog was analyzed using a mixed linear model, with factors side (left or right), covariates ΔCo-Go, ΔGo-Pog and ΔCo-Go*ΔGo-Pog (the interaction between ΔCo-Go and ΔGo-Pog) and a random effect of the factor side to deal with the correlation between the left and right side outcomes. The model was simplified by stepwise removing of the non-significant factor side and the interaction term (ΔCo-Go*ΔGo-Pog), resulting in a final model with only the covariates ΔCo-Go and ΔGo-Pog: $\Delta CoPog = 4.4633 + 0.4203 \times \Delta CoGo + 0.6928 \times \Delta GoPog$.

Table 1) Hard and soft tissue landmarks and their definitions. *(page 147-149)*

Hard tissue landmarks	Definition
A-point (A)	The point of maximum concavity in the midline of the alveolar process of the maxilla
Anterior nasal spine (ANS)	The most anterior midpoint of the anterior nasal spine of the maxilla
B-point (B)	The point of maximum concavity in the midline of the alveolar process of the mandible
C-point (C)	The most caudal point of the margin of the sigmoid notch
Condylion (Co)*	The most postero-superior point of the mandibular condyle
Frontozygomatic suture (Fz)*	The most medial and anterior point of the left frontozygomatic suture at the level of the lateral orbital rim
Gonion (Go)*	The most caudal and most posterior point of the mandibular angle
Lower incisor (LI)*	The most mesial point of the tip of the crown of the lower central incisor
Lower molar (LM)*	The most superior point of the mesial cusp of the crown of the left first lower molar in the sagittal plane
Pogonion (Pog)	The most anterior midpoint of the chin
Menton (Men)	The most inferior midpoint of the chin on the outline of the mandibular symphysis
Nasion (N)	The midpoint of the frontonasal suture
Orbitale (Or)*	The most inferior point of the inferior orbital rim
Pogonion (Pog)	The most anterior midpoint of the chin on the outline of the mandibular symphysis
Porion (Po)*	The most superior point of the meatus acusticus externus
Posterior maxillary point (PMP)	The point of maximum concavity of the posterior border of the palatine bone in the horizontal plane
Posterior nasal spine (PNS)	The most posterior midpoint of the posterior nasal spine of the palatine bone
Sella (S)	The center of the hypophyseal fossa (sella turcica)
Upper incisor (UI)*	The most mesial point of the tip of the crown of the upper central incisor
Upper molar (UM)*	The most inferior point of the mesial cusp of the crown of the first upper molar in the sagittal plane
Zygion (Zy)*	The most lateral point on the outline of the right zygomatic arch

Soft tissue landmarks	Definition
Alar curvature point (ac)*	The point located at the facial insertion of the alar base
Alare (al)*	The most lateral point on the left alar contour
Columella constructed point (c`)	The midpoint of the columella crest at the level of the nostril top points
Cheilion (ch)*	The point located at the labial commissure
Christa philitri (cph)*	The point at the crossing of the vermilion line and the elevated margin of the philtrum
Endocanthion (en)*	The soft tissue point located at the inner commissure of the eye fissure
Exocanthion (ex)*	The soft tissue point located at the outer commissure of the eye fissure
Glabella (g)	The most anterior midpoint on the fronto-orbital soft tissue contour
Glabella`(g`)	The point localized on the midline tangent of the frontal contour cranial to glabella
Gonion (go)*	The most lateral point on the soft tissue contour of the left mandibular angle, located at the same level as the 3D hard tissue cephalometric Gonion landmark
Gnathion (gn)	The most inferior midpoint of the soft tissue contour of the chin, located at the level of the 3D cephalometric hard tissue Menton landmark
Gnathion` (gn`)	The point localized on the midline tangent of the chin contour posterior to gnathion
Labiale inferius (li)	The midpoint of the vermilion line of the lower lip
Labiale superius (ls)	The midpoint of the vermilion line of the upper lip
Maxillofrontale (mf)*	The soft tissue point located at the lateral margin of the nose base at the level of endocanthion
Nasion (n)	The midpoint on the soft tissue contour of the base of the nasal root at the level of the frontonasal suture
Nostril base point (nb)*	The lowest point of the nostril or the inferior terminal point of the nostril axis
Proximal nasal root tangent (nrt1)	The proximal point on the midline of the nasal root
Distal nasal root tangent (nrt2)	The distal point on the midline of the nasal root
Nostril top point (nt)	The highest point of the nostril or the superior terminal point of the nostril axis

Soft tissue landmarks	Definition
Orbitale inferius (oi)*	The soft tissue point located at the most inferior level of the left infraorbital rim, located at the level of the 3D hard tissue cephalometric Orbitale landmark
Orbitale superius (os)*	The soft tissue point located at the most superior level of the supraorbital rim
Pogonion (pg)	The most anterior midpoint of the chin
Porion (po)*	The soft tissue point located at the level of the 3D hard tissue cephalometric Porion landmark
Pronasale (prn)	The most anterior midpoint of the nasal tip
Sellion (se)	The most posterior point of the frontonasal soft tissue contour in the midline of the base of the nasal root
Sublabiale (sl)	The most posterior midpoint on the labiomental soft tissue contour that defines the border between the lower lip and the chin
Subnasale (sn)	The midpoint on the nasolabial soft tissue contour between the columella crest and the upper lip
Subnasale` (sn`)*	The point at the margin of the midportion of the columella crest
Subspinale (ss)	The most posterior midpoint of the philtrum
Stomion inferius (sti)	The midpoint of the upper border of the lower lip
Stomion superius (stu)	The midpoint of the lower border of the upper lip
Zygion (zy)*	The most lateral point on the soft tissue contour of the zygomatic arch, located at the level of the 3D hard tissue cephalomteric Zygion landmark

*=*Bilateral landmarks*

Results

3D Hard tissue cephalometry

The outcomes of the hard tissue cephalometric measurements are presented in Supplemental Table 1 *(at http://link.springer.com/article/10.1007%2Fs11102-014-0565-x).* Nine of the 39 hard tissue measurements are significantly different between the patients in long-term remission of acromegaly and the matched healthy control subjects (Figure 1). Compared to the control subjects, the patients have both a longer and wider facial skeleton expressed by a larger total anterior facial height and lower anterior facial height (median + 12.0 and + 10.8 mm, both $p<0.01$) and an increase in the bizygomatic width (+ 2.95 mm, $p<0.01$).

Furthermore, both the maxilla and the mandible are larger in patients: the maxillary length is increased (+ 3.8 mm, p ‹ 0.01) as well as the vertical mandibular height (right side + 5.7 and left side + 6.2 mm, p=0.02 and p=0.01, respectively) and total mandibular length (right side + 7.6 mm and left side + 8.9 mm, both p ‹0.01). As can be seen in Table 2, an increase in both the vertical mandibular ramus height and the horizontal mandibular ramus length significantly contribute to increase in the total mandibular length.

Additionally, the angle of the frontal inclination of the occlusal plane to the horizontal plane is greater in the patient group (+ 1°, p‹0.01), indicating the tendency of transversal asymmetry in the lower third of the face in the acromegalic patients.

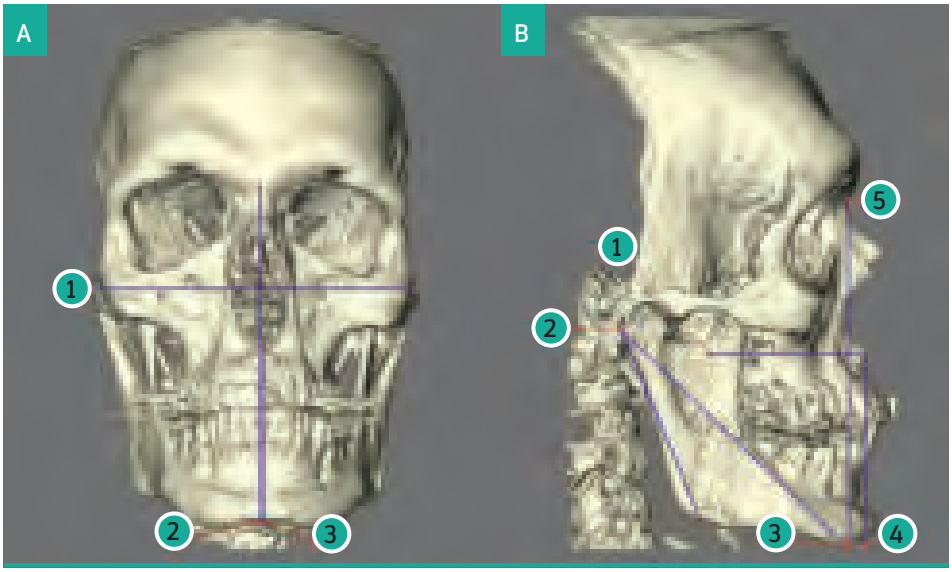

Figure 1) 3D cone beam CT scan of the scull of one of the patients in remission of acromegaly. The projected lines depict the hard tissue measurements that differ between patients and controls.

A) *1) Bizygomatic width (Zy $_r$ - Zy $_l$) 2) Anterior total facial height (N - Men), 3) Anterior lower facial height (ANS - Men). The lines are.*

B) *1) Maxillary length (ANS – PNS), 2) Mandibular vertical ramus length ColGol/CorGor), 3) Mandibular total length (ColPog/CorPog), 4) Anterior lower facial height (ANS - Men) 5) Anterior total facial height (N - Men).*

Table 2) Summary of mixed model analysis to study if the increase in length of the total mandibula can be explained by an increase in the vertical and/or horizontal dimension.

	Estimate	SE	P-value
Intercept	4.4633	1.6550	0.0173
Δ CoGo	0.4203	0.0959	0.0007
Δ GoPog	0.6928	0.1955	0.0027

3D soft tissue cephalometry

The distance color maps (Figure 2) show how the average soft tissue profile of the patients in long-term remission of acromegaly differs from the average soft tissue profile of the healthy control subjects for both men and women. The outcomes of the soft tissue cephalometric measurements are presented in Supplemental Table 2 *(at http://link.springer.com/article/10.1007%2Fs11102-014-0565-x).* Thirty of the 111 soft tissue measurements are significantly different between the patients in long-term remission of acromegaly and the matched healthy control group, all indicating growth in the patient group.

Compared to the healthy control group, the patients in long-term remission of acromegaly have a longer face: the morphological height of the face (+ 9.4 mm, $p<0.01$), the height of the upper face (+ 5.1 mm, $p=0.01$), the height of the lower face (+ 7.5 mm, $p=0.01$), the height of the lower profile (+ 5.5 mm, $p=0.02$), the lower right and left half of the craniofacial height (+ 10.5 mm, $p< 0.01$) and the right and left orbitogonial distances (+ 7.7 and 5.3 mm, $p=0.02$) are significantly larger. The face is also proportionally longer in the patient group, expressed in a greater facial index (+ 8 in the ratio total facial length and facial width, $p=0.01$) and the patients have a proportionally narrower lower third of the face (-11.5 in the ratio lower face width / morphological height of the face, $p<0.01$).

Furthermore the patients have a larger nose, characterized by a longer nasal bridge (+ 4.9 mm, $p=0.02$), a wider nasal root (+ 2.3 mm, $p<0.01$), a greater nasal tip (+ 2.8 mm, $p=0.01$) and nasal root protrusion (right side + 0.3 and left side + 1.8 mm, $p=0.18$ and $p=0.04$), a larger columella base-facial insertion depth (right side + 2.3 and left side + 2.1 mm, $p<0.01$) and a larger 3D alar length (right side + 3.6 and left side + 4.6 mm, $p <0.01$). In the patient group nasolabial angle is decreased (-9.0 °, $p=0.01$).

Figure 2) Comparison between the average A) female and B) male faces of the acromegaly patient group and control group.

Blue areas represent areas in which the average acromegalic face is more prominent than the average control face and orange areas represent areas were the average control face is more prominent than the average acromegalic face.

With regard to the orbital region, the right and left endocanthion-facial midline distance is larger in the patient group (right side + 2.0 and left side + 2.8 mm, p= 0.07 and p=0.01), resulting in a large intercanthal width. The intercanthal index is larger in the patient group which indicates a tendency to develop telecanthus (+2.9, p=0.02. The angle of inclination of the right and left orbital rim line from the vertical plane is also greater, which suggests frontal bossing (right side + 4.6° and left side + 2.15°, p=0.03 and p=0.15).

Furthermore the mandible is larger in the patient group with an increased height (+ 4.2 mm, p=0.02) and depth of the mandible (+ right side 1.7 mm, and + left side 3.2 mm, p=0.24 and p=0.03). In comparison to the control group, the patient group has a significantly wider mouth (+ 2mm, p=0.04). The lower lip of the patients is longer (+ 3.2 mm in height of lower lip, p=0.02) and tends to incline more anteriorly, making the lower lip even more pronounced in the facial profile (+ 13.5°, p=0.04). The mentocervical angle is smaller (- 8.3°, p=0.01), which indicates a more prominent chin.

Discussion

This is the first study that uses 3D cephalometry to analyze craniofacial disproportions in (former) acromegaly patients. Because of the advantages of 3D cephalometry compared to 2D cephalometry, our study is the first study that accurately describes which craniofacial disproportions of both bony structures and soft tissues persist in patients in long-term remission of acromegaly and accurately quantifies the magnitude of the disproportions.

We investigated the differences in craniofacial dimensions between 16 patients in long-term remission of acromegaly and a control group matched for gender, age, BMI and ethnicity. Because all previous studies on craniofacial changes in patients with acromegaly performed a 2D cephalometric analysis using standardized lateral cephalograms, their focus was on changes in the length of the viscerocranium, neglecting dimensional changes in the transverse plane [8,12,14]. In line with the findings of Dostálová et al. who analyzed a group of patients with active and treated acromegaly [14], we found that the total facial height and the height of the lower face are enlarged in patients in long-term remission of acromegaly. However, our study also shows that patients in long-term remission of acromegaly have an increased skeletal width at the level of the zygoma. This indicates that the facial growth takes place both in vertical and in transverse directions, although, proportionally, the vertical growth is the largest. Interestingly, with the soft tissue cephalometric analysis no differences in the bizygomatic and bigonial width are found between the patient and the control group, suggesting that patients in long-term remission of acromegaly have less soft tissue in the region of the zygoma and mandibular angles than control subjects. It is known that active GH hypersecretion is associated with a reduced fat mass due to increased lipolysis [24]. As the soft tissue in the region of zygonion and gonion is mainly composed of malar and subcutaneous adipose tissue, the reduced amount of soft tissue indicates that the facial adipose tissue volume remains reduced among patients, even after long-term remission of acromegaly.

In line with all previous studies on craniofacial changes in acromegaly, we found a marked increase in the size of the mandible (total mandibular length) among patients in long-term remission of acromegaly. We demonstrated that this increase can be explained by growth of the mandible both in the vertical and horizontal directions, although the horizontal growth of the horizontal ramus was not statistically significant. In contrast with the previous studies we found that the maxillary length was also increased in former acromegaly patients compared to control subjects. Kunzler and Herrmann found an

increased sella-nasion-B point (SNB) angle and an unchanged sella-nasion-A point (SNA) angle in patients with controlled and active acromegaly, which suggested absence of maxillary growth [8,12]. Dostálová even found retroposition of the maxilla (a diminished SNA angle) in women [14]. In the current study the SNA and SNB angles did not differ between the patient and control groups. A possible explanation for this discrepancy is the difference in imaging and cephalometry techniques. Three-dimensional cephalometry is less susceptible to magnification and landmark identification errors than 2D cephalometry. Furthermore the previous studies did not use an adequately matched control group, so their results could be influenced by differences in age, ethnicity and BMI.

With the 2D imaging technique it is much more difficult to quantify soft tissue changes than with the 3D image fusion technique. Therefore this is the first study to quantify facial soft tissue changes in acromegaly. Our results show that many typical soft tissue deformities persist even after long-term remission of acromegaly. The nose is larger in all dimensions and the lower lip is also larger. This shows that the period of GH hypersecretion has remaining effects on soft tissues, probably on both cartilage and connective tissue, in addition to the remaining effects on the bony structures.

The differences in measurements between the patients in long-term remission of acromegaly and the healthy control group may seem small, but the human eye is able to detect very small changes [25]. Furthermore, a majority of studies on facial esthetics suggests that facial measurements closest to the mean are considered to be the most attractive [26]. Therefore, patients in long-term remission of acromegaly are more at risk to be considered unattractive than healthy control subjects, although other aspects, such as complexion, posture, sex and age are also important for the judgment of attractiveness [27]. Indeed, there are indications that patients in long-term remission of acromegaly are insecure about their general appearance, which may have a large psychological impact and can affect quality of life [28]. Moreover, the craniofacial disproportions may cause functional problems such as jaw pain, mastication problems or mouth breathing. Both appearance and functional problems may be a reason for intervention, for example consultation of a specialized nurse, psychologist or a specialist in reconstructive surgery. We therefore believe that all patients in remission of acromegaly should be asked about any issues regarding their appearance and functional problems that may require interventions, and all intervention possibilities should be discussed.

A limitation of this study is the relatively small sample size, which is caused by the rareness of acromegaly and our strict in- and exclusion criteria. The major strengths of this study, besides the one-on-one matching of patients and control subjects, are the use

of the 3D imaging technique and the 3D cephalometric analysis of both soft and hard tissues. The reliability of 3D measurements is dependent on inaccuracies that are mainly related to the manual identification of 3D cephalometric landmarks. The 3D cephalometric analysis according to Swennen has a low intra- and interobserver measurement error, which does not exceed 1 mm [19,29]. In this study all measurements were performed by one experienced observer in order to reduce the magnitude of the measurement error even more. The use of a color-encoded distance map to visualize and quantify the facial morphology between patients and controls is especially advantageous as it is not influenced by landmark identification errors and provides a better representation of the clinical situation, since all image data of the facial regions are used. Beside 3D cephalometry, the employment of a distance map should therefore be an integral part of the 3D facial analysis.

Because of the advantages of 3D facial analysis it is a very promising technique for the evaluation of the effects of acromegaly on the viscerocranium in both clinical practice and research settings. For example, we know that, like soft tissue, bone is an active organ that undergoes continuous remodeling throughout life [30]. During normal growth the craniofacial bones respond to alterations in the functional matrix [31]. Therefore, although this study demonstrates that changes in craniofacial dimensions persist after long-term remission of acromegaly, it is possible that the viscerocranium keeps remodeling for many years after the patients have been cured. To investigate how the viscerocranium changes over time and whether a stable state is reached, a longitudinal prospective study is necessary . Furthermore, at present, besides determination of IGF-1 levels, there are no methods to compare the effectiveness of different treatment modalities (e.g. pituitary surgery or different kinds of medication) for acromegaly on tissue-specific endpoints. Three-D cephalometry is a promising method for the evaluation of the effect of different treatment modalities for acromegaly on soft and bony tissue.

Finally, because acromegaly only causes subtle changes in appearance in the first years of the disease which are difficult to recognize by the human eye, there is often a delay in diagnosis for up to 10 years. It has recently been demonstrated that, if 2D photographs are analyzed with a computer model, the computer could sort photographs of patients with acromegaly from photographs of normal subjects much more accurately than medical experts could [32, 33]. So potentially, relatively simple imaging techniques could be applied to risk populations for acromegaly in order to shorten the delay in diagnosis, which will presumably result in less disfiguration. It is very well possible that the use of a 3D stereophotograph, instead of a regular 2D photograph, will be even more accurate. This should be investigated in the future.

In conclusion, the application of 3D cephalometry on 3D fusion head models is a good method to investigate craniofacial disproportions in patients with acromegaly, and is a promising technique for both clinical practice and research settings. Significant craniofacial changes persist even after long-term remission of acromegaly. Longitudinal prospective studies are required to evaluate to what extent the craniofacial changes are reversible after remission, how different treatment modalities for acromegaly affect craniofacial characteristics, and to what extent (ex-)patients suffer from psychosocial inconveniences caused by the persisting craniofacial disproportions.

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Persistent centripetal fat distribution and metabolic abnormalities in patients in long-term remission of Cushing's syndrome

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Abstract

Objective: Centripetal obesity is associated with systemic low-grade inflammation and an increased cardiovascular risk. Patients in long-term remission of Cushing's syndrome (CS) report persisting abdominal fat accumulation. However, this has never been adequately objectified. Therefore we investigated the adipose tissue distribution and adipocytokine profiles of patients in long-term remission of CS.

Design: Cross sectional case-control study in a tertiary referral centre.

Patients: Fifty-eight patients, in remission of CS for at least 5 years, were compared to 58 age-, gender- and BMI-matched healthy control subjects.

Measurements: measures of body composition (assessed with clinical evaluation and Dual Energy X-ray Absorptiometry (DEXA) scanning) and serum adipocytokine profiles.

Results: Compared to the matched control subjects, patients in long-term remission of CS had a greater waist circumference ($p<0.01$), a smaller thigh circumference ($p<0.01$), a higher waist-to-hip ratio ($p<0.01$) and a higher hip-to-thigh-ratio ($p<0.01$). As measured with DEXA scanning, patients had a higher percentage of truncal fat mass ($p=0.01$) and the truncal fat mass to leg fat mass ratio was greater ($p<0.01$). Patients had lower adiponectin levels ($p<0.01$), higher leptin levels ($p<0.01$) and higher resistin levels ($p=0.04$) than control subjects.

Conclusion: Even after long-term remission, patients who suffered from CS in the past continue to have a centripetal adipose tissue distribution and an adverse adipokine profile. This is independent of etiology of CS, treatment strategies, hormonal deficiencies and comorbidity, and probably contributes to the persistently increased cardiovascular risk.

Introduction

Endogenous Cushing's syndrome (CS) is associated with significant morbidity and mortality [1]. Although the Cushingoid phenotype dramatically improves after curative treatment, quality of life remains impaired, even after long-term remission. This is regardless of etiology, presence of hormonal deficiencies and treatment strategies [2]. The cause of the remaining impaired quality of life is most likely multifactorial, but there is probably an important physical component [3].

Research in patients with obesity has shown that excess visceral adipose tissue (VAT) causes a state of systemic low-grade inflammation[4-6]. This process is triggered when macrophages infiltrate the VAT in response to microhypoxia and rupturing of adipocytes [4, 5]. As a result, the secretion of adipocytokines by the VAT changes, leading to an adverse adipocytokine profile, which causes insulin resistance, endothelial dysfunction and eventually macrovascular cardiovascular disease [4-6].

When CS is diagnosed, centripetal obesity is present in the majority of patients and it usually developed in a relatively short period of time. Even though the volume of centripetal obesity diminishes after remission, patients report persisting abdominal fat accumulation despite overall weight loss [7]. Besides the psychological burden of the changed appearance, the metabolic consequences of centripetal obesity may be a major cause of persisting impaired quality of life and increased morbidity and mortality in patients in remission of CS [8, 9]. Previously, a number of studies have investigated if centripetal obesity and systemic low-grade inflammation persist after remission of CS [10-13]. However, these studies have some methodological limitations and the results are conflicting.

The aim of this study was therefore to investigate the adipose tissue distribution, adipocytokine profiles and metabolic risk profiles of patients in long-term remission of CS.

Materials and methods

Subjects

All adult patients of the Department of Medicine, Division of Endocrinology, of the Radboud University Medical Centre Nijmegen, who had successfully been treated for CS (caused by either an ACTH-producing pituitary adenoma or a benign adrenal adenoma) and had been in remission for at least five years, were eligible for inclusion in this cross sectional case-control study. Remission was defined as absence of clinical signs and

symptoms of hypercortisolism and suppression of plasma cortisol to ≤ 50 nmol/l after 1 mg dexamethasone overnight [14] or, if a patient had received radiotherapy of the pituitary gland, a 24-hour urinary free cortisol value of <240 nmol/24h for men or <150 nmol/24h for women. The medical records of all patients were retrospectively reviewed to assess clinical data regarding the etiology of CS, the type and number of treatments that patients received, duration of remission, hormonal deficiencies and comorbidities.

Patients with untreated hormonal deficiencies or hormonal deficiencies that had not been treated adequately in the last 5 years according to international standards, except for estrogen deficiency, were excluded. Hypothyroidism was defined as free thyroxin (fT4) plasma concentrations < 8 pmol/l (institutional reference range 8-22 pmol/l). Testosterone deficiency in men was defined as testosterone levels < 11 nmol/l (reference range 11-45 nmol/l). In women, estrogen deficiency was defined as untreated secondary hypogonadotropic hypogonadism or a postmenopausal state without the use of estrogen substitution therapy. Growth hormone (GH) deficiency was investigated in all patients and defined as a maximal GH response of < 5.1 µg/l during an insulin tolerance test (ITT), or as a maximal GH response of < 4.1 µg/l during an arginine/GHRH test [15]. Glucocorticoid deficiency was defined as a maximal cortisol response < 550 nmol/l during an ITT [16].

Patients with active malignancy or systemic therapy for malignancy in the past, auto-inflammatory diseases and psychiatric pathology were also excluded. All patients underwent a new 1 mg dexamethasone suppression test (or a 24-hour urinary free cortisol measurement in case of pituitary RT) before entering the study to confirm remission**.** Each patient was matched to a control subject with the same gender, age (± 2 years) and BMI (± 2 kg/m^2). Control subjects were recruited via advertisements. They were not allowed to have a known disease (with the exception of obesity) or use of medication.

This study was performed according to the Declaration of Helsinki and was approved by the institutional Medical Ethics Committee. Written informed consent was obtained from all participants.

Clinical assessment

All participants visited our outpatient clinic for data collection. Before their visit, all subjects completed a questionnaire about medical history, current complaints and lifestyle. In order to detect unknown comorbidities extensive physical examination was performed. Furthermore, the following anthropometric measurements were performed in a standardised way: 1) weight 2) height, 3) systolic and diastolic blood pressure (average of 10 measurements, every 3 minutes with an oscillometric sphygmomanometer (Criticon model 1846; Criticon Inc., Tampa, FL)), 4) heart rate, 5) skinfold thickness (using a Harpenden skinfold calliper) at the biceps, triceps, subscapular, and suprailiac level [17] and 6) the circumference of the waist, hip and left thigh. Body mass index (BMI), waist to hip- and hip to thigh-ratios were calculated with these measurements.

Biochemical evaluation

Fasting blood samples were drawn for biochemical evaluation of the following measurements: plasma glucose, glycated hemoglobin (HbA1c), insulin, total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and creatinine, and serum fT4 and insulin-like growth factor type 1 (IGF-1). Insulin sensitivity was assessed by homeostasis model assessment (HOMA-IR). Furthermore, serum adipocytokine profiles were measured: interleukin 6 (IL-6), interleukin 8 (IL-8), macrophage chemotactic protein-1 (MCP-1), Tumour necrosis factor alpha (TNF-α), adiponectin, leptin and resistin.

DEXA evaluation

Total body dual-energy X-ray absorptiometry scanning (DEXA) was performed using a Hologic QDR 4500 densitometer (Hologic, Bedford, Zaventem, Belgium) to determine the percentage of total body fat mass and total lean body mass. Furthermore, in order to assess the presence of a centripetal adipose tissue distribution, the fat percentage of different body compartments was measured. This enabled us to calculate the trunk/leg fat ratio and the trunk/extremities fat ratio.

Assays

Serum concentrations of IL-6, IL-8, MCP-1, leptin and resistin were measured by Multiplex Fluorescent Bead Immunoassays (xMAP technology, Millipore, Billerica, MA, USA) and a Bio-plex microbead analyzer (Luminex, Austin, TX, USA) according to the manufacturers protocol. Serum concentrations of adiponectin and TNF-α were determined by enzyme-linked immunosorbent assays (RandD Systems, Minneapolis, MN, USA). Fasting plasma glucose, HbA1c, insulin, total cholesterol, triglycerides, LDL, HDL and creatinine, and serum fT4 and IGF-1 were measured by standard automated procedures of our hospital.

Statistical methods

Data were analyzed using SPSS 20.0 statistical package for Windows (SPSS Inc, Chicago, IL). Data are expressed as mean ± SD, unless otherwise mentioned. Data distribution was analyzed by means of the Kolmogorov-Smirnov test. Before statistical analyses a logarithmic transformation was performed on non-normally distributed data. Differences between patients and controls were tested by means of paired t-tests. Differences in categorical variables were analyzed using χ^2 tests. Subgroup analyses were performed to assess the effect of glucocorticoid replacement therapy and estrogen status. Stepwise backward multiple linear regression analysis was performed in the patient group in order to detect the influence of clinical patient characteristics (etiology of CS, treatment strategies, presence of hormonal deficiencies, use of alcohol, smoking and comorbidity) as predictors for the outcomes of centripetal fat distribution and adipokine profiles. Tests were two-tailed and $p<0.05$ was considered statistically significant.

Results

Subject characteristics

Table 1 shows the clinical characteristics of the patients and controls. Fifty-eight patients met all inclusion criteria and were willing to participate. Seventy-nine percent of patients were female. The age of the total group was 50.8 ± 12.3 years and BMI was 26.5 ± 4.2 kg/m^2. Data did not differ between the patient and control group, thus confirming adequate matching. Sixty-nine percent of patients had had CS caused by a pituitary adenoma and 31% CS caused by an adrenal adenoma. Patients had been in remission of CS for an average of 13.6 ± 8.0 years. At the time of investigation, 7% of all patients were treated for diabetes mellitus, 31% for hypertension and 21% for hyperlipidemia. Fifty-nine percent of all patients had one or more hormonal deficiencies which had been treated accurately for at least 5 years (except for the women with estrogen deficiency who were all over 50 years of age). At the time of the study 13 of the 21 patients with GC deficiency used hydrocortisone, 7 patients used cortisone acetate and one patient used dexamethasone. The mean dose, in equivalents of hydrocortisone, for these 21 patients was 19.7 ± 9.7 mg daily.

Patients only differed from controls with respect to smoking habits, with 14 smoking patients versus 5 controls ($p<0.024$).

Table 1) Group A: Clinical characteristics of patients in long-term remission of Cushing's syndrome and healthy controls.

	Patients (n=58)	Controls (n=58)	P-value
Gender (n): male/female	12/46	12/46	
Age: mean (± SD) (years)	50.8 (12.3)	51.2 (12.4)	0.863
BMI: mean (± SD) (kg/m2)	26.5 (4.2)	26.3 (4.1)	0.793
Duration of remission: median (± range) (years)	13.6 ± 8.0		
Smoking (yes/no)	14/44	5/53	0.024*
Pack-years (± SD)	11.5 (15.6)	6.9 (13.9)	
Alcohol consumption: yes/no	10/48	13/45	0.485
Treatment modalities: n (%)			
Unilateral adrenalectomy	19 (32.8)	-	-
Bilateral adrenalectomy	12 (20.7)	-	-
Pituitary surgery	38 (65.5)	-	-
Pituitary radiotherapy	13 (22.4)	-	-
Hormonal deficiencies: n (%)			
Glucocorticoid deficiency	21 (36.2)	-	-
Growth hormone deficiency	15 (25.9)	-	-
Thyroid hormone deficiency	25 (43.1)	-	-
Mineralocorticoid deficiency	11 (19.0)	-	-
Testosterone deficiency	6/12 (50.0)	-	-
Estrogen deficiency [1]	25/46 (54.3)	29/46 (63.0)	-
Comorbidities: n (%)			
Hypertension	18 (31.0)	0 (0)	-
Diabetes mellitus	4 (6.9)	0 (0)	-
Hypercholesterolemia	12 (20.7)	0 (0)	-
Cushing type: n (%)			
Pituitary	40 (69.0)	-	-
Adrenal	18 (31.0)	-	-

*BMI: body mass index; CS: Cushing's syndrome.; * P<0.05;*

Note [1]: *Secondary hypogonadotropic hypogonadism or a postmenopausal state without the use of chronic estrogen replacement.*

Comparisons between patients in remission of CS and healthy controls

Table 2 shows the comparisons between patients and controls with regard to the different outcome measures. Figure 1 shows the main findings of this study.

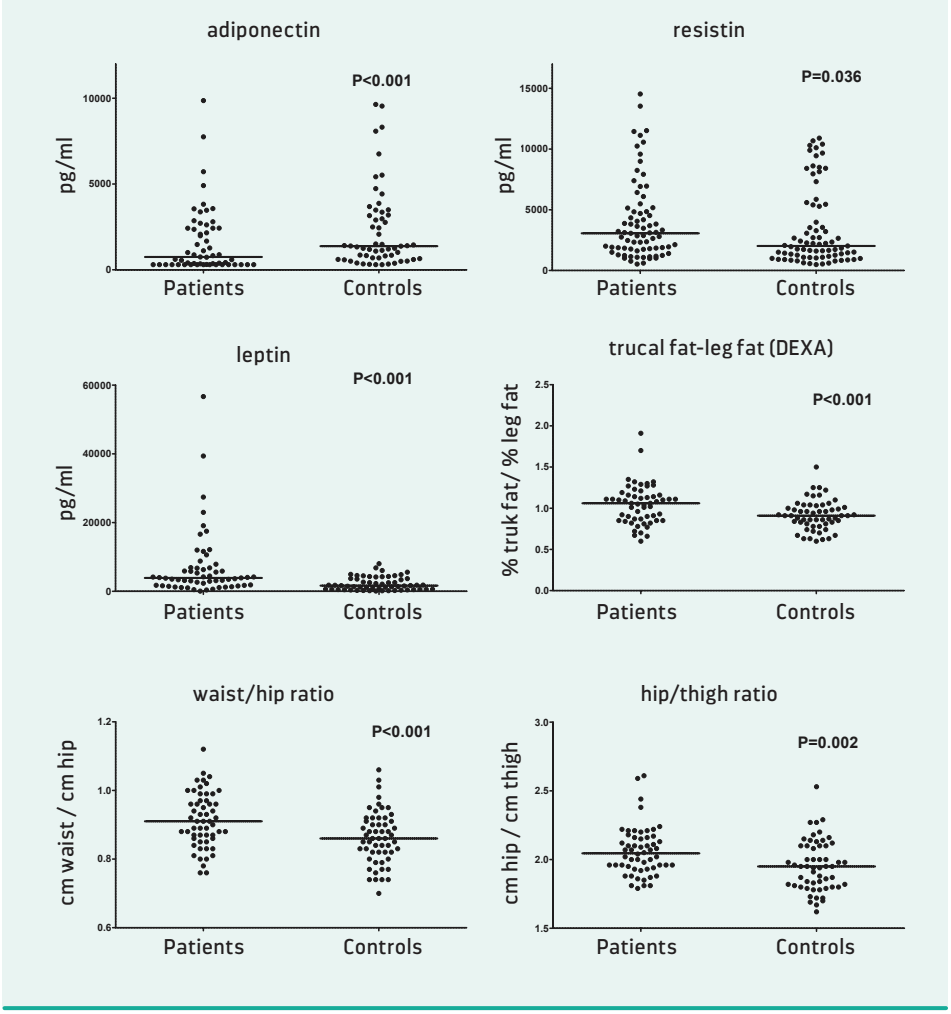

Figure 1) Scatter plots of the main differences in adipokine profiles and adipose tissue distribution and adipokine profiles between patients in remission of Cushing's syndrome (N=58) and healthy controls (N=58). Depicted are the absolute values or ratios of each individual and the result of the of the paired t-test. P<0.05 was considered statistically significant. The bars represent the mean values.

Table 2) Outcomes in patients and controls. *(page 169-170)*

Variable	Controls (n=58) (mean)	SD	Patients(n=58) (mean)	SD	P-value
Biochemical evaluation					
Total serum cholesterol (mmol/l)	5.38	0.198	5.16	0.165	0.188
HDL-cholesterol (mmol/l)	1.44	0.242	1.33	0.216	0.061
LDL-cholesterol (mmol/l)	3.38	0.255	3.05	0.236	0.055
Triglycerides (mmol/l)	1.01	0.445	1.43	0.531	‹.001***
Creatinin (µmol/l)	68.10	0.144	70.81	0.188	0.194
Insulin (mE/l)	6.51	0.552	6.51	0.722	0.933
Hba1c (mmol/mol)	37.49	0.088	39.10	0.151	0.355
Fasting glucose (mmol/l)	4.98	0.107	4.99	0.172	0.973
HOMA_IR	1.71	0.99	2.36	5.08	0.371
fT4 (pmol/l)	12.28	0.136	15.20	0.202	‹.001***
IGF-1 (nmol/l)	16.02	0.353	13.25	0.434	0.011*
Adipocytokines					
Adiponectin (pg/ml)	1478.82	0.972	915.07	1.074	‹.001***
Leptin (pg/ml)	1358.31	1.114	3739.33	1.182	‹.001***
Resistin (pg/ml)	2435.73	0.958	3001.90	0.855	0.036*
IL-6 (pg/ml)	0.58	1.118	0.61	0.994	0.602
IL-8 (pg/ml)	0.33	2.130	0.48	1.845	0.328
TNF- (pg/ml)	2.00	1.345	2.01	1.455	0.988
MCP-1 (pg/ml)	64.46	0.536	67.36	0.581	0.614
Clinical assessment					
Systolic blood pressure (mmHg)	132.37	19.06	126.04	14.55	0.095
Diastolic blood pressure (mmHg)	77.24	9.27	73.85	9.02	0.134
Heart rate (bpm)	64.03	8.42	66.81	9.48	0.151
Thigh circumference (cm)	53.68	6.17	50.57	4.14	‹.001***
Hip circumference (cm)	104.17	9.29	103.84	8.55	0.854
Waist circumference (cm)	90.13	13.10	94.79	12.89	‹.001***
Skinfold thickness (mm)	74.74	0.452	80.48	0.276	0.255
Waist: hip	0.86	0.08	0.91	0.08	‹.001***
Hip: thigh	1.95	0.19	2.06	0.18	0.002**

Variable	Controls (n=58) (mean)	SD	Patients(n=58) (mean)	SD	P-value
DEXA evaluation					
Total fat mass (%)	40.28	51.88	33.93	6.69	0.371
Total lean body mass (%)	47.10	8.40	46.81	8.79	0.830
Trunk fat (%)	32.44	8.77	34.42	7.39	0.010*
Extremity fat (%)	36.87	9.52	36.68	8.32	0.939
Leg fat (%)	35.90	8.94	34.15	7.97	0.107
Trunk: leg	0.92	0.18	1.04	0.24	‹.001***
Trunk: extremities	0.89	0.16	0.96	0.18	‹.001***

Differences were tested by means of paired t-tests. For logarithmically transformed data the geometric means were calculated using back transformation to enable clinical interpretation of the outcomes. HDL, high density lipoprotein; LDL, low density lipoprotein; Hba1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; IGF-1, insulin like growth factor type 1; IL-6, interleukin 6; IL-8, interleukin 8; TNF-α, tumor necrosis factor alpha; MCP-1, monocyte chemotactic protein 1.

** p‹0.05; ** p‹0.01; *** p‹0.001*

Body composition and anthropometry

Anthropometric measurements demonstrated that, compared to healthy control subjects, patients had a significantly larger waist circumference (p‹0.001), a smaller thigh circumference (p‹0.001) and thus a greater waist to hip ratio (p‹ 0.001) and hip to thigh ratio (p=0.02). The hip circumference and skinfold thickness did not differ between patients and control subjects. Results of the DEXA scans showed that patients had a significantly larger percentage of trunk fat than control subjects (p=0.01) and that the trunk to leg fat percentage ratio was higher (p‹0.001). Total lean body mass percentage, total body fat percentage, leg fat percentage, systolic- and diastolic blood pressure and heart rate were not significantly different between patients and controls.

Adipocytokine profiles

Compared to control subjects, patients had a significantly lower serum total adiponectin level (p‹0.001), a higher leptin level (p‹ 0.001) and a higher resistin level (p=0.036). The levels of IL-6, Il-8, TNFα and MCP-1 in serum did not differ between patients and controls.

Laboratory outcomes

Compared to control subjects, patients had a significantly higher level of plasma triglycerides, but total cholesterol, HDL-cholesterol and LDL-cholesterol levels did not

Table 3A) Outcomes in patients and controls without glucocorticoid deficiency.

Variable	Controls (n=37) (mean)	SD	Patients (n-=37) (mean)	SD	P-value
Adipocytokines					
Adiponectin (pg/ml)	1274.11	0.98	962.95	1.10	0.030*
Leptin (pg/ml)	1436.55	1.20	4146.42	1.23	0.001**
Resistin (pg/ml)	2489.91	0.94	2892.86	0.89	0.215
Clinical assessment					
Systolic blood pressure (mmHg)	133.44	19.17	125.73	12.78	0.041*
Thigh circumference (cm)	53.94	6.70	50.69	4.28	0.004**
Waist circumference (cm)	90.31	14.01	94.78	13.45	0.010*
Waist:hip	0.86	0.08	0.90	0.08	0.006**
Hip:thigh	1.95	0.18	2.07	0.18	0.004**
DEXA evaluation					
Trunk fat (%)	32.55	8.88	34.85	7.98	0.030*
Trunk:leg	0.91	0.18	1.00	0.20	0.004**
Trunk:extremities	0.88	0.16	0.93	0.15	0.010*

Table 3B) Outcomes in estrogen sufficient women.

Variable	Controls (n=14) (mean)	SD	Patients (n=14) (mean)	SD	P-value
Adipocytokines					
Adiponectin (pg/ml)	2121.76	0.88	1299.84	1.29	0.058
Leptin (pg/ml)	1141.39	1.25	3677.54	0.64	0.009**
Resistin (pg/ml)	2835.57	1.01	2779.43	0.87	0.911
Clinical assessment					
Thigh circumference (cm)	52.62	5.91	51.15	3.93	0.394
Waist circumference (cm)	80.00	10.04	85.08	7.57	0.093
Waist:hip	0.80	0.08	0.84	0.05	0.179
Hip:thigh	1.90	0.17	1.98	0.09	0.108
DEXA evaluation					
Trunk fat (%)	29.25	8.62	31.85	6.00	0.196
Trunk:leg	0.80	0.18	0.86	0.15	0.228
Trunk:extremities	0.79	0.14	0.81	0.10	0.454

Differences were tested by means of paired t-tests. For logarithmically transformed data the geometric means were calculated using back transformation to enable clinical interpretation of the outcomes
** $p<0.05$, ** $p<0.01$, *** $p<0.001$*

differ. Furthermore, fasting plasma glucose and insulin levels were not significantly different, nor were the level of HbA1c and the calculated HOMA-IR. IGF-1 levels were significantly lower in patients compared to control subjects (p=0.011), and fT4 levels were higher (p<0.001).

Effect of glucocorticoid replacement therapy

When data were reanalyzed excluding the 21 patients that used glucocorticoid replacement therapy (including their matched controls, Table 3a), the differences found were largely the same as in the total group. However, HDL-cholesterol levels and systolic blood pressure were now significantly lower in patients compared to controls (p=0.041). Furthermore, the difference in resistin levels was no longer significant (p=0.215).

Effect of estrogen status

If the 14 couples of women in whom the patient and control subject were both estrogen sufficient are compared, most differences were no longer significantly different (Table 3b), although there was a trend towards a more centripetal adipose tissue distribution with adverse adipokine profile in the patient group compared to the control group. Furthermore, leptin levels remained significantly higher in the patients (p=0.009).

Stepwise backward multiple linear regression

Stepwise backward multiple linear regression analysis in the patient group identified smoking behaviour (yes/no) as being significantly related to waist circumference (β 3.35; p=0.02). Mineralocorticoid substitution therapy (yes/no) was significantly related to trunk fat percentage (β 3.10; p=0.02). Exclusion of all couples containing a smoker in the patient or control group did not change the statistically significant difference in waist circumference between the groups (p=0.02). Furthermore, after exclusion of the patients using mineralocorticoid substitution therapy, the difference in trunk fat percentage between patients and controls remained statistically significant (p=0.03). No relationship was found between other patient-related factors (e.g. deficiency of GH, GC, testosterone or estrogen) and the outcomes of centripetal fat distribution and adipokine profiles.

Discussion

In the present study we investigated the adipose tissue distribution and adipocytokine profiles of patients in long-term remission of CS and compared the results with a healthy gender-, age- and BMI-matched control group. Previously, a small number of

studies have investigated if centripetal obesity and systemic low-grade inflammation persist after remission of CS [10-13]. However, these studies have some methodological limitations (e.g. small numbers of patients, patients with untreated hormonal deficiencies included, patients included shortly after remission), and the results are conflicting: two studies [10-11] describe persisting centripetal obesity and an adverse adipokine profile in patients in long-term remission of CS, while two other studies [12-13] concluded that adipose tissue distribution and adipokine profiles become more favourable after remission of CS and suggest that in time it might even normalise. The strengths of this study are the large group of patients (n=58) who had been in remission of CS for at least 5 years, so changes in body composition most likely had reached a steady state. In all patients presence of hormonal deficiencies had been investigated and, if present, treated for at least 5 years to make sure that untreated hormonal deficiencies would not influence our findings. Furthermore, we performed one-to-one matching of each patient to a healthy age-, gender- and BMI-matched control subject. Because of the solid methodology and adequate selection of subjects, we believe that this study provides convincing data concerning the adipose tissue distribution and adipocytokine profiles of patients in long-term remission of CS. Furthermore, this is the first study that investigates resistin and leptin values in patients in long-term remission of CS.

In line to what Barahona et al. and Colao et al. previously reported [10,11], this study confirms that patients in long-term remission of CS continue to have a centripetal adipose tissue distribution, even though total fat percentage and BMI did not differ from controls. Furthermore, patients had an adverse adipokine profile with decreased adiponectin and increased resistin and leptin levels. The concentrations of the cytokines IL-6, IL-8, TNF-α and MCP-1 did not differ between patients and controls.

We measured adipose tissue distribution by performing manual measurements of circumferences of different body parts and DEXA scanning. Unfortunately, DEXA scanning cannot distinguish between different adipose tissue depots [18]. However, because skinfold thickness, which can be seen as a measure for subcutaneous fat accumulation, did not differ between patients and control subjects, it is very likely that the abnormal centripetal adipose tissue distribution in the patients is caused by an increase in VAT. The significantly increased triglyceride values and the abnormal adipokine profiles in patients support this conclusion [18,19].

Adiponectin is an adipokine mainly produced by VAT and circulates in different forms of varying molecular weight[4, 20]. Levels are markedly decreased in visceral obesity. Adiponectin levels correlate inversely with insulin resistance. A number of studies have

investigated adiponectin levels in active CS, or (mostly shortly) after remission, but results have been conflicting [10, 12, 21-23]. In line with what Barahona et al. previously found in women, this study confirms that adiponectin levels are decreased after long-term remission of CS, in both women and men, compared to healthy matched controls.

Resistin is produced by macrophages and adipocytes predominantly in VAT [4]. Levels are elevated in obese patients. Resistin is positively associated with insulin resistance and is a contributor to atherogenesis and cardiovascular disease [4, 20]. Until now, only one study investigated resistin levels in 10 female patients with CS [24]. Resistin levels were elevated during active disease, and declined 9 months after remission. However, the difference was not statistically significant. Our study is the first that shows that resistin levels are elevated after long-term remission of CS compared to healthy matched controls.

Leptin is an anorexigenic hormone secreted by adipocytes in proportion to (predominantly subcutaneous) adipose tissue content [4, 20]. Leptin levels are typically elevated in obesity, which is considered a leptin-resistant state. Hyperleptinemia in obese individuals has been widely recognised as an independent cardiovascular risk factor associated with insulin resistance. It also has a pathogenetic role in atherothrombosis and endothelial dysfunction. Most previous studies show elevated levels of leptin in active CS, which seem to decline after remission [13, 21, 25, 26]. It has been speculated that hyperleptinemia in active CS may be a mechanism to antagonise glucocorticoid excess or to reduce the stimulatory effect of cortisol on food intake [20]. Our study is the first study that shows that leptin levels are elevated even after long-term remission of CS. Therefore, in patients in remission of CS, hyperleptinemia is more likely a result of a leptin-resistant state, like in obesity.

Another interesting finding in this study is that thigh circumference was significantly smaller (±3 cm) in patients in long-term remission of CS than in control subjects. It is well known that hypercortisolism causes sarcopenia [27]. Possibly this is not entirely reversible after remission. Indeed, patients in remission of CS still complain about muscle weakness and inability to exercise [2, 28]. Total lean body mass was not different between patients and control subjects.

At the time of investigation, 31% of patients were treated for hypertension and 21% for hyperlipidemia, which seems to be adequate, as no differences in blood pressure and (LDL) cholesterol levels between patients and controls were found. Seven percent of all patients were treated for diabetes mellitus. Measures of insulin resistance did not

differ, but this is influenced by the treatment for diabetes mellitus in the patient group. Free T4 values were slightly higher and IGF-1 values were slightly lower in the patients. This is related to relative overtreatment for secondary hypothyroidism and relative undertreatment of growth hormone deficiency in a number of patients (even though individual levels of fT4 and IGF-1 were always within the normal reference ranges), as the differences disappear if the patients who receive substitution are excluded. However, this did not affect the results in the total group, as subgroup analysis excluding the couples of whom the patients had either GH deficiency or hypothyroidism revealed essentially the same results as in the total group (data not shown).

Because a large percentage of our patients used substitution with glucocorticoids, which could potentially have contributed to the differences we observed in adipose tissue distribution and adipokine profiles between patients and controls in the total group, we performed a subgroup analysis comparing only the couples in whom the patients did not use glucocorticoids. Except for resistin, which was no longer significantly different between patients and control subjects, all other differences remained statistically significant. Furthermore, we performed a stepwise backward multiple linear regression analysis to investigate if other clinical parameters of the patients (etiology of CS, treatment strategies, presence of hormonal deficiencies, use of alcohol, smoking and comorbidity) could explain the differences we found. Only smoking behaviour had a statistically significant relation with waist circumference. Furthermore, use of mineralocorticoid substitution therapy turned out to have a statistically significant relation with trunk fat percentage. However, separate subgroup analyses excluding smokers and mineralocorticoid users did not change total group differences. No other associations were found between clinical patient characteristics and outcome parameters. We therefore strongly believe that the previous period of exposure to high levels of cortisol is the main cause of the centripetal adipose tissue distribution and adverse adipokine profile in patients in long-term remission of CS.

Barahona et al. found that the differences in adipose tissue distribution and adipokine profile between patients and controls were no longer significantly different if the estrogen sufficient women were analysed separately. They therefore suggested that the findings in the total group might be caused by estrogen deficiency. We performed a subgroup analysis in our estrogen sufficient group, even though in only 14 couples both patient and control were estrogen sufficient. In our study, the measurements for adipose tissue distribution also lose significance in this small group, although patients still had, on average, an almost 5 cm larger waist circumference and a 2.5% larger truncal fat percentage. Furthermore, leptin remained significantly higher in the patients.

A major issue in both studies is that the sample size is severely reduced in the subgroup analysis and that, hence, statistical significance is easily lost. Indeed, in the study of Barahona et al. the estrogen sufficient patients still have a centripetal adipose tissue distribution and adverse adipokine profile compared to control subjects but, like in our study, the difference is no longer statistically significant. Therefore, further research in larger groups is needed to investigate the influence of estrogen status on adipose tissue distribution and adipokine profiles after long-term remission of CS.

Further research is also needed to investigate the cause of the persisting centripetal adipose tissue distribution in patients in long-term remission of CS. We hypothesise that during the rapid expansion of visceral adipose tissue during active CS, macrophages infiltrate the adipose tissue in response to microhypoxia, and that these macrophages continue to cause a vicious circle of inflammation and centripetal adipose tissue accumulation after remission, as is observed in obesity. Furthermore, future research should investigate the consequences of the persistent centripetal obesity and adverse adipokine profile in patients in remission of CS.

In conclusion, even after long-term remission, patients that suffered from CS in the past continue to have a centripetal adipose tissue distribution and an adverse adipokine profile. This is independent of etiology of CS, treatment strategies, presence of hormonal deficiencies and comorbidity, and probably contributes to the remaining decreased quality of life and persistent increased cardiovascular risk in these patients.

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Vascular health in patients in long-term remission of Cushing's syndrome and no or adequately treated comorbidity is comparable to BMI-matched subjects

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Abstract

Context: In active Cushing's syndrome (CS), patients suffer from endothelial dysfunction and premature atherosclerosis. However, it is uncertain to what extent vascular health recovers after long-term remission. This is highly relevant as this topic relates to future development of cardiovascular disease.

Objective: To investigate whether micro- and macrovascular health is impaired after long-term remission of CS, in patients with no or adequately treated comorbidities.

Design and setting: Cross-sectional case-control study in two tertiary referral centers.

Patients and main outcome measures: 63 patients (remission of CS for ≥ 4 years) and 63 healthy, well-matched controls were compared. In group A (58 patients and 58 controls) serum biomarkers associated with endothelial dysfunction, intima-media thickness, pulse wave velocity and pulse wave analysis were studied. In group B (14 patients and 14 controls) endothelium-dependent and -independent vasodilatation was studied in conduit arteries (flow-mediated dilation of the brachial artery) and forearm skeletal muscle resistance arteries (vasodilator response to intra-arterial acetylcholine, sodium-nitroprusside and N^G-monomethyl-L-arginine using venous occlusion plethysmography).

Results: There were no significant differences between the outcome measures of vascular health in patients and controls, neither in group A nor in group B.

Conclusion: Vascular health of patients in long-term remission of Cushing's syndrome seems to be comparable to that of healthy gender-, age- and BMI-matched controls, provided that the patients have no, or adequately controlled comorbidities. Therefore, the effects of hypercortisolism *per se* on the vasculature may be reversible. This accentuates the need for stringent treatment of metabolic comorbidities in these patients.

Introduction

Patients with chronic hypercortisolism due to endogenous Cushing's syndrome (CS) have a very high mortality rate, with an estimated 5-year survival of 50% in untreated patients [1]. Cardiovascular disease is the main cause of mortality [1]. Multiple studies have shown that endothelial function is impaired in these patients [2-5], with an increased incidence of atherosclerosis [6,7]. It has been suggested that this is mainly caused by the fact that most patients with CS have centripetal obesity, impaired glucose tolerance, systemic hypertension, hypercoagulability and dyslipidemia[8]. All these factors are associated with impaired endothelial function and premature atherosclerosis, especially if they occur simultaneously [9]. In addition, one should realize that the hypercortisolism itself has a direct effect on the vasculature (via both the glucocorticoid and the mineralocorticoid receptor) [10,11].

Successful surgical treatment of CS, resulting in normalization of cortisol secretion, significantly decreases cardiovascular risk and reduces mortality rate [1,12]. However, it is unclear to what extent vascular health recovers in patients in long-term remission of CS. Full recovery is not self-evident, since centripetal obesity and an adverse adipokine profile (which is known to be associated with endothelial dysfunction and eventually macrovascular disease [13,14]) persists even after long-term remission of CS [15,16]. Furthermore, it is questionable if the direct effects of hypercortisolism on the vasculature are fully reversible.

A number of studies have previously investigated vascular health in small groups of patients in remission of CS [17-23]. These studies reported inconsistent results, which may partly be explained by the small group size and/or selection of single markers of vascular health that, therefore, cannot provide a broad insight.

The aim of this study was to investigate micro- and macrovascular health in a large group of patients in long-term remission of CS with adequately treated comorbidity if present, in comparison with a matched healthy control group. We measured serum biomarkers associated with endothelial dysfunction, performed gold standard measurements of endothelial function and investigated the presence of overt atherosclerosis.

Subjects and methods

Subjects

All adult patients of Radboud University Medical Centre Nijmegen and Leiden University Medical Centre, who had been successfully treated for CS (caused by either an ACTH-producing pituitary adenoma or a benign adrenal adenoma) and were in remission for at least four years, were eligible for inclusion in this multi-center cross-sectional matched case-control study. Remission was defined as absence of clinical signs and symptoms of hypercortisolism and suppression of plasma cortisol to ≤50 nmol/l after 1 mg dexamethasone overnight or, if a patient had received radiotherapy of the pituitary gland, a 24-h urinary free cortisol value of <240 nmol/24 h for men or <150 nmol/24 h for women. The medical records of all patients were retrospectively reviewed to assess clinical data regarding the etiology of CS, the type of treatments that patients had received, duration of remission, presence of hormonal deficiencies and comorbidities.

In our study we investigated 2 different patient groups: The first group (group A, n=58) was the same group of patients that we previously described in our study on body composition [16]. The following exclusion criteria were applied: untreated (or inadequately treated) hormonal deficiencies, active malignancy or systemic therapy for malignancy in the past, severe inflammatory diseases and psychiatric pathology. Each patient was matched to a control subject with the same gender, age (±2 years), and body mass index (BMI, ±2 kg/m^2). Control subjects, recruited via advertisements in a local newspaper, had to be healthy and without current use of medication.

For the second group of patients (group B, n=14), even stricter exclusion criteria were used: all subjects with hormonal deficiencies, except for adequately treated hypothyroidism (free T4 range 8.0-22.0 pmol/l), were excluded. Furthermore, besides the previously mentioned comorbidities, all patients with comorbidities that are known to affect vascular function or who used medication that may interfere with the cardiovascular system were excluded. In addition to gender, age and BMI, the healthy control subjects were also matched for smoking, ethnicity, and physical activity levels (estimated via metabolic equivalent of task scores and measured for one week with a SenseWear Pro Aramband™ (Body Media, Pittsburg, USA)). Female controls were matched for estrogen status and oral contraceptive use. Nine patients were included in both groups A and group B.

The Medical Ethics Committees of our institutions approved this study and all participants provided written informed consent prior to participation.

Methods

All subjects refrained from smoking, alcohol, caffeine, chocolate and vitamin C for at least 18 hours, and vigorous physical exercise for at least 24 hours before testing. Subjects fasted at least 6 hours before testing.

Biochemical markers associated with endothelial dysfunction (group A)

Serum concentrations of plasminogen activator inhibitor-1 (PAI-1), intracellular adhesion molecule-1 (ICAM-1) and soluble E-selectin were measured by Multiplex Fluorescent Bead Immunoassays (xMAP technology, Millipore, Billerica, MA, USA) and a Bio-plex microbead analyzer (Luminex, Austin, TX, USA) according to the manufacturer's protocol. Serum concentrations of vascular cell adhesion molecule-1 (VCAM-1) were determined by an enzyme-linked immunosorbent assay (RandD Systems, Minneapolis, MN, USA).

Non-invasive measurements of atherosclerosis and arterial stiffness (group A)

Measurements of carotid intima-media thickness (cIMT), pulse wave velocity (PWV) and pulse wave analysis (PWA) were performed according to a highly standardized protocol and performed by the same experienced technician (SH) in all patients [24]. Mean cIMT was calculated from the mean of four measured segments of the vessel: far wall left, far wall right, near wall left and near wall right. Subsequently the presence of plaques and size was evaluated at the level of the common, internal and external carotid arteries. Plaque was defined as any focal protrusion above the surrounding intima of at least 1.5 x mean cIMT.
PWV and PWA were measured with applanation tonometry, using SphygmoCor system version 7.1 (Atcor Medical, Sydney. Australia). Central arterial pressure (CAP) and central systolic pressure (CSP) were derived and central augmentation index (AIx) was calculated. As AIx is influenced by heart rate, an index normalized for a heart rate of 75 beats/min was used. To determine pulse wave velocity, pulse wave forms were recorded at the right carotid artery and left femoral artery sequentially. Wave-transit time was calculated using the R-wave of a simultaneously recorded ECG as a reference frame. The coefficient of variation (CV) for measuring PWV is 5-10%[25].

Endothelial function (group B)

Brachial artery flow mediated dilation (FMD) is widely accepted to reflect endothelium-dependent and largely nitric oxide-mediated function of conduit arteries [26]. Measurements were performed by two experienced vascular sonographers (DT and TS). A 10 MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, Massachusetts, USA) was used for imaging of the brachial artery in the distal third of the upper arm. Subjects rested in a supine position for at least 15 minutes to enable baseline assessment of arterial diameter and

blood flow. The arm was extended and positioned at an 80º angle from the torso. A rapid inflation pneumatic cuff (Hokanson, Bellevue, Washington, USA) was positioned on the forearm immediately distal to the olecranon to provide the forearm ischemic stimulus. After obtaining an optimal image, the probe was manually stabilized and the ultrasound parameters were set to optimize longitudinal B-mode imaging of the lumen-arterial wall interface. Continuous Doppler velocity was measured using the lowest possible insonation angle (< 60º). The forearm cuff was inflated to 220 mmHg for 5 minutes. Diameter and flow recordings resumed 30 seconds prior to cuff deflation and continued for 5 minutes thereafter. Following a 15-minute resting period, a 1-minute baseline recording of the brachial artery diameter and flow was taken. Subsequently, brachial artery endothelium-independent vasodilatation was examined after administration of a single spray of sublingual glyceryl trinitrate (GTN), which serves as a direct nitric oxide (NO) donor, to detect endothelium-independent vasodilator capacity. This was followed by 5 minutes of continuous recording of brachial artery diameter and blood flow. Post-test analysis of brachial artery diameter was performed using customized edge detection and wall tracking software [27]. Baseline diameter, flow and shear rate were calculated as the mean of data acquired across the 1-minute preceding the cuff inflation period. Peak diameter following cuff deflation was automatically detected as previously described [28]. FMD was calculated as the percentage rise of the peak diameter from the preceding baseline diameter. The time to peak diameter (seconds) was calculated from the point of cuff deflation to the maximum post-deflation diameter. According to a recent study, inadequate scaling for FMD would be present if the upper confidence limit of the regression of the relation between logarithmically transformed base diameter and peak diameter is <1.0 [29]. In such an event, FMD% is not an appropriate measure for the estimation of endothelial function. Data were checked for this phenomenon and subsequently allometric modeling was applied [29]. Furthermore, FMD% was corrected for shear rate stimulus by adding this factor as a covariate in our analysis [30]. The CV for measuring FMD with our protocol is 6.7% [30].

Forearm blood flow measurements using venous occlusion plethysmography (FBF) measures changes in blood flow (mainly determined by arteriolar resistance arteries in the muscle bed) in response to the infusion of intra-arterial vasoactive medications [25,31]. It therefore mainly assesses microvascular function. FBF was measured at the forearm using ECG-triggered bilateral strain-gauge venous occlusion plethysmography [31]. Measurements were performed at 09:00 AM in a quiet, temperature controlled room (22°C). Mercury in silastic strain gauges placed around the widest portion of the upper third of both forearms were electrically coupled to a plethysmograph calibrated to measure normalized changes in volume. For each measurement, venous flow

was occluded just proximal to the elbow by rapidly inflating a blood pressure cuff to 60 mmHg. A wrist cuff was inflated to suprasystolic (220 mmHg) pressures to exclude the hand circulation from the blood flow during the measurement, starting 30 seconds prior to each measurement. After local anesthesia (lidocaine 2%), a brachial artery catheter (angiocath 20G 1.88in, BD Angiocath) was inserted in the non-dominant arm (after local anesthesia (lidocaine 2%)), which was elevated slightly above the right atrium. Systolic blood pressure (BP), diastolic BP, mean arterial BP and heart rate were monitored continuously. The other arm was used as a control for systemic changes in vasomotor tone. To establish resting FBF, we administered 0.9% saline for 30 minutes. Vasoactive agent infusions were then started. Between each series of drug infusions, FBF was allowed to return to basal value during a 20-minute resting period, during which solvent (0.9% saline for acetylcholine (Ach) and 5% glucose for sodium-nitroprusside (SNP)) was infused to maintain a constant infusion rate. Ach (Miochol-E intraocular solution, 20 mg, BauschandLomb; 1-2-4 µg/dL forearm volume/minute) was used to explore endothelium-dependent vasodilatation. SNP (25mg/ml, 2ml, Sigma-Aldrich; 0.2-0.4-0.8 µg/dL forearm volume/minute) was used to explore non-endothelium dependent vasodilatation. Finally, the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA acetate 250 mg, Clinalfa® Basic, Bachem 0.2-0.4-0.8 µmol/dL forearm volume/minute) was infused to investigate the contribution of nitric oxide to basal vascular tone. Each substance dose was infused for 5 minutes. FBF values are reported in ml/min/ 100 ml of forearm volume. The baseline value is a mean of all measurements during the baseline measurement period. The values during drug infusion are a mean value of the last 6 measurements per drug dose during a measurement period. Besides changes in blood flow, the blood flow ratio between the infusion and control arm was also calculated to correct for possible systemic effects[32]. The CV of FBF has been reported to be 8-10% during stimulation [31,33].

Statistical methods

Data were analyzed using SPSS 20.0 statistical package for Windows (SPSS Inc, Chicago, IL). Data were expressed as mean ± SD, unless mentioned otherwise. Data distributions were analyzed and logarithmic transformation was performed before statistical testing when appropriate. Differences between patients and controls were tested with paired t-tests. Differences in categorical variables were analyzed using the χ^2 test. In group A, stepwise backward multiple linear regression analysis was performed in the patients in order to test whether clinical characteristics (etiology of CS, treatment strategies, presence of hormonal deficiencies, use of alcohol, smoking and comorbidity) were predictors of vascular function. A stepwise backward multiple linear regression analysis could not reliably be performed in group B because of the small sample size. $P < 0.05$ was considered statistically significant.

Table 1) Group A: Clinical characteristics of patients in long-term remission of Cushing's syndrome and healthy controls.

	Patients (n=58)	Controls (n=58)	P-value
Gender (n): male/female	12/46	12/46	
Age: mean (± SD) (years)	50.8 (12.3)	51.2 (12.4)	0.863
BMI: mean (± SD) (kg/m2)	26.5 (4.2)	26.3 (4.1)	0.793
Duration of remission: median (± range) (years)	13.6 ± 8.0		
Smoking (yes/no)	14/44	5/53	0.024*
Pack-years (± SD)	11.5 (15.6)	6.9 (13.9)	
Alcohol consumption: yes/no	10/48	13/45	0.485
Treatment modalities: n (%)			
Unilateral adrenalectomy	19 (32.8)	-	-
Bilateral adrenalectomy	12 (20.7)	-	-
Pituitary surgery	38 (65.5)	-	-
Pituitary radiotherapy	13 (22.4)	-	-
Hormonal deficiencies: n (%)			
Glucocorticoid deficiency	21 (36.2)	-	-
Growth hormone deficiency	15 (25.9)	-	-
Thyroid hormone deficiency	25 (43.1)	-	-
Mineralocorticoid deficiency	11 (19.0)	-	-
Testosterone deficiency	6/12 (50.0)	-	-
Estrogen deficiency [1]	25/46 (54.3)	29/46 (63.0)	-
Comorbidities: n (%)			
Hypertension	18 (31.0)	0 (0)	-
Diabetes mellitus	4 (6.9)	0 (0)	-
Hypercholesterolemia	12 (20.7)	0 (0)	-
Cushing type: n (%)			
Pituitary	40 (69.0)	-	-
Adrenal	18 (31.0)	-	-

BMI: body mass index; CS: Cushing's syndrome.; * *P<0.05;*

Note [1]***:*** *Secondary hypogonadotropic hypogonadism or a postmenopausal state without the use of chronic estrogen replacement.*

Results

Subject characteristics

Table 1 shows the clinical characteristics of the patients and control subjects for group A, and Table 2a for group B. Intra-arterial cannulation was not successful in 3 patients and therefore the vasomotor response to intra-arterial drug infusions was investigated in 11 patients and controls (Table 2b). Adequate matching was reflected by the fact that no differences between patients and controls were present in both groups in gender, age and BMI. In group A patients only differed from controls with respect to smoking habits (more smokers in the patient group, P<0.05).

Biochemical markers associated with endothelial dysfunction (Group A)

No statistically significant differences in sVCAM-1, sICAM-1, E-selectin and PAI-1 were detected between patients and controls (Table 3).

Table 2A) Group B (Flow Mediated dilation): Clinical characteristics of patients in long term remission of Cushing's syndrome and healthy controls.

	Patients (n=14)	Controls (n=14)	P-value
Gender (n): male/ female	2/12	2/12	1.00
Age at time of test: mean (SD) (years)	46.8 (11.8)	45.7 (10.9)	0.79
Duration of remission: median (range) (years)	12.9 (4.8-29.4)	-	-
BMI: mean (SD) (kg/m2)	25.6 (2.3)	25.6 (2.5)	0.98
Cushing's syndrome type: n (%)		-	-
Pituitary	7 (50.0)		
Adrenal	7 (50.0)		
Treated hypothyroidism: n (%)	4 (28.6)	-	-
Estrogen status in females (n):			
Sufficient	7 (58.3)	7 (58.3)	1.00
Insufficient	5 (41.7)	5 (41.7)	

BMI: body mass index

Table 2B) Group B (venous occlusion plethysmography): Clinical characteristics in long-term remission of Cushing's syndrome and healthy controls.

	Patients (n=11)	Controls (n=11)	P-value
Gender (n): male/ female	2/9	2/9	1.00
Age at time of test: mean (SD) (years)	45.6 (13.2)	45.8 (12.1)	0.98
Duration of remission: median (range) (years)	12.8 (4.8-28.8)	-	-
BMI: mean (SD) (kg/m2)	25.7 (1.7)	25.3 (2.7)	0.62
Cushing's syndrome type: n (%)		-	-
Pituitary	5 (45.5)		
Adrenal	6 (54.5)		
Treated hypothyroidism: n (%)	3 (27.3)	-	-
Estrogen status in females (n):			1.00
Sufficient	5 (55.6)	5 (55.6)	
Insufficient	4 (44.4)	4 (44.4)	

BMI: body mass index

Non-invasive measurements of atherosclerosis and arterial stiffness (Group A)

cIMT, PWV and CAP were not different between patients and controls (Table 3). A trend towards a statistically significant difference between the two groups was found for the AIx (P=0.056). Atherosclerotic plaques were detected in 10 patients and 10 controls. Plaque thickness was not significantly different between patients and controls.

Endothelial function (Group B)

No statistically significant differences were found between patients and controls in all FMD measurements (Table 3). Furthermore, no statistically significant differences were found between patients and controls regarding FBF or blood flow ratio responses at baseline or in response to the incremental doses of Ach, SNP and L-NMMA (all $p > 0.09$) (Figure 1).

Table 3) Micro- and macrovascular health parameters in patients in long-term remission of Cushing's syndrome and matched controls.

Variable	Patients		N	Controls		N	P-value
	Mean	95%-CI		Mean	95%-CI		
GROUP A							
Serum biomarkers							
*ICAM-1 (pg/ml)	280.4	226.7-346.7	57	314.9	234.7-422.4	57	0.545
*PAI (pg/ml)	1810.8	1505.8-2163.1	57	1940.5	1653.9-2276.7	57	0.497
*VCAM-1 (pg/ml)	670.0	615.1-729.9	57	682.4	637.3-730.6	57	0.721
*E-Selectin (pg/ml)	40.0	35.7-44.6	57	38.5	34.6-43.0	57	0.661
Non-invasive measurements of arterial stiffness and atherosclerosis							
CAP (mmHg) (HR75)	10.1	8.8-11.5	52	9.4	7.6-11.3	52	0.457
Aortic AIx (HR75)	26.0	23.2-28.8	53	23.1	19.6-26.6	53	0.056
PWV (m/s)	8.4	8.0-8.9	58	8.3	7.8-8.8	58	0.648
Mean cIMT (mm)	0.75	0.72-0.78	58	0.75	0.72-0.77	58	0.617
Plaque thickness (mm)	2.66	1.94-3.38	10	1.95	1.71-2.18	10	0.092
GROUP B							
Measurements of flow mediated dilation							
Baseline diameter (mm)	3.60	3.33-3.86	14	3.56	3.30-3.82	14	0.839
FMD (%)	5.13	4.10-6.15	14	6.22	4.72-7.72	14	0.125
GTN (%)	18.6	15.5-22.0	14	19.4	15.0-22.9	14	0.691
Time to peak diameter (s)	40.3	33.4-47.3	14	54.3	42.1-66.6	14	0.059
SR_{AUC} (s, 10^3)	30323	25530-35115	14	32164	26471-37857	14	0.597

*Note [1] **: For ln-transformed data the geometric means and back-transformed 95%-CI were calculated to enable clinical interpretation of the outcomes.*

Note [2]: For plaque thickness the comparison between the groups was performed using an unpaired t-test ICAM-1, intracellular adhesion molecule 1; PAI-1, plasminogen activator inhibitor 1; VCAM-1, vascular cell adhesion molecule 1; CAP, central augmented pressure; AIx, augmentation index; cIMT, carotid intima-media thickness; PWV, pulse wave velocity; HR75, corrected for a heart rate of 75 beats per minute. FMD, flow mediated dilation; GTN, glyceryltrinitrate; SR_{AUC}, shear rate area under the curve; CI, confidence interval

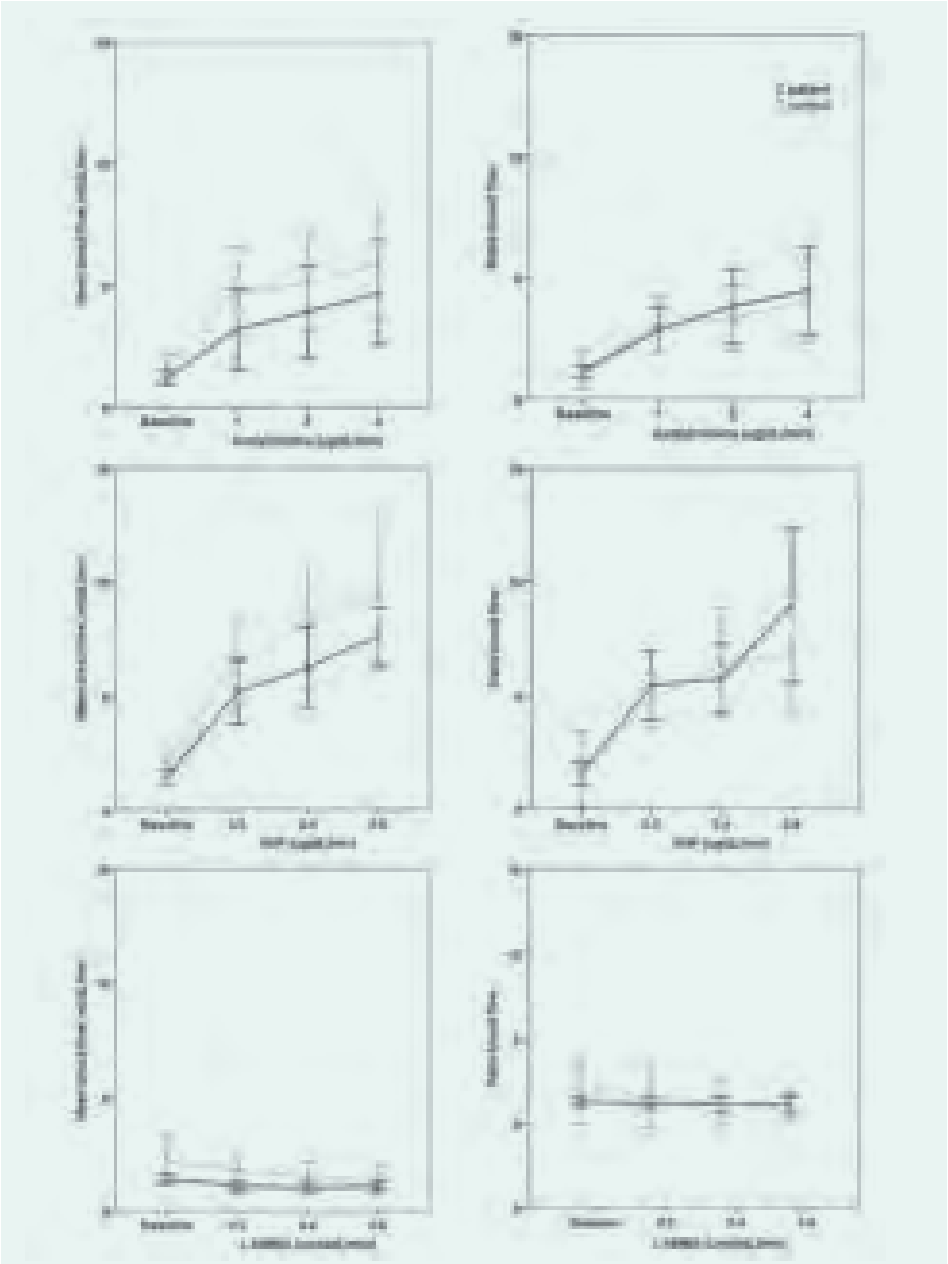

Figure 1) Change in forearm blood flow from baseline in response to infusion of different vasoactive agents in increasing dosages.

Note: On group level, no acute vasomotor responses were observed in the control arm after drug infusions. Error bars: 95% CI.

Stepwise backward multiple linear regression analysis (Group A)

Having DM predicted both a higher PWV (p=0.01) and higher sVCAM-1 levels (p<0.01). Subgroup analysis was performed for these two outcomes after exclusion of all matched patient-control couples containing a patient with DM. This did not lead to significant differences between patients and controls (PWV p=0.796; sVCAM-1 p=0.865). Being a smoker was a predictor for a higher AIx (p<0.01). Subgroup analysis, after exclusion of all patient-control couples with a smoker, did not lead to a significant difference between patients and controls (AIx; p=0.078). Mineralocorticoid replacement was a predictor for higher E-selectin levels (p<0.01). Subgroup analysis, after exclusion of all couples with mineralocorticoid users, did not lead to a significant difference between patients and controls (E-selectin; p=0.913). Thyroid hormone replacement was a predictor for higher sVCAM-1 levels (p<0.01). Subgroup analysis after exclusion of couples with thyroid hormone users did not lead to a significant difference between patients and controls (sVCAM-1; p=0.504).

Discussion

In this study we investigated micro- and macrovascular health in patients in long-term remission of CS who had no, or adequately treated comorbidities using a combination of state-of-the-art methods that has not been used in any previous study. We compared the patient group to a strictly one-to-one matched healthy control group. The main finding of our study is that the vascular health of patients in remission of CS is not significantly different from that seen in healthy control subjects matched for age, gender and BMI. This suggests that the direct effect of the period of hypercortisolism *per se* on the vasculature during the active disease is potentially reversible.

Our findings that endothelial function recovers after remission of CS are in line with the study of Akaza et al. who investigated arterial endothelial function, with FMD, in a group of 12 patients shortly after remission (>3 months) of CS [22]. They found that the impaired FMD in active CS was reversible after remission. Previous studies have shown that in vitro (cell culture) and in vivo (mouse) exposure of endothelial cells to glucocorticoids reduced the mRNA and/or protein content of endothelial NO synthase [34,35] and reduced acetylcholine induced vasodilation of mouse resistance arteries [34] and rat aortas [36]. Therefore Akaza et al. [22] proposed that endothelial dysfunction in active CS is largely accounted for by the direct effect of hypercortisolism on vascular endothelium and that this is reversible after treatment.

On the other hand, five other studies observed persistent impaired vascular health after remission of CS [17,18,20,21,23]. However, in three of these studies there was either a short period of remission [17] or a pediatric study population [20,21], so these studies are not comparable to our study. The studies reported by Colao et al. [18] and Barahona et al. [23] are more comparable. They both found a higher prevalence of atherosclerosis (measured by cIMT and presence of coronary artery disease detected by computed tomography, respectively) compared to gender-, age- and BMI-matched controls [18,23]. However, the patients in these studies had significantly more uncontrolled metabolic comorbidities than their matched controls. In our study population the comorbidities in Group A were adequately treated [16], and the patients in Group B had no known co-morbidities (except for treated hypothyroidism in 4 patients).

A more recent publication of Colao et al. [19] also supports our findings. This study measured differences in cIMT and arterial stiffness between active disease and one year after remission of CS in 25 patients. There was a significant decrease in both variables between active disease and remission. After 1 year of remission both variables did not differ from a gender-, age- and BMI-matched control group as used in our study, but they were still higher than in controls with a lower BMI, matched only for gender and age. Moreover, diastolic blood pressure, LDL and HDL cholesterol levels were not different between the patients and the BMI-matched control group, but were significantly more adverse in the patients compared to the controls with a lower BMI. This emphasizes the importance of strict matching of each patient to a healthy individual of at least the same gender, age and BMI if one wants to investigate the effect of the previous period of hypercortisolism *per se*.

Taken both our results and the previous findings into account, we conclude that patients in remission of CS, who are equally well-controlled for comorbidities as age-, gender- and BMI-matched healthy subjects, have comparable vascular health. This accentuates the need for stringent treatment of metabolic comorbidities in these patients. Interestingly, the normalized vascular health seems to be irrespective of the fact that these patients have, as we have previously shown, a more centripetal adipose tissue distribution and adverse adipokine profile than their age-, gender- and BMI-matched controls [16].

As could be expected in group A, DM was associated with a higher PWV and higher sVCAM-levels and smoking predicted a higher AIx, but this did not affect the results of the total group. Moreover the trend towards a higher AIx in the patient group disappeared after correcting for smoking. Interestingly, except for an association between mineralocorticoid replacement and E-selectin levels and the use of thyroid hormone

replacement and VCAM-1 levels, no other patient characteristic (e.g. etiology of CS, treatment strategies, hormonal deficiencies) negatively affected vascular health parameters. This is in contrast to previous studies, where for example the use of glucocorticoid replacement therapy was associated with an increased cardiovascular risk [10].

The major strength of our study is the broad spectrum of methodologies we used to investigate vascular health. All techniques are well-validated and reproducible [25,30,31]. Furthermore this is the first study that investigates endothelial function in patients in long-term remission of CS both in conduit arteries (FMD) and forearm resistance arteries (FBF, which is considered the gold standard procedure to measure endothelial dysfunction)[25]. Thus we have investigated both the macrovasculature and the microvasculature.

A possible limitation of this study is the relatively small sample size for group B. For FBF and FMD a number of about 10 patients was found to be adequate to detect a relevant difference [31,37], however the subjects within our patient group (and thus also the control group) were more heterogeneous than in most previous studies, leading to a greater SD. Therefore it is possible that we missed subtle but relevant differences. For example, there seems to be a non-significant trend towards a lower baseline FBF in the patients, which could indicate a reduction in muscle microvascular density. The latter might explain the exercise intolerance experienced by the patients [38]. As blood flow in the skin and subcutaneous adipose tissue also contribute to FBF [31], future research measuring microvascular density in muscle biopsies will have to confirm whether skeletal muscle microvascular density is indeed lower in patients in remission of CS.

A multitude of epidemiological studies reported an increased cardiovascular risk and standardized mortality rate (SMR) in patients in long-term remission of CS compared to an age- and gender- but not BMI-matched reference population [1]. As patients in remission of CS tend to have an overall higher BMI and waist circumference than the general population, this may negatively affect cardiovascular risk and SMR. Furthermore these studies did not analyze potential differences between patients with and without comorbidities. However it may be possible that cardiovascular risk is still elevated in the healthiest patients in remission of CS because of a persistent effect of the prior hypercortisolism on other organs than the vasculature, e.g. the myocardium [10,11]. However, this was not supported by a small study [39]. Therefore further research is necessary to investigate these issues.

In conclusion, vascular health of patients in long-term remission of Cushing's syndrome seems to be comparable to that of healthy gender-, age- and BMI-matched controls,

provided that the patients have no, or adequately controlled comorbidities. Therefore, the effects of the previous hypercortisolism *per se* on the vasculature may be reversible. This accentuates the need for stringent treatment of metabolic comorbidities in these patients.

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10

General discussion and summary

This thesis focuses on the treatment and long-term effects of two diseases in which the pituitary gland plays a key role: acromegaly, which is caused by overproduction of growth hormone (GH), and Cushing's syndrome (CS), caused by overproduction of cortisol.

PART 1
Treatment of acromegaly and Cushing's disease by transsphenoidal surgery

The endoscopic technique of transsphenoidal pituitary surgery (TS) was introduced in the 1990s as an alternative to the traditional microscopic technique of TS, which was considered the "gold standard". Compared to the microscopic technique, the endoscopic technique of TS theoretically has both advantages and disadvantages. Advantages include the panoramic view with increased illumination of the operating field and the possibility to use different operating angles, making it possible to reach suprasellar and parasellar regions. Disadvantages include the two-dimensional view and the easy clouding of the lens in case of bleeding [1,2]. For these reasons, there has been controversy among neurosurgeons about the position of endoscopic TS for the treatment of pituitary adenomas [1,2]. However, despite the fact that the endoscopic technique was received with skepticism soon after its introduction, a recent report by Rolston et al. that analyzed nationwide data from the USA shows that in more recent years its popularity has steadily increased among neurosurgeons. This is illustrated by a clear increase in the use of the endoscopic technique of TS at the expense of the microscopic technique [3]. But, as the report states, the underlying causes of these trends are not yet clear.

Because our centre was the first in the Netherlands to introduce the endoscopic technique of TS and has accumulated ample experience over the years with this technique, **the first aim of this thesis was to gain more insight in the role of endoscopic TS for the treatment of both acromegaly and CD by systematically analyzing the results of endoscopic TS in our hospital.**

In chapter 2 we analyzed the outcome of endoscopic TS in 40 patients with a growth hormone (GH)-secreting macroadenoma (thus causing acromegaly) treated in our hospital between 1998 and 2007. In 36 patients, the intent of TS was cure, whereas in four patients TS was aimed at debulking of the tumor. We found that of the 36 patients, remission was achieved in 20 patients (56%). The overall remission rate was 50%. However, remission was achieved in 6 out of 18 patients (33%) in the first 5 years after

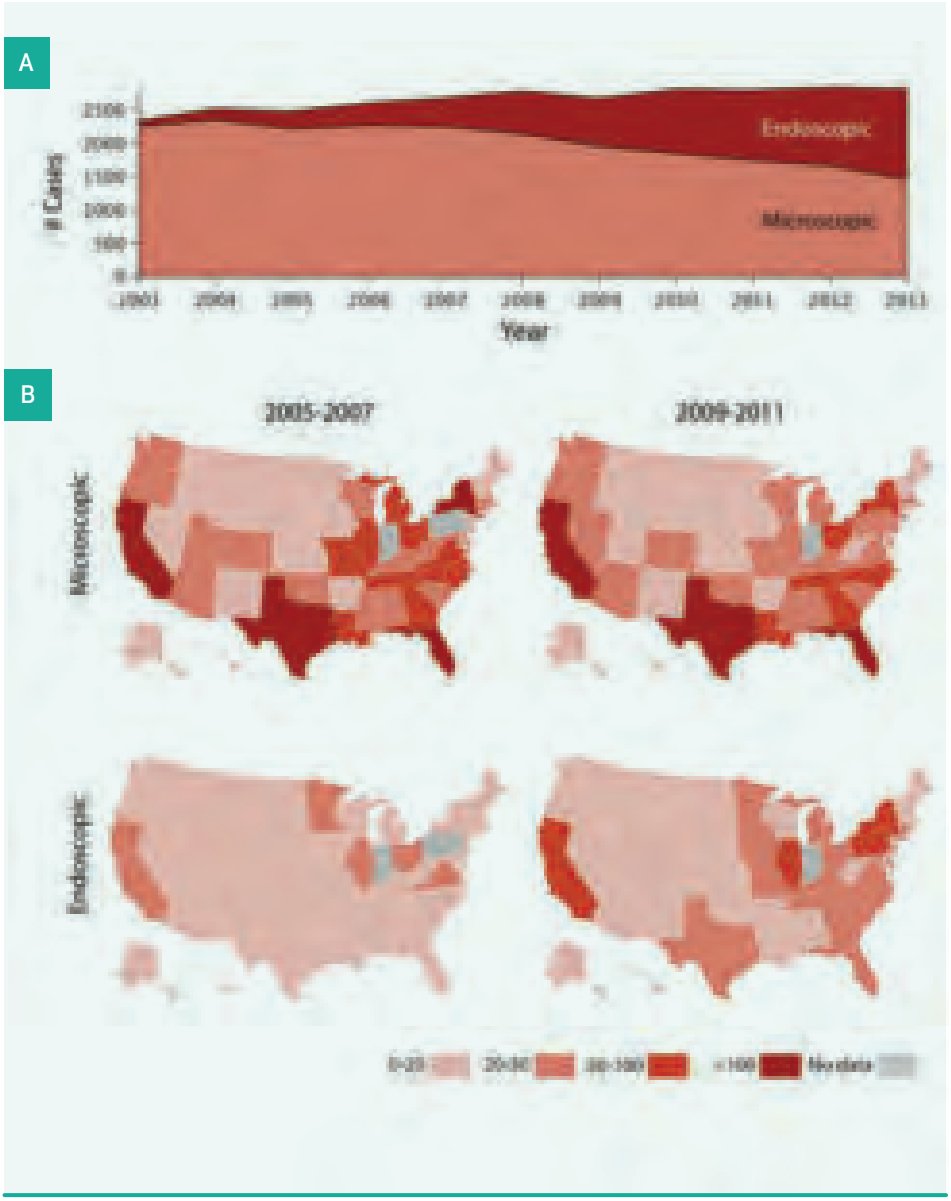

Figure 1) Trends in pituitary surgery in the United States.

A) National data from the Centers for Medicare and Medicaid Services.

B) State-level data, shown as the average number of cases per year during the periods 2005–2007 and 2009–2011 [3].

Reproduced from Rolston et al, 2015, with permission of Springer.

introduction of the endoscopic technique compared to 14 out of 22 patients (63%, or 77% if the patients who underwent debulking are excluded) in the following 5 years (p=0.06 and p=0.01, respectively). Thirteen patients had a mild transient perioperative complication. Before TS 37.5% of the patients received hormonal substitution therapy compared to 33% after TS.

In **chapter 3** we analyzed the outcome of endoscopic TS in 86 patients with Cushing's disease (CD), treated in our hospital between 1998 and 2011. The remission rate in different adenoma subclasses varied significantly: 60% in magnetic resonance imaging (MRI)-negative CD, 83% in microadenomas, 94% in noninvasive macroadenomas, and 40% in macroadenomas that invaded the cavernous sinus. The recurrence rate was 16% after 71 ± 39 (mean ± SD) months of follow-up. All perioperative complications were relatively mild and did not cause any permanent damage. Before TS 15% of the patients received hormonal substitution therapy compared to 35% after TS.

The main conclusion of both **chapters 2 and 3** was that the remission rates that were achieved by endoscopic TS in noninvasive and invasive macroadenomas were comparable with or even better than previously reported remission rates after conventional microscopic TS. Furthermore, complication rates, types of complications and recurrence rates seemed comparable between the two techniques. In order to support these conclusions an extensive literature search was performed in both studies to gain more insight in the previously published remission rates. However, the multitude of definitions of remission and recurrence that were used in studies reporting on both CD and acromegaly made it very difficult to compare the various studies. Standardization of the definitions for remission and recurrence and of the methods required to analyze results of TS is essential in order to improve clinical practice and maximize patient outcomes. In acromegaly this is already done: most recent series have used the consensus criteria of remission of either 2000 [4](as we did) or 2010 [5]. In CD consensus on the criteria for remission still has to be reached [6].

Nevertheless, recent large reviews covering the literature on TS in pituitary adenomas concluded that in macroadenomas, especially when invasive, the endoscopic technique of TS seems to result in better remission rates than microscopic TS, whereas in microadenomas the remission rates and complication rates are comparable between both techniques [7,8]. The better results achieved by the endoscopic technique in macroadenomas may be explained by the fact that different operating angles can be used with an endoscope, which makes it possible to effectively reach suprasellar and parasellar portions of the lesion, including the cavernous sinus [9].

Theoretically the endoscopic technique of TS may also have advantages compared to the microscopic technique in case of repeated TS. The wider view of the endoscopic technique may provide an advantage during repeated TS, particularly in cases in which the anatomy is altered due to the previous operation [10]. Repeated TS is theoretically of particular value in CD, as all other treatment options (bilateral adrenalectomy, radiotherapy and medication) have substantial disadvantages.

To gain more insight in the value of repeated endoscopic TS for CD, we analyzed the remission rates and complications of repeated endoscopic TS in 14 patients with persistent (n=6) or recurrent (n=8) CD who underwent surgery in our hospital between 1999 and 2007 **(Chapter 4)**. A remission rate of 71% was achieved, which compared favorably with the remission rates previously reported after repeated TS via the conventional microscopic technique. Cerebrospinal fluid leakage was the most frequent complication (6 patients). Eleven patients required hormonal substitution after the second surgery, of whom 3 already received hormonal substitution therapy after the first TS. We concluded that repeated TS via the endoscopic technique is a good treatment option for selected patients with recurrent or persistent CD following primary pituitary surgery.

In **chapter 2** we also analyzed the results of repeated endoscopic TS for persistent and recurrent CD. We confirmed that repeated endoscopic TS seems to be a good therapeutic option for patients with recurrent CD with remission achieved in four of five (80%) patients. However, repeated endoscopic TS seemed to be of less value for patients with persistent CD as remission was only achieved in three of 11 patients (27%). A possible explanation why repeated endoscopic TS was less successful in patients with persistent CD than with recurrent TS in our series is the fact that the same neurosurgeon who did the first operation also performed the second operation in case of persistent hypercortisolism. This makes it understandable that if the adenoma could not be removed the first time by the same well-trained neurosurgeon using the same operating technique, the chance that it can be removed the second time is small. However we believe that repeated TS in persistent CD should nevertheless be considered, as long as no better alternative therapy (such as effective medication) exists. Until now no other series of repeated endoscopic TS in CD have been published, so we cannot compare our results with the literature.

To accurately assess the superiority of one technique over the other, a robust large comparative study should be performed. Indeed some groups have attempted to perform such a trial [11-14], but these are all single center trials where one surgeon used one technique and another surgeon used the other. Therefore, the differences described

(if present) could possibly be explained by differences in the technique-specific skills of the surgeons. Ideally a trial should be multicenter, randomized, with standardized surgical protocols and a large number of patients included. Besides remission rates it should assess recurrence rates, complications, cost-effectiveness and patient-related outcome measures. However, it will be very difficult to perform such a trial because of several reasons: 1) Acromegaly and CD are very rare diseases, so inclusion will take a very long time and inclusion of a sufficient number of patients would require participation by a large number of centers. 2) Many neurosurgeons have their own personal preference, which makes it difficult to start a randomized trial 3) It will be difficult to obtain financial support for such a study. Therefore the second best option to gain knowledge on the results of these techniques is to encourage publication of outcomes of TS, particularly in large series of different tumor types from different centers applying the technique in a standardized way. Another possibility would be to initiate mandatory registries for all patients undergoing TS.

In chapter 2 we showed that remission rates achieved for GH-secreting macroadenomas was significantly lower in the first 5 years after the introduction of the endoscopic technique compared to the second 5 years (33% versus 63%). Because the characteristics and number of the patients operated on in the first and second 5 years were comparable, we concluded that the learning curve of the surgeons probably explains the difference in remission rates. Indeed other series have described a learning curve as well after the endoscopic technique was introduced [15-18]. But also for microscopic TS there is strong evidence that remission rates depend on the skills of the surgeon [19,20]. Although a second attempt to perform TS can be done, as we described in chapter 4, remission rates are considerably lower than after the first TS. Keeping these considerations in mind we believe that TS, particularly for rare hormone-producing tumors such as in CD and acromegaly, should only be performed in highly specialized centers. Apart from specific surgical expertise these centers should have extensive experience with the complex problems these patients are facing both in the perioperative phase and during long-term follow-up. In the Netherlands 14 hospitals currently perform TS for pituitary adenomas. Only 2 hospitals perform more than 50 operations per year while in 6 hospitals less than 7 operations per year are performed (*www.qrns.nl*). Centralization of TS could represent a valuable step towards optimization of the care of patients with pituitary adenomas in the Netherlands.

In conclusion, based on all published series, endoscopic TS is a safe and effective treatment for all patients with acromegaly and CD. In case of noninvasive and invasive macroadenomas the endoscopic technique of TS should be the treatment of choice, as

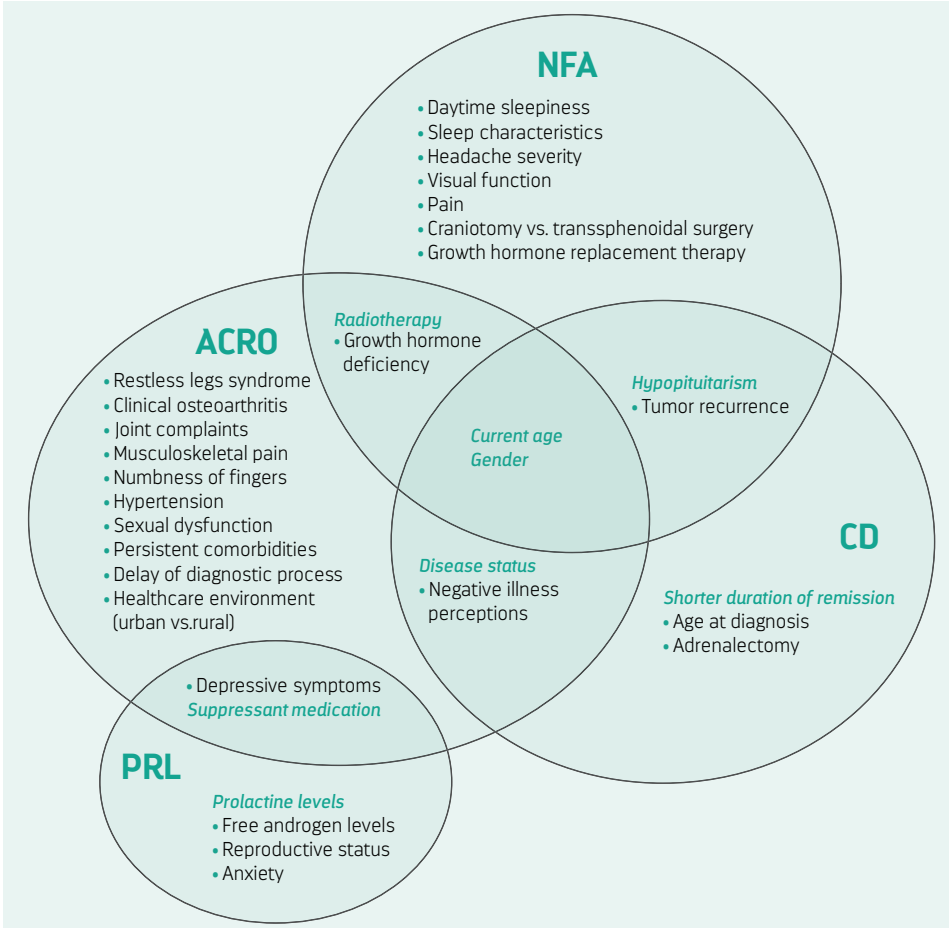

Figure 2) Factors which have been found to significantly influence QoL in pituitary adenomas.

Normal letters: consistent between studies. Italic letters: inconsistent between studies.

ACRO acromegaly, NFA non-functioning pituitary adenoma, CD Cushing's disease, PRL prolactinoma. Reproduced from Andela et al, 2015, with permission of Springer [21].

remission rates seem to be higher than those reported for microscopic TS. Furthermore repeated endoscopic TS seems to be a good therapeutic option for patients with recurrent CD. Ideally the endoscopic and microscopic techniques of TS would be compared via a comparative study but this is virtually impossible to perform. Therefore different centers should continue to publish their TS results, using consensus criteria for cure. Furthermore, because there is a clear learning curve for both techniques of TS, TS should be centralized in order to optimize treatment outcomes.

PART 2
Long-term Quality of Life after treatment of acromegaly and Cushing's syndrome

A growing number of studies show that QoL is impaired in patients with pituitary adenomas, and that successful treatment improves, but does not normalize QoL [21]. Furthermore, patients in long-term remission of CS and acromegaly generally have a more impaired QoL than patients treated for other pituitary adenomas [21]. In order to understand why QoL remains impaired in patients in long-term remission of CS and acromegaly, it is essential to determine which patient- or disease-specific characteristics negatively influence QoL. A large number of studies have described multiple factors that might influence QoL in patients with pituitary adenomas (Figure 2) [21]
In acromegaly (which causes marked changes in appearance that partially persist after remission) the influence of self-consciousness about appearance on QoL, which is well known to affect QoL in the general population [22,23], had never been adequately investigated. Furthermore in CS most previous studies had methodological limitations or only included patients with CD, which made it difficult to establish patient or disease characteristics related to impaired QoL in patients in long-term remission of CS.

Therefore the second aim of this thesis was to get more insight in patient- or disease-specific characteristics that negatively influence QoL in patients in long-term remission of acromegaly and CS.

In chapter 5 we investigated the psychological distress and dysfunction related to self-consciousness about appearance and its effect on QoL in 73 patients in long-term remission of acromegaly. To do so, participants were asked to complete the Derriford appearance scale 59 (DAS59) questionnaire, which assesses psychological distress and disruptions to everyday life associated with self-consciousness of appearance. To measure QoL they were asked to complete the research and development 36 (RAND-36) and acromegaly quality of life questionnaire (AcroQoL) and a sociodemographic questionnaire. The results of the patients were compared to the results of a gender-, age- and BMI-matched control group (n=57). We found that significantly more patients than control subjects reported self-consciousness about their appearance (46.6% versus 22.8%). Eighty-five percent of the patients that were self-conscious about appearance indicated that their face was the most prominent source of self-consciousness compared to 23 % of the controls. In the patient group, significant correlations were found between the scores of the DAS59 and the AcroQoL and RAND-36. Furthermore

there was no correlation between the DAS59 score and gender or age in the patient group. We concluded that even after long-term remission of acromegaly, a large number of patients are self-conscious about their appearance, especially about their facial appearance, and that this leads to psychological distress, disruptions of everyday life and decreased QoL.

Our results are in line with the recent findings of Tiemensma et al, who asked patients in remission of acromegaly to complete a drawing test (two retrospective drawings of their body perception before acromegaly and during the active phase of acromegaly, and one drawing on the current condition after long-term remission) [24]. Patients who drew larger (in cm) drawings to depict their current condition had a worse QoL. Moreover, in our study, the scores of our patients on the DAS-59 are comparable to scores of groups that are considered suitable for cosmetic surgery [25]. This underlines the major impact that the changes in appearance caused by acromegaly have on the patients. In retrospect, it may not be so surprising that in the present society, where the normal population is increasingly pressured to maintain beauty (as is reflected by the rapid increase in the use of botox and fillers [26]) patients in remission of acromegaly may remain concerned and self consious about their appearance. Therefore this aspect should be addressed during follow-up in order to improve QoL in these patients.

In chapter 6 we assessed the QoL of 123 patients in long-term remission of CS and compared it with the QoL of an age- and sex-matched control group (n= 105). To do so, participants completed seven validated questionnaires (both general health and disease-specific) concerning different dimensions of QoL. To investigate the influence of the etiology of CS on QoL, patients in remission of pituitary and adrenal CS were compared. Furthermore, the influence of hormonal deficiencies, treatment strategy, duration of remission, gender and age on QoL was investigated. We found that QoL in the total patient group and each patient subgroup was significantly worse on practically all dimensions of the questionnaires compared with the control group, except for patients in remission of pituitary CS without hormonal deficiencies, who had an impaired QoL on only 50% of the QoL dimensions. Subgroup analysis revealed no difference in QoL between different patient groups, especially no difference between patients in remission of adrenal and pituitary CS. Female gender and a shorter duration of remission had a negative influence on QoL in the patient group.

We concluded that QoL remains impaired in patients in long-term remission of CS, regardless of etiology, presence of hormonal deficiencies and treatment strategies. The cause of impaired QoL is probably multifactorial. However the fact that QoL is impaired in all

patient groups in long-term remission of CS is suggestive for the fact that the previous period of long-standing exposure to high cortisol levels *per se* might be an important cause of the impaired QoL.

Other factors that may influence QoL of patients in remission of CS and acromegaly

Coping strategies and illness perceptions

Coping is defined as the way a person responds (behaviorally, cognitively, and emotionally) to situations that require adjustments in dealing with an adverse event and/or its consequences, for example an illness and its treatment. It has been found that patients in remission of CD and acromegaly have less active coping strategies (and have more avoiding coping strategies, and seek less social support than a healthy sample from the Dutch population [27]. However compared with patients with chronic pain and psychological problems, patients treated for pituitary adenomas have somewhat better coping strategies [27]. Nevertheless, coaching patients in remission of CD and acromegaly toward more effective coping strategies may improve QoL.

Illness perceptions concern the pattern of beliefs that patients develop about their illness. These views are determinants of behavior directed at the illness. Tiemensma et al. found that patients in remission of CS have more negative illness perceptions than patients with other acute or chronic conditions (vestibular schwannoma, acute or chronic pain, and to a lesser extent chronic obstructive pulmonary disease) [28]. They attributed more symptoms to their disease, had stronger beliefs regarding the chronic nature of the disease, reported more negative consequences and experienced less personal controlability. Another study showed that patients in remission of acromegaly reported more negative illness perceptions than patients with acute illness, but more positive illness perceptions than patients with chronic diseases or CS [29]. In both studies illness perceptions were strongly correlated with QoL. However, it is very well possible that the perception that the disease is chronic is adequate, as many patients in remission of CS and acromegaly remain chronic patients because they require hormonal substitution or still need medication to control the disease. Indeed in CS the patients that required hormonal substitution had more negative illness perceptions than patients who did not [30] and two recent studies have shown that patients who need medication to control acromegaly reported stronger beliefs about the chronicity of the disease than patients who do not use medication, particularly when they realized the medication had to be taken lifelong, which had a strong impact on QoL [31,32]. This may explain why our group and others found that acromegaly patients who are well controlled with somatostatin analogues have a worse QoL than patients that are in remission of acromegaly after TS [33,34]. Another explanation for the fact that patients in remission of CS and acromegaly

have such negative illness perceptions, is that there is accumulating evidence that both diseases have chronic physical effects, for which there is accumulating evidence (as discussed below). However, even if the illness perceptions are adequate, it is important to be aware that patients have them. Furthermore it should be investigated if these illness perceptions can be modulated in order to improve QoL.

Effects of the previous disease on personality, behavior and the brain

There is increasing evidence that both CS and (to a lesser extent) acromegaly have marked effects on the central nervous system, which affects personality and behavior, and that these effects are (at least partially) irreversible [35,36]. This can be explained by the fact that the brain (predominantly limbic structures like the hippocampus and the prefrontal cortex) highly expresses glucocorticoid receptors, is are also rich in GH and IGF-1 receptors [37,38]. Cognitive function, reflecting memory and executive functions, has been found to be impaired in patients in long-term remission of both CD and acromegaly [35,39,40]. Furthermore, both patients in remission of CD and acromegaly have more psychopathology and maladaptive personality traits than healthy control subjects [41-43]. Moreover it has been found that patients in long-term remission of CS have structural alterations of the brain, compared to matched controls, with smaller grey matter volumes in the anterior cingulated cortex, larger grey matter volumes in the cerebellum [44], widespread reductions in white matter integrity [45], and alterations in specific neuronal metabolites in the hippocampus [36,46,47]. It is not yet proven that the structural brain alterations are the cause of behavioral phenotype of patients in remission of CS, but this seems possible. Structura l changes in the brain may also be present in patients in remission of acromegaly as two studies described larger grey and white matter volumes and larger hippocampal volumes in patients compared to matched controls. [48,49].

Patients own perception of QoL

When discussing QoL of patients with pituitary adenomas it is important to realize that all of the available questionnaires, including the disease-specific questionnaires, have been developed by physicians. For development of the disease-specific questionnaires, patients were interviewed, but the interviews were semi-structured about preselected topics. A recent study from Leiden used patient focus groups to identify factors that influence QoL in patients in remission of pituitary adenomas as perceived and discussed by the patients themselves [50]. It identified new QoL aspects from the patients' perspective, which are not included in the currently available disease-specific questionnaires (Figure 3). The most important lesson that can be drawn from this study is that patients themselves have to be inquired about their concerns and consulted when we develop strategies aimed to improve their QoL.

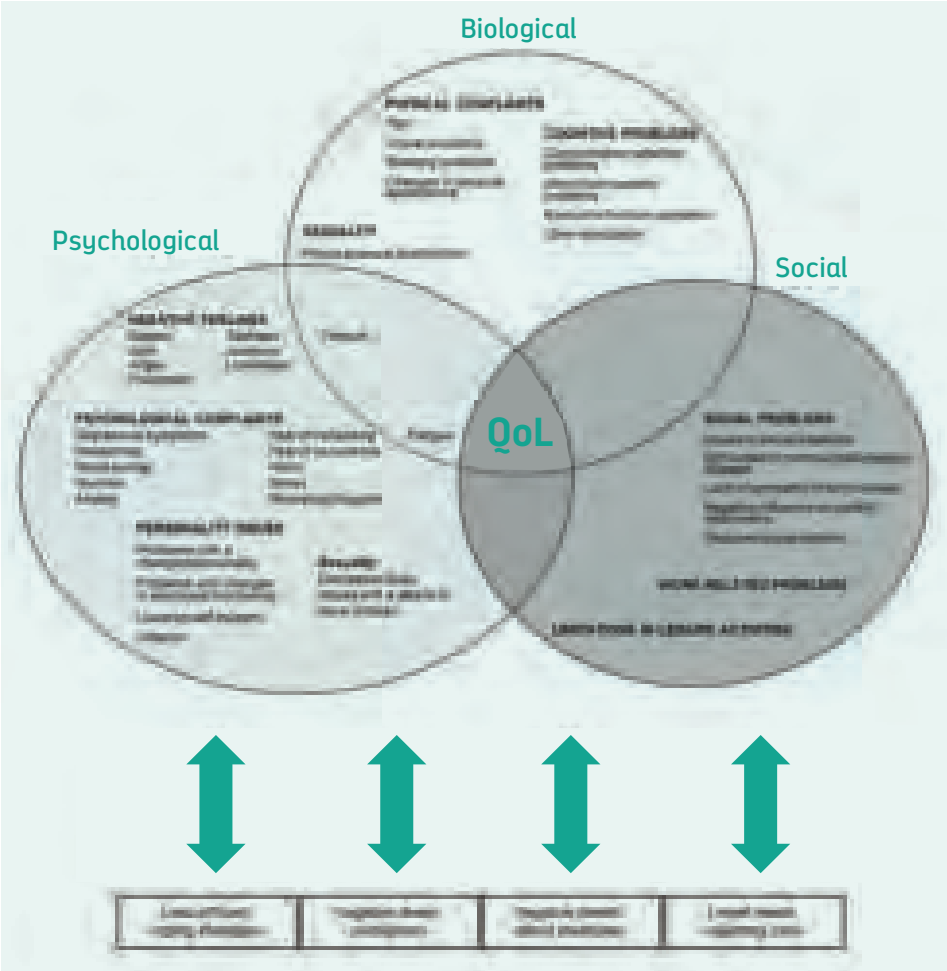

Figure 3) QoL in patients with pituitary diseases, as perceived by patients.
Bold letters: additional aspects which are not included in available disease-specific questionnaires [50]. Reproduced from Andela et al, 2015, with permission of Springer.

In conclusion, the cause of impaired QoL of patients in remission of CS and acromegaly is multifactorial. When designing interventions to improve QoL in these patients, patient-related determinations of QoL should be taken into account in addition to disease-specific factors that are identified by physicians. Furthermore, coping strategies and illness perceptions of individual patients should be taken into account. We believe that, to optimally improve QoL, each patient should be offered a personalized rehabilitation program in which each individual patient is counseled by a team consisting of at least

an endocrinologist, a psychologist and a physiotherapist. Future research should investigate the effectiveness of such an intervention.

PART 3
Long-term physical sequelae after treatment of acromegaly and Cushing's syndrome

The third aim of this thesis was to gain more insight in the long term effects of the previous period of growth hormone or cortisol hypersecretion on physical health of patients in long-term remission of acromegaly and CS.

Acromegaly

Craniofacial disproportions

The introduction and enormous development of three-dimensional (3D) imaging techniques since the 1970s has created new opportunities for researchers and clinicians to accurately assess facial deformities in three dimensions. However, although craniofacial disproportions are highly prevalent in patients with acromegaly, no studies had been performed to quantify these disproportions with 3D imaging techniques. The Department of Oral and Maxillofacial Surgery of the Radboud University Medical Centre has a 3D laboratory since 2005, with the aim to implement this technique in the daily practice of orthodontics and maxillofacial surgery. They developed methods to fuse 3D cone-beam computed tomography (CBCT) data (which images the facial skeleton) with 3D stereophotogrammetry (which captures the 3D soft tissue facial profile) into a 3D virtual head model, making it possible to quantify in detail all dimensions of the face (bone and soft tissues) [51]. With this 3D virtual head model accurate measurements can be made with 3D cephalometry [52]. Therefore this imaging method is ideal to quantify craniofacial disproportions in patients with acromegaly.

As a pilot study to test this technique in patients with acromegaly, we evaluated the differences in craniofacial dimensions with 3D cephalometry between 16 patients in long-term remission of acromegaly and 16 control subjects matched one on one for gender, age, BMI and ethnicity **(chapter 7)**. We found that the previously described (using 2D cephalometry) classical bony craniofacial deformities in patients with active acromegaly (e.g. a larger mandible and a longer total facial height) persist after long-term remission. 3D cephalometry also revealed that patients in remission of acromegaly have a wider face at the level of the zygoma and a longer maxilla compared to controls. Further-

more 3D cephalometry shows that many typical soft tissue deformities persist, even after long-term remission. We concluded that significant craniofacial changes persist even after long-term remission of acromegaly, and that 3D cephalometry is an attractive novel imaging modality to accurately investigate craniofacial disproportions of both soft tissue and bony parts of the face in patients with acromegaly.

We believe that 3D imaging is a very promising technique for patients with acromegaly, both in research settings and daily practice. Concerning research, 3D facial analysis has been shown to be very reproducible in one individual; even if a considerable amount of time has passed between two images [53]. This makes it an ideal technique to investigate how both the soft tissue and bony parts of the face of patients with acromegaly change over time. Indeed our group is already performing a longitudinal prospective study in which we regularly capture 3D images of patients in the first 2.5 years after diagnosis. Furthermore, recently a standardized method to analyze 3D stereophotographs of the hand has been developed in our hospital [54,55]. This provides a unique chance to investigate the effects of acromegaly on the soft tissue of the hands. We already found that significant soft tissue overgrowth of the hand persists in patients in long-term remission of acromegaly (article submitted). We are currently also studying changes of the hand in a prospective way.

We expect that 3D imaging will have clinical impact for patients with acromegaly who need orthognatic surgery because of jaw problems, as it is already used in daily practice for the planning of orthognatic surgery [56]. Furthermore, with the use of a virtual head model, personalized printable osteosynthetic plates or templates to guide positioning during surgery can be made. Another possible future clinical application of 3D imaging in acromegaly may be its use as a diagnostic tool. In the last few years several reports have suggested that acromegaly can be detected with facial image analysis of normal photographs. With this technique, at present, patients and control subjects can be accurately classified in up to 90% of cases [57]. It is very well possible that, if a 3D stereophotograph is used instead of a regular photograph, the accuracy will be even more. Furthermore, because 3D stereophotographs are very reproducible in a patient, they can possibly be used to detect a relapse or, if a patient uses medication to control his acromegaly, as a disease activity score along with the IGF-1 values.

Other possible long-term physical sequelae caused by acromegaly and recommendations for future research

To date, besides our pilot study on craniofacial disproportions in acromegaly, no other study has investigated the long-term physical consequences of acromegaly in a patient

group that is in remission after only TS, compared to a matched control group. Most studies have been performed in patients who are biochemically controlled with somatostatin analogues and/or pegvisomant, or in a mixed patient population of patients in remission after TS and on medication. The use of medication can have a significant effect on long-term outcomes. For example, Claessens et al. found that patients with biochemically controlled acromegaly using somatostatin analogues had a 9-fold increased risk to develop osteophyte progression compared with patients cured by surgery or additional radiotherapy [58]. Indeed, it has been postulated that somatostatin analogues may normalize serum IGF-1 levels, but that increased GH action in extra-hepatic tissues can persist [59]. Furthermore in previous studies on long-term consequences of acromegaly many patients had other hormonal deficiencies that may have affected the outcomes. Therefore we conclude that the long-term physical consequences of a period of long-standing GH excess *per se* are still unknown. In order to accurately investigate the long-term effects of GH excess, studies should be performed in patients that are in sustained remission of acromegaly after TS and do not have any (or only adequately treated) hormonal deficiencies. Major topics that should be investigated are body composition, metabolic outcomes, presence of cardiovascular disease, presence of OSAS and eventually mortality rates.

Cushing's syndrome

Adipose tissue distribution and function

Adipose tissue, and especially visceral adipose tissue (VAT), is an important endocrine organ that produces various adipokines which regulate the microenvironment of the adipose tissue and communicate with the brain, heart, vasculature, liver, and muscle [60]. These adipokines can be pro-inflammatory or anti-inflammatory, and their balance is crucial in maintaining systemic homeostasis. Common obesity has been shown to induce adipose tissue dysfunction (a process that is triggered when macrophages infiltrate the adipose tissue in response to microhypoxia and rupturing of hypertrophic lipid-laden adipocytes), which leads to a dysregulated adipokine production [61]. This induces systemic low-grade, chronic inflammation, which contributes to the development of metabolic and cardiovascular diseases [60,61].

CS almost inevitably leads to a redistribution, of body fat deposition resulting in increased abdominal adiposity with increased abdominal VAT and subcutaneous adipose tissue (SAT), but reduced peripheral SAT [62]. Although the biochemical and molecular mechanisms of the depot-specific actions of glucocorticoids (GCs) on adipose tissue remain poorly understood, several mechanisms have been suggested to play a role in the cause of centripetal adiposity in CS [62]. Firstly, hypercortisolism can affect appetite, resulting

in an increased caloric intake, especially of dietary fat [63]. Secondly, glucocorticoids regulate multiple steps in the process of adipogenesis. They increase the expression of numerous genes involved in fat deposition, which are depot specific [64]. Thirdly, CS causes increased adipocyte lipoprotein lipase activity in abdominal adipocytes with decreased lipolytic activity, which results in adipocyte hypertrophy. Furthermore, activity of 11β-hydroxysteroid dehydrogenase type 1 activity (11β-HDS-1) which converts inactive cortisone to active cortisol in peripheral tissues, may play a crucial role in the development of centripetal obesity in CS, as was demonstrated by the case of a young girl with CS who did not get the Cushingoid phenotype because of a partial defect in 11β-HSD-1 activity [65]. However, while 11β-HSD mRNA in omental adipose tissue is increased in obese individuals compared to lean controls, this was not the case in obese patients with active CS [66]. Other mechanisms that have been suggested to contribute to centripetal obesity in CS are the inhibition of adipose tissue AMPK activity (AMPK regulates carbohydrate and lipid metabolism in adipose tissue) or upregulation of LIM domain only 3 (LMO-3, a proadipogenetic factor) by glucocorticoids [67]. In addition to the centripetal adipose tissue distribution in active CS there also seems to be adipose tissue dysfunction, reflected by the fact that adverse adipocytokine profiles have also been described in active CS [67]. The role of macrophages in adipose tissue dysfunction in active CS may differ from common obesity, as hypercortisolism affects the immune system.

Patients in long-term remission of CS continue to complain about excess of abdominal fat [30]. Because a centripetal adipose tissue distribution with adipose tissue dysfunction is strongly associated with metabolic and cardiovascular diseases, we investigated the adipose tissue distribution, adipocytokine profiles and metabolic risk profiles of 58 patients in long-term remission of CS in **chapter 8**. Each patient underwent an extensive clinical evaluation with measurement of weight, height, blood pressure, heart rate, skinfold measurements and measurement of circumferences. Serum and plasma markers for cardiovascular risk and adipokine and cytokine profiles were determined, and a dual-energy X-ray absorptiometry (DEXA) scan was performed to determine body fat distribution. We compared the patients with a healthy control group matched one on one for age, gender and BMI. We found that patients in long-term remission of CS had a greater waist circumference, a smaller thigh circumference, a higher waist-to-hip ratio and a higher hip-to-thigh ratio. As measured with DEXA, patients had a higher percentage of truncal fat mass, and the truncal fat mass to leg fat mass ratio was also higher. Biochemically, patients had lower adiponectin levels, higher leptin levels, and higher resistin levels than control subjects, which indicatesan adverse adipokine profile, indicative for adipose tissue dysfunction. Furthermore patients had higher triglyceride levels. We concluded that even after long-term remission, patients who suffered from

CS in the past continue to have a centripetal adipose tissue distribution and an adverse adipokine profile. This was independent of the etiology of CS, treatment strategies, presence of hormonal deficiencies and comorbidity.

To date only three other, smaller, studies have investigated body composition after long-term remission of CS, and they are all case-control studies [68-70]. They all concluded that patients in long-term remission of CS have a centripetal adipose tissue distribution, as we did. Barahona et al. also found an adverse adipokine profile with lower adiponectin levels in patients in remission of CS, which supports our finding of an adverse adipokine profile of patients in long-term remission of CS [69].

However, no study has yet objectified if the increase in abdominal adipose tissue is caused by an increase in visceral adipose tissue (VAT) or subcutaneous adipose tissue (SAT). Colao et al. estimated the adipose distribution by solely measuring the waist-to-hip ratio and both Barahona et al. and Ragnarsson et al. used conventional DEXA scanning protocols as we did. It is important to differentiate between VAT and SAT because, although SAT and VAT are both correlated with metabolic risk factors, VAT is more strongly associated with an adverse metabolic risk profile [71]. It seems most likely that predominantly VAT is increased in patients in long-term remission of CS because this is also the case in active CS [72], and skinfold thickness (which can be seen as a measure for SAT) was not different between patients and controls in our study. However a future study should determine if this is really the case. Until quite recently VAT could only be adequately measured with computed tomography (CT) or MRI. However these techniques are not ideal for assessing VAT in larger number of subjects for different reasons (e.g. lack of availability of equipment for research, radiation (for CT), manual measurement of VAT which is labor-intensive and requires a lot of experience, costs etc.); therefore, we did not use these techniques. Recently a new fully automated method for abdominal VAT assessment by DEXA has been developed and proven to be accurate [73]. This provides an easy technique to investigate the different adipose tissue compartments in patients in Cushing's syndrome and should be used in a future study.

Furthermore the cause of the persisting centripetal obesity should be investigated. We hypothesize that after remission of CS, when the immunosuppressive effect of the hypercortisolism is resolved, macrophages will massively infiltrate the hypertrophic adipose tissue. Like in obesity, this will trigger a process of local inflammation, which will upregulate 11β-HSD1, causing persisting local CS and adipose tissue dysfunction. Our group is currently performing a study investigating this issue.

Vascular health

The endothelium is a monolayer of cells that comprises the inner lining of blood vessels. Over the past decades multiple studies have revealed the complexity of this semi-permeable membrane (which facilitates the passage of substances such as nutrients and leukocytes across the vessel wall) and its key role in maintaining vascular homeostasis. The endothelium secretes numerous mediators that are necessary for normal vascular function by regulating vascular tone, coagulation, modulating immune responses and controlling vascular cell growth[74].

The most important endothelium-derived vasodilator is nitric oxide (NO), which plays multiple roles in preventing atherosclerosis. Other important vasodilatory factors are endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin (PGI2). The endothelium is also able to secrete potent vasoconstrictors such as endothelin-1, oxygen free radicals, angiotensin-II and thromboxane. Under physiological conditions, the endothelium maintains a fine balance between anti- and prothrombotic states [74].

The term endothelial dysfunction generally refers to reduced NO bioavailability, through decreased endothelial nitric oxide synthase (eNOS) expression. Endothelial dysfunction has been documented in most conditions that are associated with atherosclerosis and is believed to be an early feature in atherogenesis [75]. This point is underscored by the fact that endothelial dysfunction can be reversed by interventions that reverse the cause of endothelial dysfunction, like lifestyle changes and modulatory drug therapies[74,75].

Multiple studies have shown that endothelial dysfunction is present in patients with active CS [76-79]. It has been suggested that this is mainly caused by the fact that most patients with CS have centripetal obesity, impaired glucose tolerance, systemic hypertension, hypercoagulability [80] and dyslipidemia [81]. However, glucocorticoids also have a direct effect on the vasculature [67]. Among other effects, glucocorticoids have been shown to downregulate eNOS and eNOS cofactor tetrahydrobiopterin (BH4) and GTP cyclohydrolase 1, the rate-limiting enzyme in the production of BH4, thus contributing to the reduced endothelium-dependent relaxation [82,83]. Furthermore CS induces vascular remodeling (patients with CS have an increase in IMT and a higher prevalence of arteriosclerotic plaques [84,85]), and hypercortisolism probably has a cardiotoxic effect ***per se*** with an increase in myocardial fibrosis [67]. After remission of CS many comorbidities that are related to endothelial dysfunction can be reversed (for instance, in active CS 55-88% of the patients have hypertension, compared to 24-56% after remission of CS [67]). However, hypertension remains more prevalent in patients who had CS in the past than in the general population.

Previously, only two small studies have investigated vascular health in patients in long-term remission of Cushing's syndrome, and both were case-control studies [68,86]. However they only investigated the presence of atherosclerotic disease, (Colao et al., by assessing intima-media thickness (IMT) and Barahona et al., by investigating the presence of coronary artery disease detected by multi-slice CT, respectively) which they both found to be more prevalent in patients than controls. However, no study has ever investigated if endothelial dysfunction is still present in patients in long-term remission of CS, although one report suggests that it may be reversible[87].

In **chapter 9** we investigated whether micro- and macrovascular health is impaired after long-term remission of CS in 63 patients with no or adequately treated comorbidities. We measured serum biomarkers associated with endothelial dysfunction, IMT, performed pulse wave velocity (PWV) and performed pulse wave analysis. Furthermore endothelium dependent and independent vasodilation was studied in both conduit arteries (flow mediated dilation (FMD) of brachial artery) and forearm skeletal muscle resistance arteries (vasodilator response to intra-arterial acetylcholine, sodium-nitroprusside and NG-monomethyl-L-arginine using venous occlusion plethysmography). The results of the patients were compared to the results in healthy, one to one, well-matched, control subjects. We found no significant differences in outcome measures of vascular health between patients and controls. We concluded that after long-term remission, vascular health of patients treated for CS, who have no or adequately controlled comorbidities, does not differ from healthy gender-, age- and BMI-matched controls. Therefore, the effects of hypercortisolism *per se* on the vasculature seem to be reversible in these patients, which strengthens the need for stringent treatment of metabolic comorbidities.

The techniques we used in our study to investigate arterial function are all well validated[88]:
PWV represents aortic stiffness, which is determined by structural aspects of the aorta, with a minor impact from endothelium-dependent NO. PWV can be assessed with an easy to perform, non-invasive procedure, that has been found to be highly reproducible with a coefficient of variation of 5-10% [88]. As demonstrated by a recent meta-analysis of 16 studies with 17,635 subjects, PWV is an independent predictor for cardiovascular events, with a reported hazard ratio of 1.2-1.3 for each SD increase in PWV [89].

FMD in general terms describes any vasodilatation of an artery following an increase in luminal blood flow and internal-wall shear stress. However it has conventionally come to describe the technique that assesses the peripheral conduit artery diameter following a period of distal limb ischemia [90]. It therefore investigates macrovascular function. FMD

is widely believed to reflect endothelium-dependent and largely nitric oxide-mediated arterial function.

FMD is a well-validated non-invasive technique, but it requires a high degree of operator skill [88]. The investigators that performed the FMD measurements in our study have been highly trained in performing this technique and the coefficient of variation while using our protocol was found to be only 6.7% [91], which is lower than most reported coefficients of variation for FMD [88]. FMD has been found to be an independent predictor of future cardiovascular events in some but not all studies [88].

With venous occlusion plethysmography changes in tissue blood flow (mainly in muscle resistance arteries) can be measured which is reflected as a change in total tissue volume[88]. This technique therefore mainly assesses microvascular function. We assessed the vasodilator response to the infusion of three intra-arterial vasoactive medications, which makes it possible to identify the mechanisms that are responsible for a possible attenuated response. Acetylcholine investigates endothelium-dependent (mainly NO-dependent) vasodilation. Sodium nitroprusside investigates non-endothelium-dependent vasodilation. The nitric oxide synthase inhibitor N^G-monomethyl-L-arginine investigates the contribution of nitric oxide to basal vascular tone. The main disadvantage of this technique is that the insertion of an intra-brachial arterial line is required for the infusion of the vasoactive medications, which makes the technique invasive. However, the technique is well validated, and reproducible with reported coefficients of variation of 8-10% during stimulation (however, resting forearm blood flow is less reproducible) [92,93]. It has been shown to be a strong independent predictor of future cardiovascular events [88,94].

Our finding that the vascular health of patients in long-term remission of CS, who have no or adequately controlled comorbidities, is not different from healthy gender-, age- and BMI-matched controls, is in contradiction with the findings of all previously published studies. Both Barahona et al. and Colao et al. found an increased incidence of atherosclerosis in patients in long-term remission of CS [68,86], and large epidemiological studies suggest that cardiovascular risk remains increased after long-term remission [95-97]. In contrast to our study, all previous studies included patients with uncontrolled comorbidities, so this may explain the differences between their results and ours. However, because we also showed that the same patients that participated in our vascular study had a centripetal adipose tissue distribution and adverse adipokine profile compared to healthy age-, gender- and BMI-matched controls, our finding is also in contrast to what we hypothesized. It is possible that we missed subtle differences because of the rela-

tively small sample size of our study population due to the rareness of the disease and the stringent exclusion criteria. For example, there seems to be no significant trend towards a lower baseline FBF in the patients, which could be an indication of less muscle arterioles or capillaries and this might explain the exercise intolerance that the patients complain about. Furthermore, because all techniques used remain surrogate markers for the presence of atherosclerosis in vivo, it is possible that there are differences in vascular health between patients and controls but that we could not detect them with the techniques we used. However, at the moment, there are no more accurate techniques to evaluate vascular health are not available.

Other possible long-term physical sequelae caused by CS and recommendations for future research

Patients in long-term remission of CS continue to complain about fatigue and the inability to perform exercise, suggesting a reduced level of physical fitness [98,99]. Physical fitness level is of particular importance, as previous studies have demonstrated that the physical fitness level represents a strong and independent risk factor for all-cause mortality as well as cardiovascular disease [100]. To objectify if physical fitness levels are indeed reduced in patients in long-term remission of CS, our group is performing a study that investigates the physical fitness level as measured by peak oxygen uptake ($VO_{2\,peak}$) during a maximal exercise stress test in 17 healthy patients (i.e. without comorbidities or hormonal deficiencies) in long-term remission of CS and 17 healthy controls matched for sex, estrogen status, age, BMI, smoking, ethnicity, and physical activity level. Furthermore, in order to hopefully establish the mechanism of decreased physical fitness levels, we are taking muscle biopsies to investigate mitochondrial and vascular density and eNOS content of the muscles, amongst others. Future research should also investigate if CS has long-term effects on cardiac function, as this may also explain some of the differences we found.

The question remains why some patients seem to suffer from more long-term effects of CS than others. Both our group, and Ragnarsson et al. found that differences in polymorphisms of the glucocorticoid receptor might partially explain this phenomenon [70, 101]. However, because of the relatively small sample sizes for genetic studies (58 and 50 patients, respectively) the results of these studies need to be interpreted with caution and further research in larger CS populations to replicate these findings is needed before drawing definitive conclusions.

In conclusion, both acromegaly and CS have significant long-term physical effects after remission, even in the healthiest of patients. In acromegaly we have shown that

craniofacial disproportions persist after long-term remission. Patients in remission of CS have a persisting centripetal adipose tissue distribution with an adverse adipokine profile. Their vascular health seems to normalize to that of an age-, sex- and BMI-matched reference population. However there are still many aspects that have not been adequately investigated. Furthermore underlying causes of long-term physical effects have to be identified in order to develop targeted interventions (like physical rehabilitation programs and medication) that will improve health and thus QoL in these patients. Because both acromegaly and CS are very rare diseases it would be advisable to perform future research in a multicenter setting, so larger numbers of patients can be included in studies with stringent in- and exclusion criteria. Prospective trials to investigate the long-term consequences would provide important new information about the effects of the diseases in individual patients. This information is not only important for patients that have or have had acromegaly or CS, but also for patients that use exogenous glucocorticoids or growth hormone.

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Appendix

Nederlandse samenvatting voor niet-medici

Dit proefschrift gaat over de behandeling en lange termijn gevolgen van twee hormonale aandoeningen: 1) acromegalie en 2) het syndroom van Cushing. Zowel acromegalie als het syndroom van Cushing zijn zeer zeldzame aandoeningen als ze door het lichaam zelf worden veroorzaakt (het syndroom van Cushing kan namelijk ook veroorzaakt worden door medicijnen zoals prednison, wat vaak voorkomt) maar ze hebben grote gevolgen.
Acromegalie wordt veroorzaakt door teveel aan groeihormoon. Groeihormoon zorgt voor groei van veel weefsels. In kinderen zorgt dit voor snelle en blijvende lengte groei waardoor ze "reuzen" kunnen worden. Pijpbeenderen van volwassenen kunnen echter niet meer in de lengte groeien. Volwassen patiënten zullen dus niet langer worden, maar handen, voeten, de onderkaak, neus, wenkbrauwbogen, lippen, tong en huid groeien wel. Het uiterlijk wordt dus veel grover en mannelijk. Juist om dat uiterlijk worden patiënten met acromegalie regelmatig gecast in films (onder andere "Jaws" van James bond en "Lurch" in de Adams Family). Behalve de veranderingen in uiterlijk worden ook veel andere organen door het teveel aan groeihormoon aangetast: veel patiënten krijgen gewrichtsproblemen met vroegtijdige slijtage, het hart wordt te groot en dit kan hartritme stoornissen en hartfalen veroorzaken en ze hebben een grote kans om suikerziekte en een te hoge bloeddruk te ontwikkelen net als obstructief slaapapneu syndroom (snurken met grote adempauzes). Acromegalie zorgt dan ook voor een fors verminderde kwaliteit van leven en een 3 x verhoogd risico op overlijden, met name door hart- en vaatziekten, in onbehandelde patiënten.
Het syndroom van Cushing wordt veroorzaakt door een teveel aan het stresshormoon cortisol. Het syndroom van Cushing veroorzaakt, net als acromegalie, ook forse uiterlijke veranderingen: patiënten worden dikker, waarbij het vet vooral op de buik, in de hals en het gezicht gaat zitten. Het syndroom van Cushing kan ook zorgen voor een rood gezicht, veel blauwe plekken, een dunne kwetsbare huid, brede rode striae (huidstriemen), meer haargroei of juist haaruitval en vocht vasthouden. De spieren worden veel dunner en zwakker. Daarbij ontwikkelen veel patiënten suikerziekte, een hoge bloeddruk, een te hoog cholesterolgehalte, botontkalking enz. Dit alles zorgt ook voor een fors verminderde kwaliteit van leven en een strek verhoogd risico op overlijden, ook met name door hart- en vaatziekten, in onbehandelde patiënten.

Bij zowel acromegalie als het syndroom van Cushing speelt de hypofyse (in lekentaal ook wel het hersenaanhangsel genoemd) een centrale rol. De hypofyse is een klier met de grote van een erwt die onderaan de hersenen hangt, vlak achter de aanhechting van de neusbrug. De hypofyse geeft veel hormonen af: groeihormoon en ACTH, wat de bijnieren aanzet tot het maken van cortisol, maar ook hormonen die de geslachtsorganen, de

schildklier en de melkproductie van de borsten aansturen. Daarmee vervult de hypofyse een centrale rol in de regulatie van onze hormoonhuishouding. Zowel acromegalie en het syndroom van Cushing wordt in de meerderheid van de gevallen veroorzaakt door een goedaardig gezwel in de hypofyse (een hypofyse adenoom). Indien dit het geval is, is de eerste keus van behandeling bij beide ziektes dan ook het weghalen van dit gezwel door een operatie van de hypofyse. Tijdens deze operatie wordt de hypofyse transsphenoidaal benaderd, via de neus en de wiggenbeensholte (de sinus sphenoidalis). Vroeger gebeurde dit altijd met een microscoop, maar sinds de jaren 90 van de vorige eeuw wordt de endoscoop (een slang met een cameraatje aan het uiteinde) steeds meer gebruikt. Als het syndroom van Cushing wordt veroorzaakt door een adenoom in de hypofyse noemen we het de ziekte van Cushing.

Hoofdstuk 1, de introductie van dit proefschrift, geeft een uitgebreid overzicht van de werking en de anatomie van de hypofyse. De werking en regulatie van groeihormoon en cortisol worden verder besproken. Er wordt een overzicht gegeven van mogelijke aandoeningen van de hypofyse. De symptomen en gevolgen van de ziektes acromegalie en het syndroom van Cushing worden besproken en de verschillende behandelingsmogelijkheden (transsphenoidale hypofysechirurgie, radiotherapie van de hypofyse, medicamenteuze therapie en, voor het syndroom van Cushing, verwijdering van beide bijnieren) worden besproken. De doelen van dit proefschrift worden besproken en de studies worden geïntroduceerd.

DEEL 1

Het eerste doel van dit proefschrift was om meer inzicht te krijgen in de rol van de endoscopische techniek van trassphenoidale hypofysechirurgie voor de behandeling van acromegalie en de ziekte van Cushing.

Daarom hebben we systematisch de resultaten van endoscopische transsphenoidale hypofysechirurgie in het RadboudUMC geanalyseerd. Het RadboudUMC was het eerste ziekenhuis in Nederland dat de endoscopische techniek van hypofysechirurgie ging gebruiken en we hebben er dus relatief veel ervaring mee.

In hoofdstuk 2 hebben we de resultaten van endoscopische hypofysechirurgie in 40 patiënten met acromegalie geanalyseerd. Alle patiënten waren geopereerd tussen 1988 en 2007 en hadden een hypofyse-adenoom dat 1centimer of groter was (een macro-ad-

enoom). In 36 patiënten was het doel van de operatie om ze te genezen, en in 20 van deze patiënten is dat gelukt (dus 56% van de patiënten was genezen na de operatie). In de overige 4 gevallen wisten we voor de operatie al dat het adenoom te groot was (en ingegroeid in omliggende structuren) om genezing te bereiken en was het doel van de operatie om zoveel mogelijk van de adenoom weg te nemen. We vonden dat de ervaring van de neurochirurg van belang was om de kans op genezing te vergroten: In de eerste 5 jaar nadat de endoscopische techniek geïntroduceerd was in ons ziekenhuis werden er 18 patiënten geopereerd waarvan er slechts 6 waren genezen na de operatie (dus 33%). In de tweede 5 jaar werden er 22 patiënten geopereerd van wie er 14 waren genezen (dus 63%). In de 2de 5 jaar werden echter ook de patiënten geopereerd van wie we vooraf wisten dat genezing niet mogelijk was. Als die patiënten niet worden meegeteld was zelfs 77% van de patiënten genezen na de operatie. In 13 mensen was er een complicatie (een onvoorziene en ongewenste gebeurtenis) van de operatie maar alle complicaties waren mild en van voorbijgaande aard. Voor de operatie was er al sprake van uitval van productie van hypofysehormonen in 37,5% van de patiënten (waardoor patiënten deze hormonen in medicijnvorm toegediend kregen). Na de operatie was dit het geval 33% van de patiënten.

In **hoofdstuk 3** hebben we de resultaten van endoscopische hypofysechirurgie in 86 patiënten met de ziekte van Cushing geanalyseerd. Deze patiënten werden in ons ziekenhuis geopereerd tussen 1998 en 2011. De kans op genezing bleek afhankelijk van de grootte van het adenoom op de MRI-scan die voor de operatie werd gemaakt: Zestig procent van de patiënten waarbij er op de MRI geen adenoom was gezien was genezen na de operatie. Van de patiënten met een adenoom dat kleiner was dan 1 cm (een micro-adenoom) op de MRI was 83% genezen. Van de patiënten met een macroadenoom dat niet was ingegroeid in de omliggende weefsels was 94% genezen en van de mensen met een macroadenoom dat wel ingroeide was slechts 40% genezen. In 16% van de patiënten kwam de ziekte van Cushing terug na mediaan (een soort gemiddelde) 71 maanden. Alle complicaties waren mild en van voorbijgaande aard. Voor de operatie had 15 % van de patiënten hormonale uitval en na de operatie was dit 35%.
We hebben de onze resultaten vergeleken met alle resultaten die andere ziekenhuizen hadden gepubliceerd over de microscopische methode van hypofysechirurgie. De belangrijkste conclusie van **hoofdstuk 2 en 3** was dat de kans op genezing bij hypofyseoperaties met de endoscopische techniek in patiënten met macroadenomen (vooral als ze ingroeien) waarschijnlijk groter is dan bij operaties met de microscopische techniek. Dit is theoretisch gezien ook logisch omdat je met een endoscoop ook om een hoek kan kijken en opereren, terwijl dat met de microscopische techniek niet mogelijk is. Wij vinden dan ook dat de endoscopische techniek de voorkeur heeft in patiënten

met macroadenoom. In patiënten met microadenomen lijken de resultaten tussen de endoscopische en microscopische techniek vergelijkbaar. Ook het aantal, de aard en ernst van de complicaties lijkt vergelijkbaar tussen beide technieken.
In theorie heeft de endoscopische techniek van hypofysechirurgie ook voordelen ten opzichte van de microscopische techniek als patiënten voor de tweede keer aan de hypofyse geopereerd moeten worden, omdat de anatomie dan al veranderd is. Een tweede operatie is met name bij de ziekte van Cushing wel eens een goede optie omdat er nog geen goede medicamenteuze therapie bestaat. In **hoofdstuk 4** hebben daarom de resultaten van een tweede hypofyseoperatie in 14 patiënten met de ziekte van Cushing, die in ons ziekenhuis waren geopereerd tussen 1999 en 2007, geanalyseerd. Zes van deze patiënten was niet genezen na de eerste operatie en in 8 patiënten was de ziekte van Cushing teruggekomen nadat ze een tijd genezen waren. Eenenzeventig procent van deze patiënten was genezen na de tweede operatie, wat een hoger percentage is dan wat gerapporteerd wordt over microscopische hypofysechirurgie. De belangrijkste complicatie was lekkage van het hersenvocht in 6 patiënten. Elf patiënten hadden hormonale uitval na de tweede operatie, van wie er in 3 patiënten al uitval was voor de operatie. We concludeerden dat een tweede hypofyseoperatie via de endoscopische techniek een goede behandeling kan zijn voor de ziekte van Cushing.

Als het lukt om acromegalie en het syndroom van Cushing te genezen, dan hebben meerdere studies aangetoond dat de kwaliteit van leven fors verbeterd. Maar de kwaliteit van leven blijft duidelijk minder dan in mensen die de ziekte niet hebben gehad. Het is nog niet duidelijk wat hier de precieze oorzaak van is.

DEEL 2

Daarom was het tweede doel van dit proefschrift om meer inzicht te krijgen in de oorzaken van de blijvende verminderde kwaliteit van leven in patiënten die langdurig genezen zijn van acromegalie en het syndroom van Cushing.

Omdat het uiterlijk van patiënten met acromegalie fors veranderd tijdens de ziekte, hebben we in **hoofdstuk 5** onderzocht of onzekerheid over het uiterlijk zorgt voor psychologische problemen in patiënten die langdurig genezen zijn van acromegalie, en of dit van invloed is op hun kwaliteit van leven. Om dit te doen hebben we 73 patiënten gevraagd om de Derriford Appearence Scale (de DAS) vragenlijst in te vullen. De DAS vragenlijst is ontwikkeld door de plastischechirurgie om te meten wat voor een invloed onzeker-

heid over iemands uiterlijk heeft op hun psychisch functioneren en dagelijks leven en over welk lichaamsdeel mensen dan onzeker zijn. Ook vroegen we de patiënten twee bekende vragenlijsten in te vullen die kwaliteit van leven meten: de RAND-36 (een generieke vragenlijst) en de AcroQol (een ziekte specifieke vragenlijst). De resultaten van de patiënten hebben we vergelijken met de resultaten van 57 gezonde controle personen die vergelijkbaar waren qua geslacht, leeftijd en BMI (Body Mass Index, een verhouding tussen lengte en gewicht die aangeeft of er sprake is van een normaal gewicht of juist ondergewicht of overgewicht). We vonden dat significant meer patiënten onzeker waren over hun uiterlijk, namelijk 46,6% van de patiënte vergeleken met 22,8% van de gezonde controle personen. Vijfentachtig provent van de patiënten die onzeker waren over hun uiterlijk waren onzeker over hun gezicht, vergeleken met 23,1 % van de gezonde controle personen. In de patiëntengroep was er een duidelijk verband tussen onzekerheid over uiterlijk en een slechtere kwaliteit van leven. Er was in de patiëntengroep geen verband tussen onzekerheid over uiterlijk en leeftijd of geslacht. We concludeerden daarom dat zelfs na langdurige genezing van acromegalie patiënten onzeker blijven over hun uiterlijk, met name over hun gezicht, en dat dit zorgt voor psychische problemen en dat het een negatieve invloed heeft op hun dagelijks functioneren en kwaliteit van leven.

In **hoofdstuk 6** onderzochten we de kwaliteit van leven in 123 patiënten die langdurig genezen waren van het syndroom van Cushing, vergeleken met de kwaliteit van leven van 105 gezonde controle personen met een vergelijkbaar geslacht, leeftijd en BMI. Om dit te kunnen doen vulden deelnemers aan het onderzoek 7 gevalideerde (zowel generieke als ziekte specifieke) vragenlijsten in die kwaliteit van leven meten. Om te onderzoeken of de oorzaak van het syndroom van Cushing van invloed was op de kwaliteit van leven vergeleken we ook de resultaten van patiënten bij wie het syndroom van Cushing was veroorzaakt door een afwijking in de bijnieren met de resultaten van patiënten bij wie het syndroom van Cushing was veroorzaakt door een adenoom in de hypofyse. Verder onderzochten we of hormonale uitval, de verschillende vormen van behandeling die patiënten hadden gehad, de duur van genezing, geslacht en leeftijd van invloed waren op de kwaliteit van leven van de patiënten. We vonden dat de kwaliteit van leven van de gehele patiëntengroep significant lager was dan de kwaliteit van leven van de gezonde controle personen op alle onderdelen van alle vragenlijsten, behalve in patiënten die de ziekte van Cushing hadden gehad (dus veroorzaakt door de hypofyse) en geen hormonale uitval hadden: in deze groep hadden de patiënten voor 50% van de onderdelen van de vragenlijst een slechtere kwaliteit van leven dan de gezonde controles. Tussen de verschillende patiëntengroepen werd geen verschil gevonden, maar vrouwen hadden een slechtere kwaliteit van leven dan mannen en ook de duur van genezing was van invloed op de kwaliteit van leven. We concludeerden dat kwaliteit van leven verminderd

blijft na langdurige genezing van het syndroom van Cushing, en dat dit onafhankelijk is van de oorzaak van het syndroom van Cushing, de uitval van hornomen en de soorten behandelingen die mensen hebben gehad. De verminderde kwaliteit van leven heeft waarschijnlijk diverse oorzaken. Maar omdat de kwaliteit van leven verminderd is in alle patiëntengroepen is het waarschijnlijk dat de langdurige blootstelling aan te hoge cortisolwaardes op zich een belangrijke oorzaak is.

DEEL 3

Het derde doel van dit proefschrift was om meer inzicht te krijgen in de langdurige fysieke gevolgen van acromegalie en het syndroom van Cushing.

Zoals eerder genoemd ondergaan vrijwel alle patiënten met acromegalie behoorlijke veranderingen van het gezicht. Na genezing worden deze veranderingen meestal minder uitgesproken, maar ze blijven zeker bestaan. Het was vroeger moeilijk om deze veranderingen te meten en te kwantificeren. Met nieuwe driedimensionale (3D) technieken, die ontwikkeld zijn door de mond-, kaak-, aangezichtschirurgie, is dit wel mogelijk. Deze techniek was echter nooit gebruikt in patiënten met acromegalie. In **hoofdstuk 7** onderzochten we daarom de verschillen tussen het gezicht van 16 patiënten die langdurig genezen waren van acromegalie en 16 gezonde controle personen. De patiënten en controle personen waren een op een gekoppeld qua geslacht, leeftijd, BMI en etniciteit. Van alle deelnemers maakten we 3D foto's en 3D cone-beam CT-scans. Deze foto's en CT-scans kunnen gecombineerd worden tot een virtueel hoofd, dat zeer gedetailleerd informatie geeft over de botten en weke delen en waarop zeer nauwkeurig metingen kunnen worden verricht. Met deze techniek bevestigden we dat de klassieke veranderingen van het de schedel die ontstaan tijdens acromegalie (zoals de grotere onderkaak en een langer gezicht) blijven bestaan na genezing. We vonden echter ook dat patiënten een breder gezicht hebben ter hoogte van de jukbeenderen en dat de bovenkaak langer is. Ook vonden we dat veel veranderingen van de weke delen blijven bestaan na langdurige genezing van acromegalie. We concludeerden dat patiënten die langdurige genezen zijn van acromegalie nog altijd duidelijke veranderingen hebben van het gezicht en dat de nieuwe 3D technieken veel belovend zijn om de veranderingen van zowel de weke delen als de botten van het gezicht te onderzoeken in acromegalie, ook in een persoon in verloop van tijd.

Zoals eerder al aangegeven zorgt het syndroom van Cushing voor gewichtstoename waarbij het vet met name op de buik gaat zitten. Studies in mensen met overgewicht hebben laten zien dat met name dit buikvet ongezond is. Het geeft ongezonde vethormonen af (adipokines) en zorgt voor een chronische milde ontsteking in het gehele lichaam, waardoor de kans op hart en vaatziekten toeneemt. Na genezing van het syndroom van Cushing verliezen veel patiënten veel gewicht, maar het was onduidelijk of ze in verhouding meer buikvet houden dan gezonde controle personen.

In **hoofdstuk 8** onderzochten we daarom de vetverdeling, de adipocytokineprofielen en metabole risicoprofielen van 58 patiënten die langdurig genezen waren van het syndroom van Cushing. Elke patiënt werd uitgebreid onderzocht door een arts waarbij lengte, gewicht, bloeddruk, de hartslag, de dikte van de huiplooien en de omtrek van buik, heupen, armen en benen werd gemeten. Verder werd er bloed afgenomen om de adipocytokine profielen en andere bloedwaardes die geassocieerd zijn met een verhoogd cardiovasculair risico te bepalen. Ook kregen ze een dual-energy X-ray absorbomery (DEXA) scan waarmee de hoeveelheid vetmassa, botten en spieren gemeten kan worden in verschillende lichaamsdelen. Voor elke patiënt werd een gezonde controle persoon gezocht met hetzelfde geslacht, leeftijd en BMI en we vergeleken de resultaten van de patiëntengroep met de controlegroep. We vonden dat patenten die langdurig genezen waren van het syndroom van Cushing een grotere middelomtrek hadden, een kleinere dijomtrek, een grotere middel-heup ratio en een grotere heup-dij ratio. Met de DEXAscan stelden we vast dat patiënten een hoger vetpercentage van de romp hadden, en dat de verhouding tussen rompvetpercentage en extremiteitvetpercentage(dus arm en been) groter was. Veder hadden de patiënten een ongezonder adipokine profiel met lagere adiponectinewaardes, hogere leptinewaardes en hogere resistinewaardes (precies andersom als gezond is). Ook hadden patiënten een hoger trigliceride gehalte (een ongezond vet). We concludeerden dat zelfs na langdurige genezing van het syndroom van Cushing de ververdeling veranderd blijft, met in verhouding meer buikvet, en dat dit samengaat met een ongezond adipokine profiel. Dit bleek verder onafhankelijk van de oorzaak van het syndroom van Cushing (dus hypofyse of bijnier), hormonale uitval, de behandelingen die patiënten hadden gehad en of ze nog andere ziektes hadden, zoals suikerziekte of een hoge bloeddruk.

Het syndroom van Cushing zorgt dat de gezondheid van de bloedvaten snel wordt aangetast. Dit komt door andere aandoeningen (onder andere suikerziekte en hoge bloeddruk) die patiënten krijgen maar ook door het hoge cortisol zelf. Patiënten krijgen snel aderverkalking. Verder gaat het endotheel disfunctioneren. Het endotheel is een aangesloten laag cellen aan de binnenkant van de bloedvaten dat er onder andere voor zorgt,

door stofjes zoals stikstofoxide af te geven, dat bloedvaten verwijden en vernauwen als dat nodig is (bijvoorbeeld tijdens inspanning en extreme kou). Het was echter nooit goed onderzocht of de gezondheid van de bloedvaten zich zou kunnen herstellen na genezing van Cushing en of er nog sprake is van endotheeldisfunctie en vroegtijdige aderverkalking. In **hoofdstuk 9** onderzochten we dus de gezondheid van de bloedvaten van 63 patiënten die langdurig genezen waren van het syndroom van Cushing maar verder gezond waren of alleen goed behandelde andere ziektes hadden. We maten stoffen in het bloed die geassocieerd zijn met endotheel disfunctie, maten de dikte van de vaatwand in de halsslagader (de intima-media dikte) en de stijfheid van de grote slagaderen met Pulse Wave Velocity en Pulse Wave Analysis. Verder onderzochten we de endotheelfunctie door te onderzoeken hoeveel een van de slagaderen in de arm kan uitzetten, nadat de doorbloeding van onderarm een voor een kwartier was afgesloten, met Flow Mediated Dilation. We onderzochten ook de endotheelfunctie van de kleine slagadertjes en haarvaatjes in reactie op verschillende medicijnen (met intra-arteriële infusie van acetylcholine, natrium-nitroprusside en NG-monomethyl-L-arginine) met veneuze occlusie plethysmografie. Voor elke patiënt werd een gezonde controle persoon gezocht met in ieder geval hetzelfde geslacht, leeftijd en BMI en voor de endotheelmetingen nog meer variabelen zoals fitheid en roken. We vergeleken de resultaten van de patiëntengroep met de controlegroep. We vonden, in tegenstelling tot wat we verwachtten, geen verschillen tussen de patiëntengroep en controlegroep voor alle uitkomsten van alle metingen. We concludeerden dat na langdurige genezing van het syndroom van Cushing, de gezondheid van de bloedvaten van gezonde patiënten of patiënten die goed behandeld zijn voor andere ziekten (zoals suikerziekte, hoge bloeddruk of uitval van hormonen) vergelijkbaar is met de gezondheid van bloedvaten van gezonde controle personen met hetzelfde geslacht, leeftijd en BMI. Het lijkt er daarom op dat het effect van cortisol op zich op de bloedvaten teruggedraaid kan worden. Dit benadrukt het belang van het goed behandelen van andere ziektes in patiënten die het syndroom van Cushing hebben gehad.

Hoofdstuk 10 van dit proefschrift is een samenvatting en een algemene discussie. In dit hoofdstuk worden de resultaten van dit proefschrift in perspectief geplaatst. Ook worden er aanbevelingen gedaan voor toekomstig onderzoek.

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Curriculum Vitae

Margreet Wagenmakers werd geboren op 22 september 1980 in Nijmegen. Omdat haar vader een goede postdocfunctie kreeg aangeboden in Liverpool, verhuisde ze op haar vierde naar Engeland. Hier beleefde ze samen met haar hele gezin de mooiste jaren van haar jeugd. In 1988 verhuisde het hele gezin terug naar Nederland, naar Zuid-Limburg. In 1999 behaalde Margreet haar gymnasium diploma aan het Stella Maris college in Meerssen. Omdat ze in eerste instantie was uitgeloot voor geneeskunde in Nederland, studeerde ze het eerste jaar geneeskunde aan het Limburgs Universitair Centrum te Diepenbeek, België, waar ze haar kandidaatsexamen behaalde. Hierna maakte ze de overstap naar de Katholieke Universiteit Nijmegen (later Radboud Universiteit). In 2004 ging zij voor haar wetenschappelijke stage tijdelijk naar de School of Sport and Exercise Sciences van de University of Birmingham, United Kingdom. Zij verrichte daar onderzoek onder supervisie van prof. dr. P. Stewart, endocrinoloog en haar vader. Hier is haar liefde voor de endocrinologie ontstaan. In juli 2007 behaalde zij haar artsexamen. In het jaar na haar afstuderen heeft zij als arts-onderzoeker op de afdeling Endocriene Ziekten gewerkt onder supervisie van Prof. dr. A.R.M.M. Hermus. Dit bleek achteraf de start van haar promotietraject te zijn. In 2008 startte zij met de opleiding tot internist. De eerste drie jaar van haar opleiding werkte zij in het Canisius-Wilhelmina Ziekenhuis in Nijmegen (hoofdopleider dr. A.S. Dofferhoff). Hierna vervolgde zij haar opleiding in het Radboud universitair medisch centrum (hoofdopleiders prof. dr. J. de Graaf en prof. dr. J.W.M. van der Meer). Zij heeft haar opleiding een aantal malen onderbroken voor het verrichten van het onderzoek dat staat beschreven in dit proefschrift. Momenteel werkt zij als internist-endocrinoloog in opleiding op de afdeling algemeen interne geneeskunde van het Radboud universitair medisch centrum (opleiders prof. dr. A.R.M.M. Hermus en prof. dr. C.J.J. Tack.)

Margreet is in 2007 getrouwd met Erik Vegt en samen hebben zij twee kinderen, Veerle (2012) en Joris (2015).