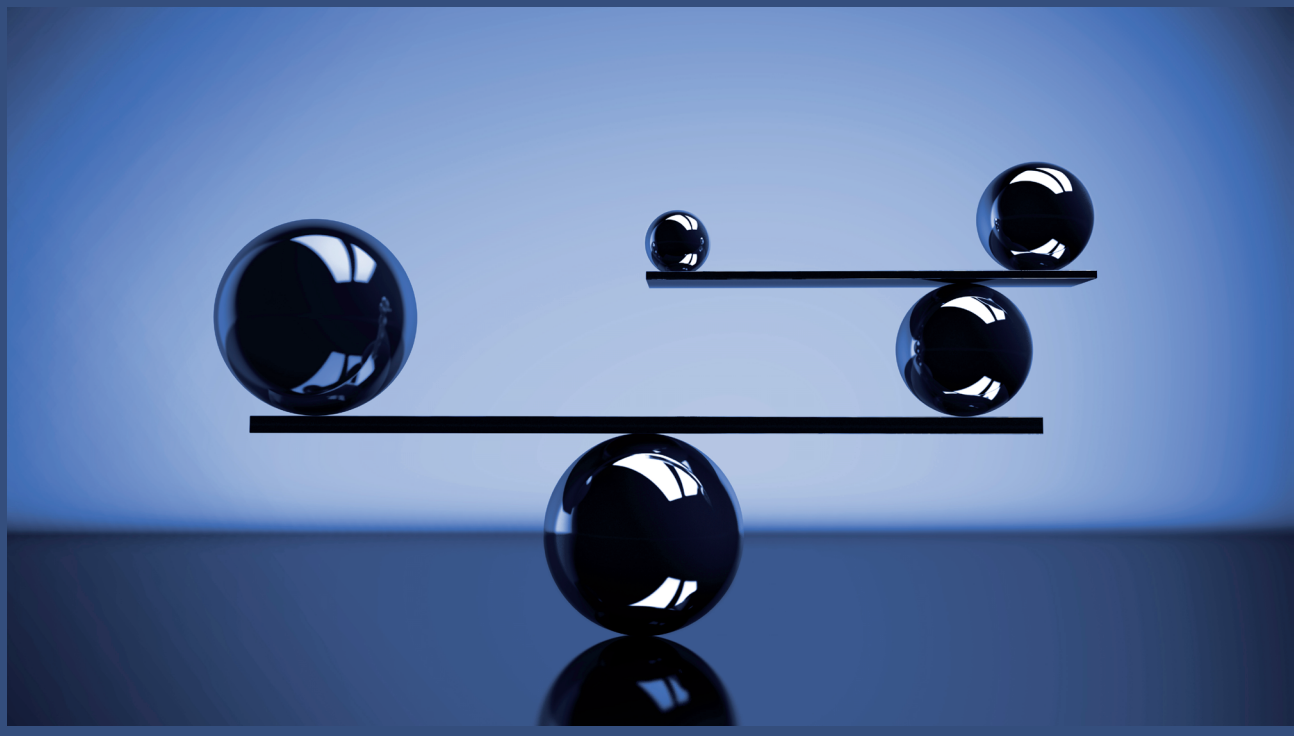
Novel Insights in the Modern Medical Management of Acromegaly



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Novel Insights in the Modern Medical Management of Acromegaly
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Novel Insights in the Modern Medical Management of Acromegaly

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Chapter 1

General introduction and aims of the thesis

Partly based on:
Review of current and emerging treatment options in acromegaly.
A. Muhammad, A.J. van der Lely, S.J.C.M.M. Neggers
The Netherlands journal of medicine. 2015;73:362-367.

Combined treatment of somatostatin analogues with pegvisomant in acromegaly.
S.E. Franck*, A. Muhammad*, A.J. van der Lely, S.J.C.M.M. Neggers
Endocrine. 2016;52:206-213.

How to position pasireotide LAR treatment in acromegaly?
E.C. Coopmans*, A. Muhammad*, A.J. van der Lely, J.A.M.J.L. Janssen, S.J.C.M.M. Neggers
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This introductory chapter of the thesis will focus on the medical treatment of acromegaly. This is preceded by a brief description of the central and peripheral regulation of the GH-IGF-I axis with a focus on dysregulation of this axis in acromegaly.

PITUITARY GLAND PHYSIOLOGY

General characteristics

The pituitary gland is often called the "master gland" of the endocrine system, because its hormones play a central role in a vast array of physiological feedback functions such as growth, metabolism, energy balance, osmoregulation, and stress response (1). The normal pituitary gland is a bean-shaped gland with an average weight of 0.6 grams (figure 1). The gland is located inferior the hypothalamus within the sella turcica and linked to the hypothalamus via the pituitary stalk which contains a highly vascularized portal system. The pituitary is comprised of three anatomically and functionally distinct lobes, the anterior lobe (adenohypophysis), the intermediate lobe, and the posterior lobe (neurohypohysis). The anterior lobe contains five different hormone-secreting cell lineages which produce the following hormones:

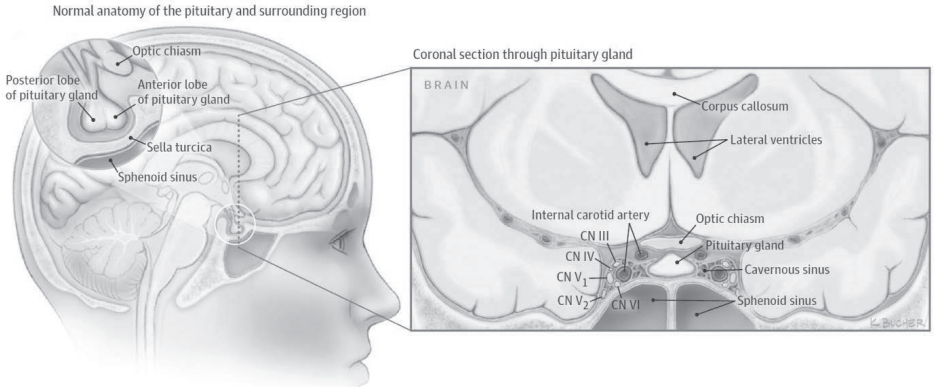

Figure 1. Normal anatomy of the pituitary gland.
Normal anatomy of the pituitary gland with its surrounding structures. The borders of the pituitary are composed of the sella turcica below (retrosellar), the optic chiasm above (suprasellar), and the cavernous sinuses on both sides (parasellar). The sella turcica is situated above the sphenoid sinus. The cavernous sinuses constitute a thin-walled venous network that receive blood from the superior and inferior ophthalmic veins, the sphenoparietal sinus, and the superficial middle cerebral veins. Structures crossing the cavernous sinuses lateral to the pituitary gland include the internal carotid artery, cranial nerve III (oculomotor), cranial nerve IV (trochlear), cranial nerve VI (abducens) nerve, two branches of cranial nerve V (trigeminal nerve branch 1 and 2), the ophthalmic branch V1, and the maxillary branch V2. Source: Molitch ME, Diagnosis and Treatment of Pituitary Adenomas: A Review, JAMA. 2017;317:516-524. Image reproduced with permission of the rights holder, JAMA.

the corticotrophs that produce adrenocorticotrophin (ACTH), the gonadotrophs that produce the gonadotrophins luteinizing hormone (LH) and follicle-stimulating hormone (FSH), the somatotrophs that produce growth hormone (GH), the lactotrophs that produce prolactin (PRL) and, the thyrotrophs that produce thyroid-stimulating hormone (TSH). The somatotrophs may also produces both GH and PRL. In addition, the anterior lobe also contains folliculo-stellate cells or pituicytes that are support cells (2). The intermediate lobe contains only α-melanotrophin (α-MSH). In humans this lobe is considered rudimental as it regresses at the 15th week of gestation. In contrast to the anterior lobe, the posterior consists of mainly neural projections from the hypothalamus that produces arginine, antidiuretic hormone (ADH, or vasopressin) and oxytocin.

Growth hormone

The somatotrophs, which synthesize, store and secrete GH, are the predominant cell type of the anterior pituitary, constituting about 45% of the pituitary cell population. GH is a single-chain 191-amino acid polypeptide and over 90% of GH in the circulation constitutes a 22 kilodaltons (kDa) molecule (3). About 50% of circulating GH is bound to GH binding protein (GHBP), which is the soluble version of the extracellular domain of the GHR, and is important for the bioavailability and bioactivity of GH (4, 5). Binding of GH to the GHR induces dimerization of the GHR and sets a intracellular signaling cascade in motion which leads to activation of the tyrosine kinase JAK2, which activates the STAT5, PI3K, and MAP kinase pathways (6-8).

Normal GH secretion occurs in a pulsatile circadian rhythm with the majority of its secretion occurring during deep sleep. During hypoglycemia and physical exercise GH secretion is amplified, while hyperglycemia and leptin suppress GH secretion (9-13). Circulating GH is high at 20 weeks of gestation, and subsequently falls during the neonatal stage and childhood until puberty, when GH secretion peaks to 2 mg/day, after which it declines progressively through aging (14).

GH is a pleiotropic hormone due to its involvement in the regulation of many physiological processes, such as growth, glucose, lipid and bone metabolism, reproduction, osmoregulation and the immune system. GH has both anabolic and catabolic actions. Most of the anabolic actions of GH are mediated via IGF-I. GH is the primary regulator of IGF-I production (15). GH is mainly a catabolic hormone during fasting and in the absence of IGF-I but becomes anabolic in the presence of IGF-I after food intake. The most well-known (anabolic) action of GH is stimulation of cellular growth and differentiation. The catabolic actions of GH derive from its lipolytic effects on adipose tissue. GH administration stimulates lipolysis and inhibits lipogenesis which results in

elevated free fatty acids (FFAs), whereas GH deficiency is associated with increased lipid mass. GH is a counter-regulatory hormone that antagonizes the effects of insulin, while IGF-I promotes insulin sensitivity (16, 17). Therefore, GH and IGF-I display opposing effects on insulin homeostasis.

Growth hormone-pituitary-hypothalamus axis

Central GH secretion is positively regulated by the hypothalamus through GH releasing hormone (GHRH) and acylated ghrelin (AG) which stimulate GH secretion, and negatively regulated by somatostatin (somatostatin release inhibiting factor, SRIF) which inhibits GH secretion (Figure 2). The peripheral actions of GH are

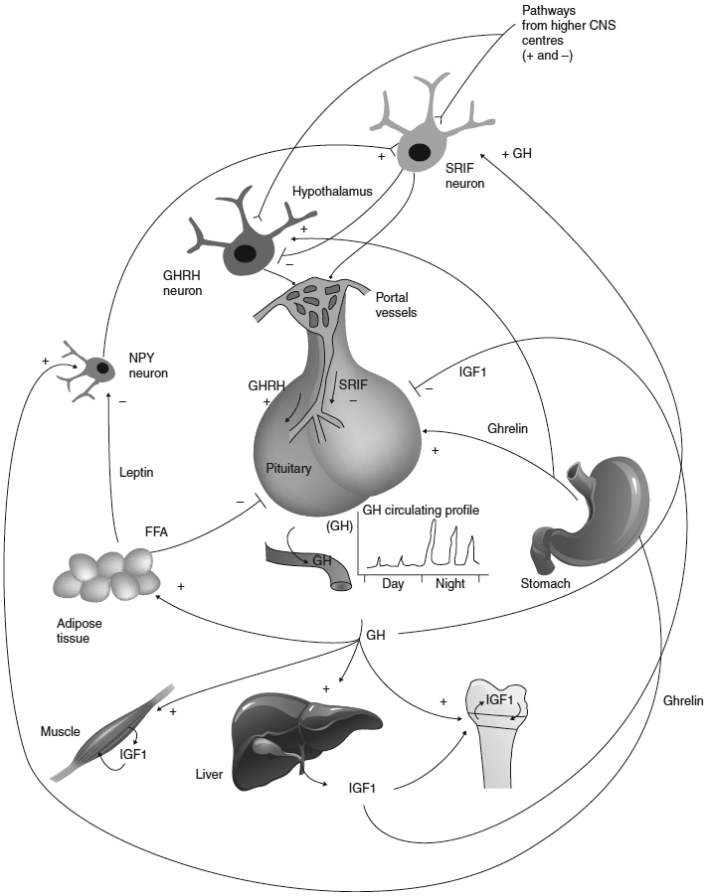

Figure 2 Central and peripheral components that regulate the GH axis.
Central and peripheral components that regulate the GH axis. NPY, neuropeptide Y; FFA, free fatty acids; GH, growth hormone; IGF1, insulin-like growth factor 1; GHRH, GH-releasing hormone; SRIF, somatotrophin release inhibitory factor. Image reproduced with permission of the rights holder, JAMA.

predominantly mediated via hepatic IGF-I production, but also through direct actions on local tissues.

Ghrelin

Ghrelin was discovered in 1999 by the group of Kojima and Kangawa as the endogenous ligand of the GH secretagogue receptor type 1a (GHSR1a). Ghrelin is a 28-amino acid peptide hormone secreted mainly from neuroendocrine X/A cells in the stomach (18, 19), circulating as both acylated ghrelin (AG) and unacylated ghrelin (UAG) isoforms (20). Unique to AG is the *n*-octanoyl acylation by the enzyme ghrelin O-acyl transferase (GOAT) at its serine-3 residue. In the circulation AG is rapidly deacylated by carboxylesterases into UAG (21). After binding to GHSR1a, AG potently stimulates pituitary GH secretion and food intake (orexigenesis). Inversely, GH itself inhibits AG secretion (22-25). But there are also conflicting reports on the interaction between ghrelin and GH, indicating that the physiological role of ghrelin in the regulation of GH secretion is not fully understood (26-29). Although AG was initially discovered as a GH secretagogue, it is now recognized as a multifunctional hormone, as it can modulate gut motility, stress response, sleep, learning, memory, cellular proliferation, differentiation and survival, immune response, energy homeostasis, glucose homeostasis, and insulin secretion (29, 30). UAG has several biological functions which are mediated independently from the GHSR1a receptor, and metabolic actions that counteract AG action. For example, AG displays diabetogenic actions such as suppression of glucose-stimulated insulin secretion and insulin sensitivity (31-38), while UAG shows the opposite (30, 31, 39). Although much work has been done, the cognate receptor(s) for UAG has yet to be identified. More about ghrelin is written in chapter 6 of this thesis.

ACROMEGALY

History

Acromegaly is a severe systemic disease most commonly caused by a benign GH-secreting (somatotroph) pituitary adenoma leading to excessive GH and IGF-I levels (40). Excessive GH secretion causes gigantism if it occurs prior to epiphyseal fusion and acromegaly after this time. The term acromegaly is from Greek meaning “large extremities”, and was originally named by the French neurosurgeon Pierre Marie in 1886, who provided the characteristic clinical description the disease (41). However, many other physicians before Marie have described acromegaly (42, 43). In 1567 the Dutch surgeon Johannes Wierus provided probably the first reliable medical description of acromegaly and gigantism (44).

Epidemiology

According to recent epidemiologic data, the estimated incidence of acromegaly is 1.8-13.7 cases per 100.000 people and the annual incidence is 0.2-1.1 cases per 100.000 people (45-51). The diagnosis of acromegaly is usually made in the fifth decade of life. The duration of symptoms until diagnosis is considerable with a median diagnostic delay of 5 years with some delays of over 15 years have been reported (47, 48, 52, 53). Many cases go unreported due to the insidious presentation and the lack of awareness of acromegaly among physicians.

Pathology

Acromegaly is almost always (≥95%) caused by a somatotroph adenoma. In very rare cases acromegaly may be caused by extrapituitary GH or GHRH hypersecretion from a pancreatic islet-cell tumour, bronchial carcinoid, or non-Hodgkin's lymphoma (54-59). Somatotroph adenomas can be classified as densely and sparsely granulated subtypes based on their appearance under an electron microscope. Sparsely granulated somatotroph adenomas (SGSA) are characterized by a more aggressive clinical behavior than densely granulated somatotroph adenomas (DGSA), as they are more likely to represent invasive macroadenomas which express lower somatostatin receptor subtype 2 (SST_2) protein expression and are resistant to first-generation long-acting somatostatin receptor ligand (SRL) therapy (60-63).

Clinical manifestations

The manifestations of acromegaly are due to the local effects of the pituitary tumour or the peripiheral actions of chronic GH and IGF-I hypersecretion (table 2). Central manifestations are mainly caused by large invasive macroadenomas and include headache, visual field defects due to impingement of the optic chiasm, cranial nerve palsy due to impingement of cranial nerves III, IV, and VI causing diplopia, or nerve V leading to trigeminal facial pain. Peripheral manifestations include soft tissue hypertrophy leading to characteristic enlargement of the hands, nose, forehead, eyebrow, jaw and feet. Clinicians should be aware of the possibility of acromegaly in patients with two or more of the following comorbidities: new-onset diabetes, diffuse arthralgias, new-onset or difficult-to-control hypertension, cardiac disease including biventricular hypertrophy and diastolic or systolic dysfunction, fatigue, headaches, carpal tunnel syndrome, sleep apnea syndrome, diaphoresis, and loss of vision (64).

Active acromegaly unmasks the diabetogenic effects of GH. Prolonged exposure to GH and IGF-I leads to insulin resistance and overt diabetes. In the general population insulin resistance and diabetes are associated with increased body fat, which is hallmark of the metabolic syndrome. However, many patients with acromegaly present

with insulin resistance and diabetes despite having a “favourable” body composition with a decreased body fat mass and increased muscle mass (65, 66).

Uncontrolled acromegaly is associated with a reduced life expectancy. If patients achieve biochemical control life expectancy is similar to the general population, despite this many patients with acromegaly have a reduced quality of life (QoL) (40, 67). Active acromegaly is associated with increased mortality. Normalization of GH and IGF-I levels largely reduces mortality to that of the general population (68).

Diagnosis

The biochemical diagnosis of acromegaly is based on demonstration of autonomous GH hypersecretion and elevated serum IGF-I levels. Due to the pulsatility and the short half-life of GH, concentration of GH may be undetectable in acromegaly patients (69). Measurement of single random GH levels is therefore not recommended. In contrast, serum IGF-I levels are relatively stable and show a log-linear correlation with GH (70), and have a long half-life of about 15 hours. In addition, IGF-I can be measured independent of time of the day and food intake. IGF-I serves as the integrative biomarker of GH secretion. A normal IGF-I within the age adjusted normal limits effectively excludes the diagnosis of acromegaly. However, in non-clear cut cases with IGF-I levels just above or around the upper limit of normality, confirmation is required by showing a lack of suppression of GH to less than 1 µg/L following a documented hyperglycemia during an oral glucose tolerance test (OGTT) (64). In acromegaly patients with poorly controlled diabetes mellitus the oral glucose tolerance test is not reliable and serum IGF-I levels should be re-assessed when glycemic control has been established. Systemic illnesses, hepatic or renal failure, malnutrition, diabetes mellitus and oral oestrogens may decrease IGF-I levels which might result in false-negative interpretations (64, 71, 72). False-positive elevated IGF-I levels can occur during pregnancy. Large variability inter-individual and intra-individual variability exists between the different IGF-I immunoassays (73). When monitoring IGF-I levels over time, it is recommended that the same immunoassay is used from laboratories that adhere to the international performance standards and use the appropriated normative data (74).

Following biochemical diagnosis, contrast enhanced magnetic resonance imaging (MRI) of the sellar region is required to assess tumour size, localization and invasiveness. A clinically relevant distinction which affects the surgical cure rates is microadenomas (≤1 cm) and macrodenomas (≥1 cm), invasiveness in the cavernous sinus, and impingement of the optic chiasm. In adenomas close to the optic chiasm visual fields assessment should be performed.

Treatment modalities

Surgery

Transsphenoidal surgery is the primary treatment for patients with small and, therefore, curable tumours or for large adenomas causing impingement of the optic chiasm (75, 76). Surgical results depend on preoperative GH and IGF-I levels, tumour

Table 1. Different clinical manifestations of acromegaly

Clinical manifestations of acromegaly	
Local tumour effects	*Somatic*
Visual field defects	Acral enlargement
Headache	Frontal bossing
Cranial nerve palsy	Prognathism
	Macrognathia
Metabolic	Diastema
Impaired glucose tolerance	Carpal tunnel syndrome
Hyperinsulinemia and insulin resistance	Arthralgias
Diabetes mellitus	Osteoarthritis
	Decreased bone quality
Lipids	Vertebral fractures
Hypertriglyceridemia	Myopathy
Endocrine - hypopituitarism	*Visceromegaly*
Erectile dysfunction	Goitre
Galactorrhoea	Macroglossia
Amenorrhea	Hepatomegaly
Secondary thyroid deficiency	Splenomegaly
Secondary adrenal deficiency	Renal hypertrophy
Cardiovascular	*Mineral*
Hypertension	Hypercalciuria
Arrhythmias	Hyperphosphaturia
Cardiomyopathy	Increased levels 25-hydroxyvitamin D3
Ventricular hypertrophy	
Congestive heart failure	*Renal*
	Increased aldosterone levels
Pulmonary	Nephrolithiasis
Central and obstructive sleep apnoea	
	Psychological
Dermatological	Impaired quality of life
Skin tags	Decreased self-esteem
Hyperhidrosis	Depression
Acanthosis nigricans	
Gastrointestinal	
Adenomatous colon polyps	
Dolichocolon	
Neuropsychological	

invasiveness and surgical skills. Surgery is the treatment of choice for microadenomas and well-defined intrasellar macroadenomas. In these cases experienced surgeons can achieve remission rates of about 80%, defined as postoperative normalization of IGF-I levels and suppression of GH levels to < 1 mg/l after an oral glucose load. These rates can drop to 20-30% for macroadenomas. For macroadenomas that are not entirely surgically resectable (eg, those with cavernous sinus extension) surgery may be considered with the goal of debulking the tumour mass. Debulking seems to increase the efficacy of postoperative treatment with SRLs (77-79), although conflicting data were reported as well (80). Preoperative treatment of macroadenomas with SRLs has been shown to improve surgical outcome (81-84). Successful surgery is accompanied by a rapid fall in GH and IGF-I levels and the costs are relatively low compared to life-long drug therapy, although the efficacy of surgery might be overestimated as the data on surgical outcome are almost exclusively reported from high specialist centers. In the United Kingdom, an efficacy rate for microadenonomas < 37% and for macroadenomas < 20% has been reported, for all centres together (85).

Radiotherapy

During the early 1900s radiotherapy was the treatment of choice for acromegaly (86, 87). To date, radiotherapy (RT) is considered a third line of treatment for acromegaly in most centres. Currently, RT is recommended for patients with residual tumour mass following surgery and medical therapy, and if medical therapy is not tolerated, unsuccessful, or unavailable (88-90). The method with the longest experience is conventional radiotherapy (CRT). CRT is administered in 20-30 fractions with a total dose of 40-45 Gray (91). CRT induces remission in about 50% of patients after a follow-up of 10 years. However, radiation-induced hypopituitarism is the main side-effect of all types of CRT, as 50-80% of patients develop pituitary insufficiencies within 5 to 10 years (92-94). Another drawback is that it takes sometimes years before RT induces biochemical remission, which comes along with a negative impact on quality of life (95). Analyses of the UK acromegaly database showed that CRT was associated with an increased mortality risk, and cerebrovascular disease as the main cause of death (96). Additionally, studies assessing QoL observed a lower QoL in patients treated with CRT that further decreased during follow-up (95, 97, 98). Joint problems are important factors affecting the QoL after radiotherapy (97). Other rare but severe side-effects of CRT include, optic neuropathy, radiation-induced cerebral tumours, cerebrovascular disease, and regrowth of pituitary adenomas. Stereotactic radiosurgery (SRS) methods has been developed with the aim to provide more precise targeting of the tumour and therefore less targeting of healthy brain tissue. SRS may be more safe than CRT, but long-term studies evaluating safety are lacking at the moment (99).

Medical therapy

Dopamine agonists

Under normal physiological conditions dopamine stimulates GH secretion (100, 101). However, in the 1970s it was discovered that administration of L-DOPA paradoxically reduced GH hypersecretion in patients with acromegaly (102). Subsequently, dopamine receptors (DR) were found on somatotroph adenomas (103). Of the 5 distinct DR subtypes, the D2 receptor (DR2) is the predominantly expressed DR subtype (104, 105). Until the 1980s dopamine agonists (DA) have been the only class of pharmaceutical agents available for acromegaly. Bromocriptine is a first-generation DA which normalized IGF-I levels in only about 10% of patients (106). Cabergoline is a second-generation ergot derivative DA with a high affinity for DR2, and has been used as monotherapy and in combination with SRLs (107). Due to its higher efficacy and better tolerability it has replaced bromocriptine as the DA of choice for treatment of hyperprolactinemia. Because cabergoline has a modest efficacy of about 30% in normalizing only IGF-I levels (< 1.5 x ULN) (88, 108). Cabergoline monotherapy is considered a good add-on therapy in patients with partial response to SRLs, and for patients with no access to pegvisomant (107, 109). Cabergoline can be taken orally and is inexpensive. It has a favourable safety profile, the adverse effects of cabergoline are considered mild and include postural hypotension, headache and nausea (110). Seldomly, psychological symptoms such as psychosis and impulse control disorders have been reported (111-113). Furthermore, long-term use of very high doses of cabergoline (3.5 mg/day) has been linked to valvular heart disease in patients with Parkinson's disease (114, 115). Although patients with acromegaly use much lower doses of cabergoline, active acromegaly itself is associated with increased risk of valvular heart disease (116-119). However, latest data from a longitudinal study showed no increased risk between cabergoline-treated acromegaly patients and a matched control population with untreated acromegaly (120).

Somatostatin receptor ligands

Somatostatin (SST or SRIF) was discovered in 1973 by the group of Roger Guillemin as the first hypothalamic hormone to regulate GH secretion (121). Somatostatin exerts inhibitory effects on hormone secretion from the pituitary, pancreas and the gastrointestinal tract (122). SST is a cyclic peptide with two active isoforms present in the circulation, SRIF-14 and SRIF-28. The biological effects of SST are mediation by its binding to five G protein-coupled receptor (GPCR) subtypes of somatostatin receptors (SSTs) (123, 124). SST are widely expressed in tissue specific patterns. In the normal human foetal pituitary gland all five SSTs expressed, but SST_2 and SST_5 are most important for regulation of hormone secretion (125). Somatotroph

adenomas mainly express SST_2, SST_5 and to a lesser extent SST_1 (126-131) (table 1). In the pancreas SST is an important regulator of glucose homeostasis, as it tightly regulates the secretion of insulin, glucagon and GLP-I. α-cells which produce glucagon express mainly SST_2, ß-cells which produce insulin express predominantly SST_5 and to a lesser degree SST_1, and δ-cells which produce SSTR-14 express mainly SST_5. Furthermore, enteroendocrine L-cells located in the ileum and colon, which produce GLP-I express mainly SST_5 (132-135).

Although native SST has potent anti-proliferative and anti-secretory effects on different tissues, it is clinically not useful due to its very short half-life of less than 3 minutes and the post-infusion rebound phenomenon (136). These limitations have been overcome by the development of more stable somatostatin receptor ligands (SRLs) which have a longer half-life and increased affinity for SSTRs. The short-acting immediate release SRLs, octreotide (137) and lanreotide (138) were initially developed. These first-generation SRLs show preferential binding to SST_2 and have an increased half-life of about 2 hours allowing for multiple daily injections. To date, more practical long acting depot formulations octreotide Long Acting Release (octreotide LAR) and lanreotide autogel (lanreotide ATG) are used which allow for 4 weekly injections. Both formulations are equivalent in terms of efficacy, but differ in their mode of administration; lanreotide is available in pre-filled syringes injected deep subcutaneously and octreotide LAR requires reconstitution before being injected intramuscularly (139, 140). First-generation SRLs represent the first line medical treatment for acromegaly (141). High SST_2 protein expression of the somatotroph adenoma is the main pathophysiological rationale for their efficacy in acromegaly. Biochemical normalization of IGF-I and GH levels can be achieved in about 40% of treatment-naïve patients with SRLs (142, 143). Clinically relevant tumour volume reduction of over 20% is frequently observed (40-63%) during SRL treatment and the decrease in GH levels generally occurs within the first 4 months (143-147). SRLs have a favourable safety and tolerability profile. Relatively few side effects do occur; in the first few weeks transient mostly self-limiting gastrointestinal complaints such as abdominal discomfort, nausea and steatorrhea occurs in most patients (148). Asymptomatic gallbladder stones or bladder sludge can develop in the first 18 months in up to 20% of patients (149). In 2018, Cozzolino and co-workers published a large meta-analysis of 47 prospective interventional trials studying the effect of first generation SRLs on glucose homeostasis in 1297 patients with acromegaly (150). The authors show that both lanreotide ATG and octreotide LAR significantly reduced insulin secretion which was not (completely) counterbalanced by the reduction in GH and IGF-I levels (150). In general, the net effect of SRLs on glucose metabolism is considered marginal, but it is clinically relevant in patients with insulin resistance and overt diabetes.

Pegvisomant

The GH molecule has two sites which interact with the extracellular region of the GHR performed dimer. Site 1 of the GH molecule interacts with one the GHRs and then GH site 2 interacts with the other GHR which leads to a functionally dimerized complex that induces GH-dependent intracellular signalling (152). Glycine at position 120 of the third alpha helix in binding site 2 of GH is crucial for GH action. In the early 1990s it was discovered that substitution of this glycine by lysine blocks intracellular signalling, converting GH into a GHR antagonist (153). Additional mutations in binding site 1 increased the affinity of PEGV to the GHR. Due to the short half-life of GH, the GHR antagonist was PEGylated by covalent addition of 4 to 6 5-kDa linear polyethylene glycol (PEG) molecules, extending the half-life to about 70 hours, which resulted in the marketed drug pegvisomant (PEGV; Somavert®) (154). PEGV itself can form dimers with the GHR and is internalized by the GHR, but does not induce GH-dependent intracellular signalling (155). Recently it has been shown that PEGV also inhibits GHR nuclear localization (156).

PEGV was approved in the United States in 2003 followed by approval in Europe in 2004 (154, 157). It is currently used as a second line therapy in patients that are inadequately controlled with SRLs monotherapy (88). Early clinical trials demonstrated IGF-I normalization in over 90% of patients resistant to SRL (158-160). Because PEGV is a competitive GHR blocker, virtually all patients with acromegaly can be controlled provided that the treating physicians adequately titrate the dose of PEGV. More recent real-life registry studies showed lower IGF-I remission rates of 60-70% (161-163), which is mainly attributed to an inadequate dose titration of PEGV.

Table 1. SST expression in normal pituitary and in somatotroph adenoma. Binding affinities of the difference somatostatin analogues to the five somatostatin receptor subtypes (SST_{1-5})

	SST_1	SST_2	SST_3	SST_4	SST_5
Somatotroph adenomas	60% +	95% +++	45% +	<5% −	90% ++
Somatostatin receptor ligands					
SRIF-14	0.1-2.26	0.2-1.3	0.3-1.6	0.3-1.8	0.2-0.9
Octreotide	280	0.38	7.1	>1000	6.3
Lanreotide	180	0.54	14	230	17
Pasireotide	9.3	1.0	1.5	>100	0.16

The percentage somatostatin receptor subtype (SSTR) expression and the quantitative estimation of receptor expression in somatotroph adenomas. In the rows below the binding affinities of endogenous somatostatin and the different somatostatin analogues is shown, expressed as IC_{50} values nmol/L (124, 136, 151).

Treatment with PEGV results in a rapid fall of serum IGF-I levels which usually leads to a paradoxical rise of serum GH levels, due to the negative feedback loop via the hypothalamus and the pituitary (159, 164). Cross-reactivity between PEGV and endogenous GH in commercial assays disables proper assessment of the endogenous GH levels (165). For these reasons GH cannot be reliably assessed in patients treated with PEGV, unless specific assays are used (166). Therefore, the key biomarker during the treatment of PEGV are serum IGF-I level with specific signs such as ring-size and body fat mass.

SRL and PEGV combination treatment

Because the majority of acromegaly patients are resistant to SRLs, additional therapies are required to achieve control of disease activity. Combining SRLs with PEGV combination treatment has several advantages because of their different mode of action. The first advantage of combination treatment is the much lower (around 50%) required weekly dose of PEGV (164). Because SRLs inhibit GH secretion, PEGV encounters less competition of endogenous GH around the GHR resulting in a lower necessary dose of PEGV to block all GHRs during combination therapy and additionally reduces the number of GHRs on the hepatocytes (164, 167, 168). In addition, combination treatment induces a 20% increase in PEGV serum levels than during PEGV monotherapy (169). The underlying mechanism for the observed rise in PEGV serum levels remains unknown (170). Besides direct inhibition of pituitary GH secretion, which leads lower hepatic IGF-I production, SRLs have also non-pituitary action on IGF-I production. SRLs can indirectly suppress hepatic IGF-I production by reducing portal insulin levels, which leads to a reduction in hepatic GHR expression (171). In addition, data from rodent studies has demonstrated that somatostatin can also directly suppress hepatic IGF-I production and possibly receptor-mediated clearance of GH (172). Taken together, these data suggest that SRLs induce a state of GH resistance in the liver, while peripheral extra-hepatic tissues might still be exposed to GH excess. Blocking peripheral GH action using PEGV can therefore be useful in treating extra-hepatic acromegaly (173). Based on this concept *Neggers et al* demonstrated in a prospective double blind placebo controlled crossover study that low doses of PEGV may improve quality of life in patients controlled with SRL monotherapy (174). Furthermore, PEGV is the treatment of choice for acromegaly patients with insulin resistance or diabetes, as it has beneficial effects on glucose metabolism. PEGV improves hepatic and peripheral insulin sensitivity, reduces hepatic gluconeogenesis and reduces free fatty acids (175-180).

Safety

The most common adverse events associated with the use of PEGV are transient elevated liver transaminases (TETs) >3 times the upper limit of normal which seemed to occur more frequently during combination treatment (162, 164, 167, 168, 181). Although many risk factors have been brought forward, the underlying pathophysiology of PEGV-induced TET development remains unclear (167, 168, 182, 183). The incidence of lipodystrophy at the injection site was reported in 3% of patients, but is clinically not significant as it regresses in most patients when PEGV is discontinued (184). Initially there were concerns that the reduced pituitary feedback inhibition from GH blockade would stimulate growth of the pituitary tumour (remnant). However, there is currently is no clear evidence that PEGV causes a clinically significant increase in tumour size. Therefore, PEGV can be considered as a safe approach, especially when combined with SRLs

Pasireotide

Pasireotide long-acting release (PAS-LAR) was approved by the FDA and EMA in 2014 and is the only second-generation SRL on the market. Pasireotide is a stable cyclohexapeptide showing high affinity to multiple SSTRs, with the highest affinity to SST_5 followed by SST_2, SST_3 and SST_1 compared with SRIF, octreotide and lanreotide (151). The higher affinity for SST_5 (and to other SSTRs) forms the pathophysiological rational for the use of pasireotide in acromegaly. Besides its broader SSTR binding profile, pasireotide has unique functional post-receptor effects. SSTR activation and trafficking are modulated in a manner distinct from SRIF and octreotide. Pasireotide shows lower SST_2 internalization, less ß-arrestin mobilization and lower activation of second messenger pathways such as lower activation of ERK pathway, less increase of intracellular Ca^{2+} and less cAMP inhibition (185-187). Therefore, pasireotide is considered to be a biased agonist for SST_2.

Efficacy

Two phase III studies reported the efficacy of PAS-LAR treatment in acromegaly (188, 189). The C2305 trial was double blind randomized head-to-head superiority study between PAS-LAR and octreotide LAR in medically naïve acromegaly patients (190). After one year of treatment, IGF-I normalization was achieved in significantly more patients using PAS-LAR (38.6%) than octreotide LAR (23.6%). However, GH reduction (≤2.5 g/L) was similar between patients using PAS-LAR (48.3%) and octreotide LAR (51.6%) (190). In the PAOLA study patients with acromegaly who were inadequately controlled despite ≥ 6 months treatment on maximum doses of first-generation SRLs were randomly assigned to pasireotide LAR at 40 mg, 60 mg, or continued on their treatment with octreotide LAR or lanreotide ATG (active control) (189).

After 24 weeks, biochemical control was achieved by 15% of patients using pasireotide LAR 40 mg and 20% using PAS-LAR 60 mg, compared with no patients in the active control group (189).The extension phase of both studies showed comparable response rates (191, 192). Also, about 17% of the patients with inadequate biochemical control after 12 months of treatment with octreotide LAR, achieved biochemical control after switching to PAS-LAR treatment (193). Although the effects of PAS-LAR and octreotide LAR treatment on the reduction of GH levels were superimposable in the C2305 study, PAS-LAR treatment was more effective in lowering IGF-I levels (188, 194).

Tumour volume reduction

Results from a phase II study in 61 patients with active acromegaly showed that treatment with short-acting pasireotide resulted in clinically significant (>20%) tumour volume reduction in 39% of patients after 3 months of treatment, which increased to 54% after 6 months of treatment (195). However, in the C2305 study PAS-LAR and octreotide LAR treatment were equally effective in reducing tumour volume (188). In the PAOLA study, tumour volume reduction occurred more frequently in patients using PAS-LAR 40 mg (19%) and 60 mg (11%), than in patients in the active control group (2%) (189). This finding is not entirely surprising because it is plausible that patients who were inadequately controlled with first-generation SRLs are more likely to respond to PAS-LAR treatment than medically naïve patients. The mean decrease in tumour volume during the extension study was 25% for crossover to PAS-LAR and 18% for crossover to octreotide LAR, while 54% of PAS-LAR treatment and 42% of octreotide LAR patients achieved significant tumour volume reduction (193). Taken together, these clinical studies suggest that PAS-LAR might exert a greater effect on tumour control in patients that show no tumour shrinkage after first-generation SRL treatment. In addition, patients with large tumours due to genetic causes such aryl hydrocarbon receptor-interacting protein (AIP) mutations and X-linked acrogigantism are frequently resistant to first-generation SRL, and could therefore be more responsive to PAS-LAR treatment (196-198).

Quality of life and symptoms

In medically naïve patients, PAS-LAR and octreotide LAR showed similar improvements in acromegaly symptoms score (188). In the PAOLA study, the patients in the PAS-LAR treatment groups showed more improvement in acromegaly symptoms than patients in the active control group (189). As mentioned above, the observed improvement in acromegaly symptoms in the PAS-LAR treatment groups was to be expected. Nevertheless there is evidence that pasireotide has an analgesic effect on headache symptoms of acromegaly patients (190, 199-201).

Safety

PAS-LAR is generally well-tolerated and has a comparable safety profile to first-generation SRLs, except for a greater frequency and degree of hyperglycemia-related adverse events (188, 189). Hyperglycemia-related adverse events were reported in 57.3% of patients treated with PAS-LAR and in 21.7% of patients treated with octreotide LAR in the C2305 study (188). In the PAOLA study, hyperglycaemia-related adverse events occurred in 65% of patients treated with pasireotide LAR and 30% of patients in the active control group (189). Hyperglycaemia occurred early after drug initiation, with fasting plasma glucose (FPG) and glycosylated haemoglobin (HbA1c) levels increasing in the first 3 months, and plateauing thereafter with additional antidiabetic medication (192). In the ACCESS study, designed to assess the safety of PAS-LAR treatment in patients with acromegaly, hyperglycaemia-related adverse events were reported in 46% of patients and about 9% discontinued PAS-LAR because of hyperglycaemia (202). Predictors for pasireotide-induced hyperglycaemia were higher baseline glucose levels (FPG >5.55 mmol/l) and patients receiving antidiabetic medication (203). Mechanistic studies in healthy volunteers have revealed that pasireotide inhibits secretion of insulin, glucagon-like peptide I (GLP-I) and gastric inhibitory polypeptide (GIP), and modestly suppresses glucagon secretion. However, pasireotide did not affect hepatic or peripheral insulin sensitivity as evaluated with the hyperinsulinaemic-euglycaemic clamp test and OGTT (204, 205). The effects of pasireotide on insulin, glucagon and incretin secretion can be explained from its SSTR binding profile. SST_5 is known to be expressed on pancreatic ß-cells which mediate insulin secretion, but also on enteroendocrine L-cells which produce GLP-I (132-134). In contrast SST_2 is mainly expressed on pancreatic α-cells which mediate glucagon secretion (206, 207), which may explain the modest effect of pasireotide on glucagon secretion.

Scope and Aims of the Thesis

Over the last decade SRL and PEGV combination treatment has been established as an effective and safe second line treatment modality for acromegaly patients with inoperable pituitary adenomas and who are refractory to medical treatment. In the Rotterdam cohort over 90% of patients achieved IGF-I normalization with combination treatment. Despite this high efficacy, PEGV poses an economic burden due to its high treatment costs. Furthermore, 4 weekly SRL injections and daily to weekly PEGV injections may negatively impact long-term adherence and quality of life of patients. With the goal to improve patient adherence we hypothesized that it is possible to temporarily withdraw SRL treatment in patients using combination treatment. Therefore, in **Chapter 2** we aimed to determine the efficacy of temporary withdrawal of SRL treatment and switch to (twice) weekly PEGV monotherapy in patients controlled with combination treatment. For how long can biochemical control be maintained without a need to increase the PEGV dose?

As PAS-LAR has demonstrated superior efficacy over first-generation SRL and is approved a second line medical treatment for acromegaly, the question arises what the position is of PAS-LAR in relation to PEGV in the modern medical management of acromegaly. Switching from SRL and PEGV combination treatment to PAS-LAR could lead to a reduction in the PEGV dose required to control disease activity. A lower PEGV dose and/or less PEGV injections (i.e. PEGV sparing effect) could contribute to an improvement in quality of life and patient convenience. What is the safety of PEGV and PAS-LAR combination treatment? Especially with respect on glucose metabolism? What are predictors for pasireotide-induced hyperglycaemia? To address these questions we conducted the Pasireotide LAR and PEgvisomant (PAPE) study, a prospective single-centre open-label trial to study the efficacy and safety of switching to PAS-LAR alone or in combination with PEGV in patient who were well-controlled with SRL and PEGV combination treatment. In **Chapter 3** the 24-weeks efficacy and safety results (PAPE core study) are presented.

Chapter 4 focuses on the results of the extension phase until 48-weeks results. The main goal of the extension phase was to find the optimal PAS-LAR and PEGV dose to achieve IGF-I normalization. In addition, we aimed to investigate whether baseline insulin secretion as measured with the OGTT correlates with hyperglycaemia after PAS-LAR treatment during the study.

Based on the results of PAPE study we aimed to investigate in active acromegaly, whether the responsiveness to PAS-LAR treatment during the PAPE study correlated with the previous responsiveness to SRL monotherapy in the same patients. Secondly,

we assessed to what extent SST_2 and SST_5 protein expression on somatotroph adenoma were correlated to responsiveness to PAS-LAR treatment. It has previously been suggested that SST_5, and not SST_2, protein expression of the somatotroph adenomas predicted responsiveness to PAS-LAR treatment (208, 209). In **Chapter 5** aimed to confirm whether this is indeed the case in our cohort of patients included in the PAPE study.

Ghrelin is an important peptide hormone regulated by the GH-axis and is modulated by medical treatment. In acromegaly SRL treatment reduces ghrelin levels, while PEGV treatment increased ghrelin levels. It is however unknown what the net effect of SRL and PEGV combination treatment is on ghrelin levels in acromegaly patients. Previous studies have only measured total ghrelin using unspecific radioimmunossay methods and have not distinguished between AG and UAG levels. Therefore, in **Chapter 6** we aimed to assess plasma AG and UAG levels, and the AG/UAG ratio in acromegaly patients on SRLs and PEGV combination treatment, compared with the control groups using PEGV monotherapy and medically naïve patients.

PEGV is approved for the medical treatment of acromegaly since 2003. Patients are long-term exposed to high doses of PEG compared with other PEGylated biological drugs. Little is known about how PEGV is cleared from the body and which type of cells are involved. We postulated that PEGV is cleared by the mononuclear phagocyte system (MPS) because PEG is considered to be a non-biodegradable molecule. Kupffer cells are the most important macrophages of the MPS and have been reported to clear PEGylated nanomaterials in a previous study. In **Chapter 7** we presented the finding of a pilot study in which we assessed in two acromegaly patients whether PEGV is expressed in the liver, and if so whether Kupffer cells are involved in the clearance of PEGV using immunofluorescence studies.

Chapter 8 provides a summary of the generated data, critically reviews the strengths and limitations of the aforementioned studies and brings forward future perspectives of outstanding questions. The discussion section is ended by presenting our clinical recommendations on the position of PAS-LAR in the modern medical management of acromegaly.

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Chapter 2

What is the efficacy of switching to weekly pegvisomant in acromegaly patients well controlled on combination therapy?

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ABSTRACT

Context: While combination therapy of acromegaly with long-acting somatostatin analogues (LA-SSAs) and pegvisomant (PEGV) normalizes insulin-like growth factor-I (IGF1) levels in the majority of patients, it requires long-term adherence. Switching from combination therapy to monotherapy with weekly PEGV could improve patient comfort, but the efficacy is unknown.

Objective: to assess the efficacy of switching to PEGV monotherapy in patients well controlled on combination therapy of LA-SSAs and PEGV.

Design: Single center, open-label observational pilot study. LA-SSA therapy was discontinued at baseline and all patients were switched to PEGV monotherapy for 12 months. If IGF1 levels exceeded 1.0 times upper limit of normal (ULN), PEGV dose was increased by 20 mg weekly.

Subjects and methods: 15 subjects (8 males) were enrolled, with a median age of 58 years (range 35-80) on combination therapy of high-dose LA-SSAs and weekly PEGV for >6 months, and IGF1 levels within the normal range. Treatment efficacy was assessed by measuring serum IGF1.

Results: After 12 months of weekly PEGV monotherapy, 73% of the subjects' IGF1 levels remained controlled. In one patient LA-SSA had to be restarted, due to recurrence of headache. IGF1 levels increased from a baseline level of 0.62 x ULN (range 0.30-0.84) to 0.83 x ULN (0.30-1.75) after 12 months, while the median weekly PEGV dose increased from 60 (30-80) mg to 80 (50-120) mg.

Conclusion: Our results suggest that switching from combination therapy of LA-SSAs and PEGV to PEGV monotherapy can be a viable treatment option for acromegaly patients without compromising efficacy.

Introduction

Acromegaly is a rare disease characterized by somatic overgrowth and endocrine dysfunction due to excessive secretion of growth hormone (GH) and subsequent elevation of insulin like growth factor-I (IGF1) levels. In more than 90% of patients it is caused by a benign growth hormone-secreting pituitary adenoma (1). The main causes of death in acromegaly patients are cardiovascular and respiratory diseases (2, 3). Normalization of GH and IGF1 levels will result in normal mortality rates, reduced morbidity and a reduction in symptoms (3).

The growth hormone receptor antagonist pegvisomant (PEGV) is the medical treatment that has the highest reported efficacy (4). PEGV can be administered with or without long-acting somatostatin analogues (LA-SSAs) (5, 6). Administration of PEGV alone at a mean weekly dose of 130 mg can normalize IGF1 levels in over 90% of patients (7). If LA-SSAs and PEGV are used together, a similar efficacy of over 90% is achieved. However, a considerably lower mean weekly dose of 77-80 mg PEGV is required, this may result in a more cost-effective treatment.

Both LA-SSAs and PEGV are administered, respectively as intramuscular and subcutaneous injections. LA-SSAs are injected every four weeks, while PEGV can be injected either as a daily or as a weekly injection. While most studies on efficacy have been based on clinical trials that evaluated the daily injection (7, 8), only one study has assessed the weekly injection. Higham et al reported a normalization of IGF1 in 71% of acromegaly patients (n = 7) treated with weekly PEGV monotherapy (9).

Switching patients from combined therapy of LA-SSAs and PEGV to monotherapy with PEGV can reduce the total number of injections, and therefore improve patient adherence. In addition, PEGV monotherapy allows for easier dose adaptation.

In this pilot study, we assessed the effect of switching from combination therapy to weekly PEGV monotherapy. The main outcome parameters were the proportion of patients with normalized IGF1 levels after 12 months follow-up with weekly PEGV monotherapy.

SUBJECTS AND METHODS

Patients

In 2009, a prospective observational study enrolled 15 subjects (8 males) with acromegaly from a single centre, who were treated with a combination of LA-SSAs

Table 1: Baseline characteristics of all subjects (n = 15)

	N (%)
Demographics	
Sex, female	8 (53.3)
Age, median (range)	58 (35 – 80)
Diabetes mellitus type 2	3 (20)
Previous treatments	
Transsphenoidal surgery	3 (20)
Transsphenoidal surgery and radiotherapy	6 (40)
Primary medical therapy	6 (40)
Weekly PEGV dose (mg) – median (range)	60 (9.3 – 33.4)
Serum assessments	median (range)
IGF1 (nmol/L)	15.7 (9.3 – 33.4)
IGF1 x ULN	0.62 of ULN (0.30 – 0.84)
GH (µg/L)	3.03 (0.19 – 15.95)
Glucose (mmol/L)	5.4 (3.4 – 20)
HbA1c (mmol/L)	6.1 (5.1 – 9.2)
Long-acting somatostatin analogues	
Lanreotide Autogel	13 (87)
Octreotide LAR	2 (13)

(13 Lanreotide Autogel, 2 octreotide LAR) and PEGV (table 1). These patients received combination therapy because their serum IGF1 levels exceeded the normal range or because their acromegaly symptoms persisted during high dose of LA-SSA monotherapy (5). Inclusion criteria for the present study were a stable LA-SSA dose, the use of PEGV as a weekly injection and biochemical remission for over 6 months before enrolment, defined by an IGF1 within the normal range for sex and age of < 1.0 ULN (8). The cause of acromegaly needed to be a GH secreting pituitary adenoma and the allowed dose of weekly PEGV was ≤ 80 mg. The median age of the study group was 58 (range 35 – 80) years. Three patients had received surgery of the pituitary adenoma in the past, while 6 patients had been treated with both surgery and radiotherapy. Radiotherapy was administered at least 5 years before study entry. Six patients had only received medical treatment. Three patients had diabetes and were using oral medication, while one diabetes patient also used insulin. All patients gave their written informed consent, and the study was approved by our local IRB.

One patient had to be restarted with LA-SSAs during the study period due to a recurrence of symptoms and therefore did not want to continue with the PEGV alone. This patient was reported as uncontrolled and was censored from the results after withdrawal, but was counted as treatment failure.

Study

At the start of the study LA-SSAs were discontinued for 12 months and only PEGV was continued. Every 6 weeks patients visited our outpatient clinic for measurements of IGF1 levels, HbA1c, glucose, insulin, cholesterol, free fatty acids (FFA), and triglycerides. During the visits, data on symptoms and safety assessments were collected and the dose of PEGV was adjusted if necessary. IGF1 levels were measured by immunometric assays (Diagnostic Products Corp., Los Angeles, CA) and were interpreted according to the sex and age dependent ranges (10). GH and serum PEGV levels were measured at the start of the trial, after 6 months and after 12 months. Since endogenous GH was in the presence of PEGV, GH levels were measured using a specific assay free of interference of the drug (11). When IGF1 levels exceeded the normal range, the PEGV dose was further increased by 20 milligrams per week. This was continued until the IGF1 levels were within the normal range for sex and age and PEGV dose was not changed. Doses were divided into two equal portions per week when PEGV exceeded 80 mg. Efficacy of PEGV monotherapy was assessed 12 months after discontinuation of the combination therapy. Safety assessment included measurement of serum alkaline phosphatase, γ-glutamyl transpeptidase (γ-GT), alanine aminotransferase (ALT), aspartate aminotransaminase (AST), lactate dehydrogenase and total bilirubin. A pituitary MRI was performed before study entry and after the study.

Statistics

Prism version 5.00 for windows (Graphpad Software, San Diego CA) was used for the statistical analyses of the data. Although the nature of the study was mainly descriptive, pertinent data were analysed by Wilcoxon's signed-rank test. Statistical significance was defined by $P < 0.05$ (two-tailed). Data are expressed as median ± (range), unless specified otherwise.

RESULTS

Efficacy

Fifteen patients were enrolled in this study. At baseline the IGF1 levels of all patients were controlled with a median IGF1 level of 62% of the upper limit of normal (ULN) (range 30 % to 84 %), (figure 1).

At 6 months, IGF1 levels started to increase to 100% of the ULN (range 43–220%). The slow increase of IGF1 started 12 weeks after cessation of the LA-SSA. At baseline, patients were using PEGV at a median dose of 60 mg (range 30–80 mg).

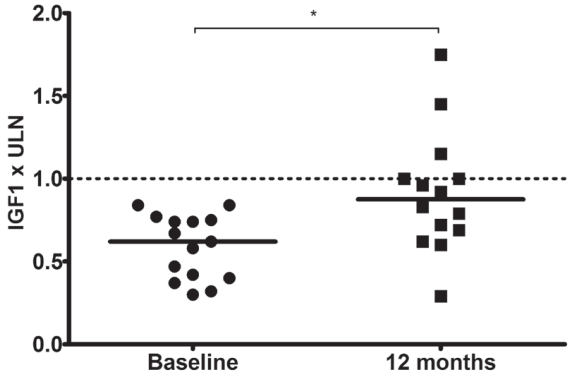

Figure 1. IGF1 levels at baseline and after 12 months of pegvisomant therapy. IGF1 levels expressed as times upper limit of normal, at baseline (n = 15) and after 12 months (n = 14). One patient restarted somatostatin analogues due to recurrence of symptoms.

After 12 months, median IGF1 levels increased to 83% (30–175%) of the ULN ($P < 0.05$) (figure 1). The median PEGV dose had been increased to 80 mg (50 – 120 mg) ($P < 0.05$). IGF1 remained controlled in 11 of 15 subjects (73 %) after 12 months of weekly PEGV monotherapy.

At the start of the study median serum PEGV levels were 3.00 mg/L (0.25 – 19.44). After an initial decrease in serum PEGV levels 2.23 (0.44 – 12.10) at 6 months, PEGV levels increased to 4.36 mg/L (range 1.12 – 21.28) at 12 months. Between 6 and 12 months the serum PEGV levels were significantly higher ($p = 0.01$), but no difference was found between baseline and 6 or 12 months.

We compared PEGV levels between the subjects in whom IGF1 levels were elevated and those who had levels that remained within the normal range after 12 months. We observed that after 12 months, acromegaly patients with an elevated IGF1 tended to have lower PEGV levels, this was 3.85 mg/L (1.30 – 4.39 mg/L) in non-controlled patients compared with 5.54 mg/L (1.12 – 21.28 mg/L) in controlled patients ($p = 0.25$). Weekly PEGV doses and increases in doses were not different between controlled and non-controlled patients: the baseline median PEGV dose was respectively 60 (30 – 80 mg) in controlled versus 60 (40 – 80 mg) in non-controlled patients. After 12 months the respective median PEGV dose increased to 80 (50 – 120 mg) in controlled versus 100 (80 – 120 mg) in non-controlled patients. The median increase in PEGV dose was +10 (0 – 60 mg) in those who were controlled after 12 months, while this was +40 (20 – 40 mg) for those who were non-controlled.

Five subjects did not require changes in PEGV dosing, since their IGF1 level remained within the age adjusted normal limits. All 5 had received combination therapy longer (5.88 (2.26 – 6.01) years) than the other 10 subjects (2.52 (1.48 – 6.39) years).

Over time the GH levels tended to increase during PEGV monotherapy. At baseline we measured 2.99 µg/L (range 0.19 – 15.95 µg/L) compared with 3.22 µg/L (0.12 – 79.14) at 6 months, and 6.36 µg/L (range 0.180 – 31.45 µg/L) at 12 months. The GH levels at baseline, 6 months and 12 months were significantly different ($p<0.05$), except for baseline vs. 12 months ($p = 0.055$).

Safety

No significant safety issues were observed during PEGV monotherapy. After 12 months glycosylated haemoglobin (HbA1c) decreased significantly from 6.0 % (5.1 – 9.2 %) to 5.9 % (5.0 – 9.1 %) ($p = 0.02$). The patients with diabetes had no alterations to their anti-diabetic medication during the study. No significant elevation of liver enzymes was observed and the pituitary MR did not show any change in tumour-size. The one patient who withdrew from the study had severe headaches that resolved after reinitiating LA-SSAs.

DISCUSSION

In this study we followed 15 acromegaly patients who previously were biochemically controlled with LA-SSAs and weekly PEGV. These patients had been switched to combination therapy since their IGF1 levels could not be controlled by LA-SSA monotherapy. In this study, we discontinued LA-SSAs and continued with weekly PEGV monotherapy. At baseline, 100% of the subjects had IGF1 levels within the normal limits during combination therapy. After 12 months follow-up 73% remained controlled.

The efficacy of 73% is in the same range as the efficacy of 68% reported by the ACROSTUDY group. However, we achieved this proportion of IGF1 normalization at a median PEGV dose of 80 mg/week after 12 months, whereas the ACROSTUDY group reported this at a median PEGV dose of 17.2 mg/day after 5 years, which corresponds to approximately 120 mg/week(12). The subjects enrolled in our study used relatively low doses of PEGV and had used the combination with LA-SSAs for at least 1.5 years. Since this may have had an impact on our results, these results cannot be extrapolated to all patients using combination therapy.

The slow and gradual increase in serum IGF1 levels that we observed sixteen weeks after discontinuation of LA-SSAs is in line with the expected wash-out time of the LA-SSAs and the carry-over effect of the LA-SSAs of about 15-20 weeks(5, 13, 14). During 12 months of follow-up five of 15 patients did not need any dose adaptation. Although IGF1 levels also increased in these patients, they remained within the age-adjusted normal range. Combination therapy in these 5 patients lasted for longer, than for the other patients in the study. A longer period of treatment with LA-SSA might result in prolonged "suppression" of GH, which may improve the efficacy of PEGV, a competitive inhibitor of the GH receptor(4). In the total population, single measurements of GH tended to increase at 6 and 12 months during the study. However, subjects in whom IGF1 remained within the normal range tended to have lower GH levels than those with elevated IGF1 levels.

Subjects in whom IGF1 levels increased after the discontinuation of the LA-SSAs tended to have lower PEGV serum levels, despite treatment with similar doses of PEGV. One would expect that PEGV levels would decrease after discontinuation of LA-SSAs, since LA-SSAs increase the PEGV serum levels by approximately 20% compared with an identical dose of PEGV alone(6, 11). This observation explains the difference in PEGV levels between controlled and uncontrolled patients at 6 months, but not the difference in PEGV levels at 12 months. A potential explanation is that uncontrolled patients do not increase in PEGV serum levels as much as controlled patients when the weekly dose of PEV is increased. The large difference in serum PEGV levels between subjects has been reported before(15).

Possibly due to the small sample size of our study however, we found no association between PEGV dose and previously reported characteristics such as adiposity and body weight.

The one patient who was discussed in the safety analysis was withdrawn from the study because she suffered from severe headaches, even though IGF1 was controlled (98% of ULN). After recommencing LA-SSA co-treatment the headache disappeared. The pituitary MRI scan showed no explanation for the headache. In this specific case, we suspect that there was a causal relationship between discontinuation of LA-SSA and the headache. As expected, HbA1c levels decreased significantly in these patients in our study. However, the change was very small, and therefore of dubious clinical relevance.

This was a pilot study on monotherapy with weekly PEGV to assess whether ceasing LA-SSAs is a viable option in a selection of our patients on combination therapy.

A drawback of this pilot is the small sample size. Nevertheless, our results suggest that a significant number of acromegaly patients on low-dose PEGV in combination with LA-SSAs can temporarily stop their LA-SSA treatment for at least a year. These treatment interruptions may improve patient adherence to the medication and reduce the economic burden of long-term use of expensive medications.

We conclude that discontinuing LA-SSAs and continuing with PEGV may be an interesting alternative for a selected group of patients that are well-controlled with a combination of LA-SSAs and relatively low-doses of PEGV. It is possible to maintain biochemical control up to 12 months in a substantial number of patients without raising the dose of PEGV. In patients who have previously been treated for an extended time with LA-SSAs, there is no need to adjust therapy even after 12 months. It seems sensible to determine the usability of structured interruptions in medical therapy in patients with acromegaly in a larger study.

Declaration of interest

A.J. van der Lely is consultant for Novartis Pharma, Pfizer International and received grants from Novartis Pharma, Ipsen Pharma International and Pfizer International. SN received research grant from Ipsen and Pfizer. JOLJ is a consultant for Pfizer and Novartis and has received lecture fees and unrestricted research grants from Novartis, Pfizer and IPSEN. The other authors have nothing to disclose.

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Chapter 3

Efficacy and safety of switching to pasireotide in acromegaly patients controlled with pegvisomant and first-generation somatostatin analogues (PAPE study)

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ABSTRACT

Aim: To assess the efficacy and safety of pasireotide long-acting release (PAS-LAR) alone or in combination with pegvisomant by switching acromegaly patients who were well-controlled with somatostatin analogues (LA-SSAs) and pegvisomant to PAS-LAR with or without pegvisomant.

Methods: 61 acromegaly patients were enrolled in a prospective open-label study. We included patients with an IGF-I ≤1.2 x Upper Limit of Normal (ULN) during treatment with LA-SSAs and pegvisomant. At baseline, the pegvisomant dose was reduced by 50% up to 12 weeks. When IGF-I remained ≤1.2 x ULN after 12 weeks, patients were switched to PAS-LAR 60 mg monotherapy. When IGF-I was >1.2 x ULN, patients were switched to PAS-LAR 60 mg and they continued with the 50% reduced pegvisomant dose.

Results: At baseline, mean IGF-I was 0.97 x ULN, while median pegvisomant dose was 80 mg/week. At 12 weeks, mean IGF-I increased to 1.59 x ULN, while IGF-I levels ≤1.2 ULN were observed in 24.6% of participants. At 24 weeks, IGF-I levels were reduced into the reference range in 73.8% of patients. Between baseline and 24 weeks the pegvisomant dose was reduced by 66.1%. PAS-LAR was well tolerated, while hyperglycemia was the most frequent adverse event. The frequency of diabetes increased from 32.8% at baseline to 68.9% at 24 weeks.

Conclusions: Switching to PAS-LAR, either as monotherapy, or combination with pegvisomant, can control IGF-I levels in the majority of patients. PAS-LAR demonstrated a pegvisomant sparing effect of 66% compared to the combination with LA-SSAs. Hyperglycemia was the most important safety issue.

INTRODUCTION

Acromegaly is an endocrine disorder predominantly caused by a pituitary adenoma characterized by excess growth hormone (GH) and insulin-like growth factor I (IGF-I) secretion. Persistently elevated GH and IGF-I levels result in reduced life-expectancy, reduced quality of life and multisystem comorbidities (1). Normalization of IGF-I and GH normalizes life-expectancy and improves quality of life and comorbidities (2). Current medical treatment regimens focus on inhibiting pituitary GH hypersecretion and blocking peripheral GH actions.

First-generation long-acting somatostatin analogues (LA-SSAs) such as octreotide long-acting repeatable (LAR) and lanreotide Autogel (ATG) are the cornerstones of pharmacological treatment of acromegaly. They inhibit pituitary GH secretion by preferential binding to somatostatin receptor subtypes 2 and 5 (SST_2, SST_5). In clinical practice only about 40% of patients treated with monotherapy LA-SSAs achieve biochemical normalization of GH and IGF-I. Therefore, most patients treated with LA-SSAs need additional therapies (3-5).

The GH receptor antagonist pegvisomant (PEGV) is a competitive GH receptor blocker, and the most effective drug to normalize IGF-I levels in acromegaly. IGF-I normalization can be achieved in 60 to over 90% of patients, provided that the dose is sufficiently increased (6-11). Importantly, PEGV improves glucose homeostasis by increasing hepatic and peripheral insulin sensitivity, even after normalization of GH and IGF-I levels (12-15). Combination treatment of LA-SSA and PEGV shows similar high efficacy of over 90%, with the added benefit of lowering the required PEGV dose (16, 17).

Pasireotide LAR (PAS-LAR) is a second-generation LA-SSA which targets multiple somatostatin receptors, with the highest affinity for SST_5, followed by SST_2, SST_2 and SST_1. Compared with octreotide and lanreotide, pasireotide has a higher affinity for SST_5 and lower affinity for SST_2 (18). PAS-LAR has been shown to provide superior clinical efficacy over octreotide LAR. This has been shown in two clinical trials, in treatment-naïve acromegaly patients and in patients inadequately controlled with LA-SSAs (19, 20). Although the effect of PAS-LAR and octreotide LAR on the reduction of GH levels is superimposable, PAS-LAR appears to be more effective in lowering IGF-I levels (19).

PAS-LAR has a similar safety profile to the LA-SSAs, with the exception of a higher frequency and degree of hyperglycemia-related adverse events, which have been reported in about 60% of patients (19). These are presumably caused by a suppression of insulin secretion and a reduced incretin response (21-23).

The combination treatment of PEGV with PAS-LAR has not been extensively studied yet. A potential advantage of this combination may be a reduction in the PEGV dose to control acromegaly activity. This PEGV sparing effect of PAS-LAR could lead to a reduction in the number of patients that need PEGV co-treatment, or a reduction in the number of injections of PEGV and, thereby, a reduction of costs of this treatment.

The effects of combination treatment of PEGV and PAS-LAR on glucose homeostasis are unknown. We hypothesized that the improvement of glucose homeostasis with PEGV may offset pasireotide LAR-induced hyperglycemia.

The aim of the present study was to assess the efficacy and safety of PAS-LAR alone, or in combination with PEGV by switching acromegaly patients who were well-controlled with LA-SSAs and PEGV to PAS-LAR with or without PEGV in a prospective open-label trial.

METHODS

Study design

The PAPE study is a 24 weeks, prospective, single-center, open-label, investigator-initiated conversion study. We recruited patients from the Erasmus University Medical Center outpatient clinic. After enrolment, LA-SSA treatment was continued, and the PEGV dose was reduced by 50% in the first 12 weeks (run-in phase, figure 1).

When IGF-I levels remained within the reference range (IGF-I ≤1.2 x ULN) after 12 weeks, patients were switched to PAS-LAR 60 mg monotherapy every 4 weeks. When IGF-I levels exceeded 1.2 x ULN after 12 weeks, patients were switched to PAS-LAR 60 mg, while the 50% reduced PEGV dose was continued. Between 12 and 24 weeks dose adaptations of PEGV were not permitted unless IGF-I decreased below the age adjusted normal limits. In that case, the dose of PEGV was decreased by 20 mg weekly, until IGF-I increased to age-specific reference ranges. PAS-LAR injections were administered at home by endocrine nurses, and PEGV injections were administered by patients themselves.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and reported in accordance with the International Conference on Harmonisation Harmonised Tripartite Guideline for Good Clinical Practice. The study protocol was approved by the medical ethics committee of the Erasmus University Medical Centre. An independent Data Safety Monitoring Board (DSMB) was established for the

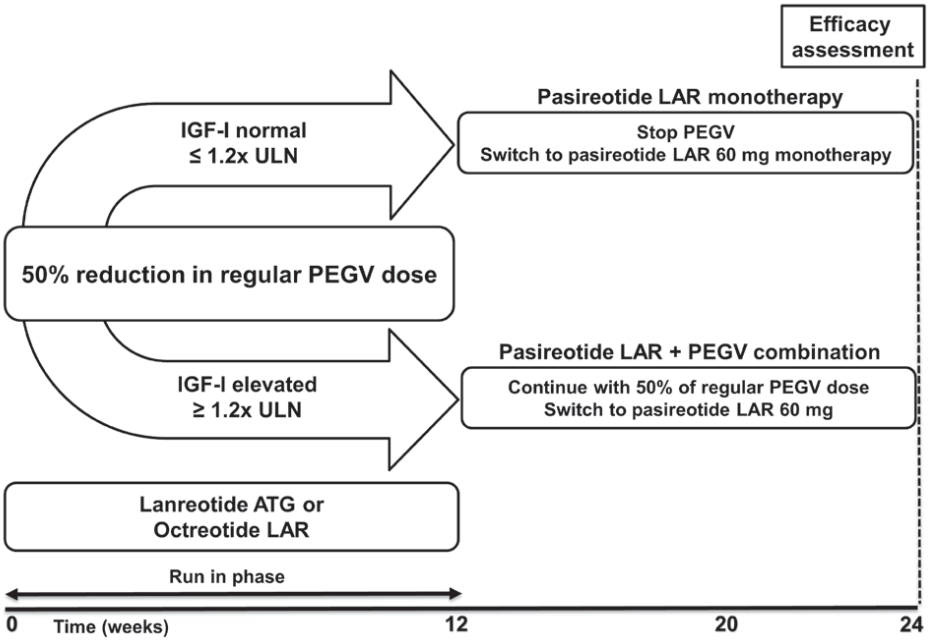

Figure 1. PAPE study design.

purpose of safety surveillance, to perform interim analyses on the safety and efficacy data, and to evaluate patient safety and scientific integrity. The DSMB consisted of a clinical expert in endocrinology and a clinical epidemiologist. All patients provided written informed consent to participate in the PAPE study. This study was registered with ClinicalTrials.gov, number NCT02668172, the Dutch Trial Register and the WHO International Clinical Trials Registry Platform, number NTR5282.

Patients

We enrolled 61 (32 male) patients, aged 18 years or older, with well controlled acromegaly (defined as IGF-I concentration ≤1.2 times the age and sex-adjusted Upper Limit of Normal [ULN]). Eligible patients had received entry combination treatment with weekly PEGV and monthly injections of either 30 mg octreotide LAR or 120 mg lanreotide ATG for at least 6 months prior to study. Eight patients received LA-SSAs every three weeks. Two patients who also used the dopamine-receptor agonist cabergoline were also included, but their dose of cabergoline was not changed during the study period. Key exclusion criteria included; diabetic patients with poor glycemic control, defined as HbA1c levels ≥9.0%; pituitary surgery or radiotherapy within the previous 6 months; patients with compression of the optic chiasm causing any visual field defect that required acute surgical intervention; patients with risk factors for

torsades de pointes, i.e. patients with a baseline QTcF >450 ms in males and >460 ms in females; patients who had congestive heart failure (NYHA Class III or IV), unstable angina, sustained ventricular tachycardia, clinically significant bradycardia, advanced heart block, history of acute MI less than one year prior to study entry or clinically significant impairment in cardiovascular function; patients diagnosed with liver disease such as cirrhosis or chronic active hepatitis; patients with an abnormal hepatic function, defined as alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase, alkaline phosphatase, or total bilirubin > 3 times the upper normal limit; patients with symptomatic cholelithiasis and or chronic pancreatitis; patients with renal insufficiency defined as clearance < 50 ml/min; patients with hypothyroidism or adrenal insufficiency who are not on adequate replacement therapy.

Procedures

Blood measurements

Total IGF-I, IGFBP3, GH concentrations were measured with the commercially available immunometric assay (IDS-iSYS; Immunodiagnostic Systems Limited; Boldon, United Kingdom). IGF-I intra-assay and inter-assay coefficients of variation (CVs) were respectively 5.7-8.1% and 1.5-2.1%, and IGF-I was interpreted according to the sex and age-dependent ranges by Bidlingmaier *et al* (24). PEGV does not interfere with the GH immunoassay that was used (25). Serum cortisol was measured using chemiluminescence immunoassay (Immulite 2000 XPi, Siemens AG). Testosterone was measured using the LC-MS/MS method (Thermo Fisher Scientific BV). TSH and FT4 levels were determined using chemiluminescence immunoassays (Vitros ECI, Ortho Clinical Diagnostics, Rochester, NY). Fasting plasma glucose, insulin, HbA1c levels, serum concentrations of (ALT), aspartate aminotransferase (AST), alkaline phosphatase, glutamyl transpeptidase, total bilirubin, coagulation and kidney function were determined with standard laboratory methods.

Study assessments

Safety assessments included: electrocardiogram at baseline, and 24 weeks. The severity of adverse events was evaluated at every study visit as mild (grade 1), moderate (grade 2), severe (grade 3), or life-threatening (grade 4) according to the Common Terminology Criteria for Adverse Events (CTCAE) (26). With respect to hyperglycemia-related adverse events, grade 1 was defined as a fasting glucose value of >6.1–8.9 mmol/l, grade 2 >8.9 – 13.9 mmol/l, grade 3 >13.9–27.8 mmol/l or requiring hospitalization, grade 4 >7.8 mmol/l.

Outcomes

The primary efficacy endpoint was descriptive in nature and was defined as the percentage of responders at 24 weeks in the intention-to-treat population in each treatment arm (PAS-LAR monotherapy, LA-SSA/PEGV combination treatment), with an exact 95% confidence interval (CI). Response was defined, similar to the inclusion criteria, as IGF-I ≤1.2 x ULN. For missing data imputation based on last observation carried forward (LOCF) was used. Secondary descriptive endpoints for efficacy included the cumulative PEGV dose reduction at 24 weeks compared with baseline. Secondary endpoints for safety included the incidence of adverse events, incidence of hyperglycemia and diabetes, vital signs, clinical examination, electrocardiogram, standard hematology, kidney function, endocrine function tests, serum concentrations of (ALT), aspartate aminotransferase (AST), alkaline phosphatase, glutamyl transpeptidase, and total bilirubin.

Changes in diabetic category during the study were determined using the following American Diabetes Association (ADA) criteria (27):

- Normal glucose tolerance: fasting plasma glucose < 5.7 mmol/l and/or HbA1c < 5.7%.
- Prediabetes: fasting plasma glucose ≥ 5.7 < 7 mmol/l and/or HbA1c ≥ 5.7 < 6.5%.
- Diabetes: patients taking antidiabetic medication, or having fasting plasma glucose ≥ 7.0 mmol/l, or HbA1c ≥ 6.5%.

Statistics

The power calculation for the primary endpoint was based on a prospective randomized double blind phase III study of Colao *et al* (19). In this study, medically naïve or post-surgery patients with acromegaly were randomized to PAS-LAR 40 mg or octreotide LAR 20 mg every 28 days. The results revealed that after one year 38.8% of patients on PAS-LAR achieved normal IGF-I levels compared to 23.6% on octreotide LAR. In this head-to-head superiority study, PAS-LAR showed an overall response rate of about 60% more in the pasireotide group (19). Therefore, we estimated that PAS-LAR had 40% higher efficacy than first-generation LA-SSAs. The 95% CI was calculated using a percentage ranging from 25% to 40%. Statistical analyses were performed using SPSS software (version 24 for Windows; SPSS Inc., Chicago, Illinois) and GraphPad Prism® Version 6.04 (GraphPad Software, San Diego, USA). Categorical data were represented as observed frequencies and percentages. Continuous data were represented as mean and 95% confidence interval (CI) or median and range. The Kolmogorov-Smirnov and the Shapiro-Wilk test were used to test normality of variables. If assumption of normality was met, the paired t-test was used. For non-normally distributed variables the Wilcoxon signed-rank test was used. We considered *P*-values of <0.05 to be statistically significant.

RESULTS

Between August 24, 2015 and July 26, 2016, 100 acromegaly patients were screened and 61 patients were enrolled in the PAPE study (figure 1 appendix page 74). These patients comprised the intention-to-treat (ITT) set. Table 1 shows the patients' baseline clinical and biochemical characteristics. The mean age was 53 years, the median time since diagnosis was 8.9 years, and the median time since start of combination treatment was 6.1 years. 44.2% of patients had previously received surgery and 6 patients (9.8%) had previously received radiotherapy after surgery. The prevalence of diabetes was 32.8% at baseline and 20% of these patients were managed with oral antidiabetic therapy, while 3 patients were on insulin therapy.

Table 1. Baseline patient characteristics

Characteristics	Patients (n = 61)
Age (median, range)	53 (26 – 80)
Female patients	29 (47.5%)
Time since diagnosis (mean, 95% CI)	9.7 (8.0 – 11.4)
Time since LA-SSA/PEGV treatment (mean, 95% CI)	6.2 (5.3– 7.1)
Previous surgery (%)	27 (44.2%)
Time since surgery (mean, 95% CI)	12.0 (9.0 – 15.1)
Previous surgery and radiotherapy (%)	6 (9.8%)
Time since radiotherapy (mean, 95% CI)	20.3 (10.5 – 30.1)
Prior acromegaly medication	
Lanreotide Autogel	35 (57.4%)
Octreotide LAR	26 (42.6%)
Cabergoline	2 (3.3%)
Pegvisomant dose (mg/week, mean, 95% CI)	134 (103–166)
IGF-I (x ULN, mean, 95% CI)	0.97 (0.91 – 1.02)
Presence of diabetes	20 (32.8%)
Oral antidiabetic therapy	12 (19.7%)
Insulin therapy	3 (4.9%)

Efficacy

In figure 2A IGF-I levels and the PEGV doses are shown. At baseline, the mean IGF-I was 0.97 x ULN (CI; 0.91 – 1.02) with a mean PEGV dose of 134 mg/week (CI; 103 – 166). At 12 weeks, as by protocol, the mean PEGV dose was decreased to 61 mg/week (CI; 44 – 78), and IGF-I levels increased to 1.59 x ULN (CI; 1.45 – 1.73). In 15 (24.6%) patients IGF-I levels remained within the reference range, so they were switched to PAS-LAR 60 mg monthly as monotherapy, while the remaining 46 (75.4%) of patients with elevated IGF-I levels continued with their reduced dose of PEGV treatment but

now in combination with PAS-LAR 60 mg monthly. After 24 weeks from baseline, IGF-I levels dropped back to within the normal range in 45 (73.8%) patients, (mean IGF-I of 1.04 x ULN (CI; 0.91 – 1.17)). Stratified by treatment, IGF-I levels within the reference range could be induced in 14/15 (93.3%) patients in the PAS-LAR monotherapy group, and in 31/46 (67.4%) of patients in the PAS-LAR /PEGV combination group. After 24 weeks from baseline, the mean PEGV dose could be further decreased to 48 mg/week (CI; 21–74), while PEGV could be discontinued in 67.8% of patients. The cumulative reduction in PEGV dose was 66.1% in all patients at 24 weeks compared to baseline.

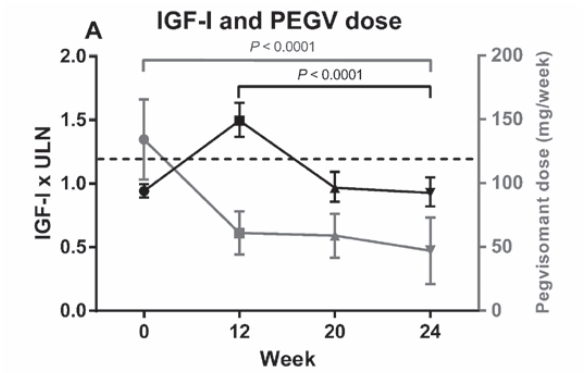

Figure 2A. IGF-I serum levels and the weekly pegvisomant (PEGV) dose are expressed as mean (95% CI). Dotted straight black line reflects the 1.2 x ULN cut-off. The first 12 weeks patients continued with first generation LA-SSAs treatment, and the PEGV dose was reduced by 50%. From week 12 until week 24 patients received in total 3 injections of pasireotide LAR 60 mg in combination with or without PEGV.

GH levels decreased significantly during the study (figure 2B). At baseline, the mean GH level was 9.3 µg/l (CI; 4.3 – 13.9), and significantly decreased to 7.4 µg/l

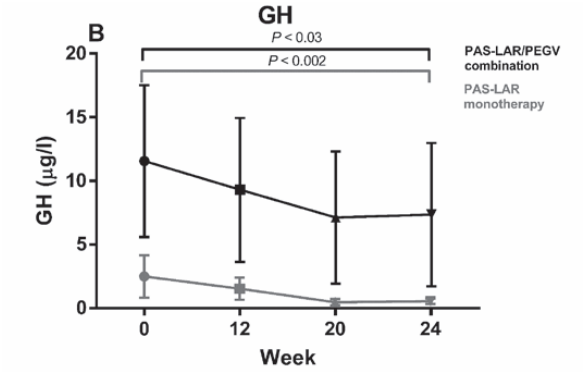

Figure 2B. Growth hormone (GH) levels are expressed as mean (95% CI), and are stratified to patients using pasireotide LAR monotherapy (grey line) and patients using pasireotide LAR and pegvisomant combination treatment (black line).

(CI; 3.1 – 11.7; *P* < 0.0001) after 12 weeks, whereas IGF-I levels increased significantly in the same time period. After 24 weeks, after subsequent treatment with PAS-LAR, mean GH levels further decreased significantly to 5.5 µg/l (CI; 1.3 – 9.6; *P* < 0.0001). Stratified to treatment group; in the PAS-LAR monotherapy group mean GH levels decreased from 2.5 µg/l (baseline) (CI; 0.9 – 4.2) to 0.6 µg/l (24 weeks) (CI; 0.3 – 0.8). In the PAS-LAR/PEGV combination group, mean GH levels decreased from 11.5 µg/l (baseline) (CI; 5.6 – 17.5) to 7.3 µg/l (24 weeks) (CI; 1.7 – 3.0).

In table 1 of supplementary data the differences are shown between patients who did achieve and who did not achieve IGF-I normalization at 24 weeks. Patients who were biochemically controlled at 24 weeks were using a significantly lower baseline PEGV dose, and had a lower baseline bodyweight. Between biochemically controlled and non-controlled patients, we found no difference in age, sex, BMI, HbA1c, and presence of diabetes. After correction for age and baseline bodyweight, the baseline PEGV dose was the most important predictor for IGF-I levels at 24 weeks with an Odds Ratio of 0.986 (CI; 0.976 – 0.996) (table 2 supplementary data). In clinical terms, a lower weekly PEGV dose at baseline increased the chance of reaching normal IGF-I levels within the reference range after 24 weeks (for each 10 mg PEGV reduction, the increase of IGF-I levels within the reference range was 14.0%).

Hyperglycemia and diabetes

The most common recorded adverse event (AE) during the study was hyperglycemia (table 2). We noted a total of 54 (88.5%) AEs were hyperglycemia-related, of which 40 (65.6%) were grade 1 and 2. Grade 3 and 4 hyperglycemias were reported in 14 (23.0%) patients, and were all related to PAS-LAR treatment.

Table 2. Adverse events regardless of study-drug relationship (> 5%) first 24 weeks

Adverse events	All grades	Grades 3/4
Hyperglycemia	54 (88.5%)	14 (23.0%)
New-onset diabetes	22 (36.1%)	0
Diarrhoea	18 (29.5%)	0
Myalgia	16 (26.2%)	0
Fatigue	13 (21.3%)	0
Headache	10 (16.4%)	0
Arthralgia	10 (16.4%)	0
Dizziness	10 (16.4%)	0
Nausea	6 (9.8%)	0

Data are expressed as mean (95% confidence interval), median (interquartile range), or n (%).

Mean fasting plasma glucose and HbA1c concentrations rose slightly between baseline and 12 weeks, but increased significantly after start of PAS-LAR treatment (figure 3).

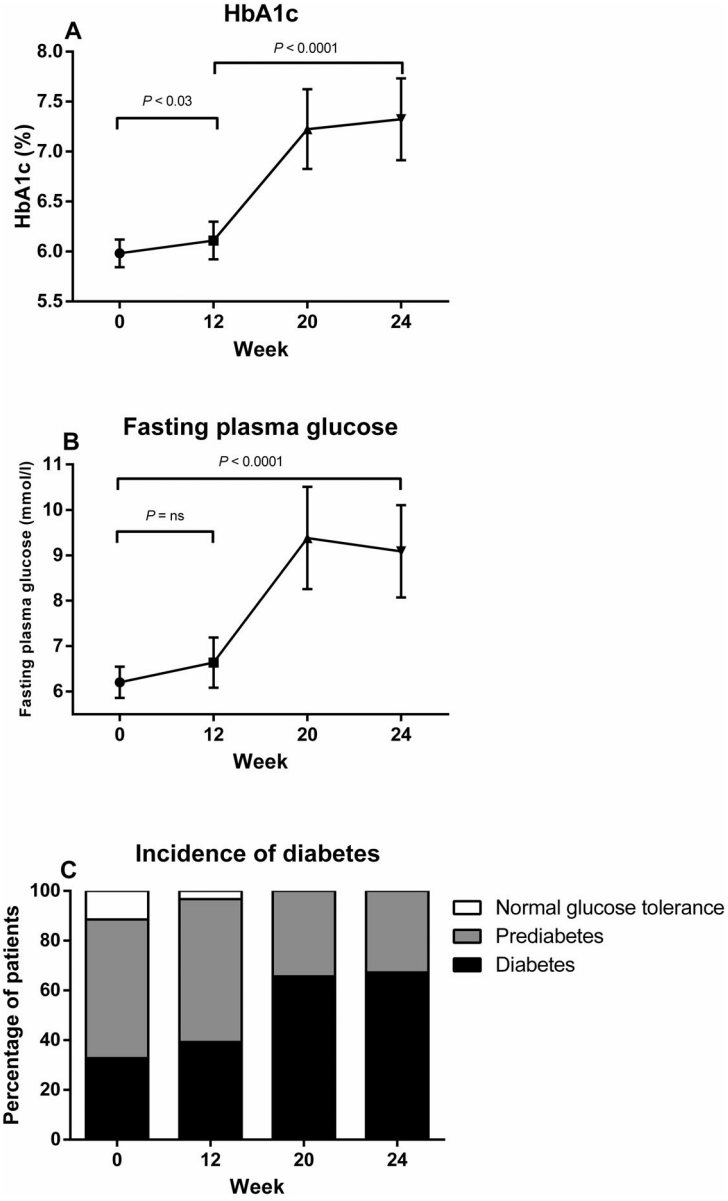

Figure 3. A. HbA1c concentrations are expressed as mean (95% CI). **B.** Fasting plasma glucose levels are expressed as mean (95% CI). **C.** Incidence normal glucose tolerance, prediabetes and diabetes over time according to the American Diabetes Association (ADA) criteria.

Between 12 and 24 weeks, mean fasting plasma glucose rose from 6.1 mmol/l (95% CI 5.9 – 6.3) to 9.1 mmol/l (95% CI 8.1 – 10.1) ($P < 0.0001$), and HbA1c from 6.1% (95% CI 5.9 – 6.3) to 7.3% (95% CI 6.9 – 7.7) ($P < 0.0001$). While 20 (32.8%) patients had diabetes at baseline, this number doubled to 42 (68.9%) patients at 24 weeks.

At 24 weeks, diabetic patients had a significantly higher baseline HbA1c compared to non-diabetic patients (table 3 supplementary data). Diabetic patients were insignificantly slightly older. Between diabetic and non-diabetic patients, we found no differences in sex, PEGV dose at baseline, IGF-I at 12 weeks, weight, or BMI. After correction for fasting plasma glucose levels and age, baseline HbA1c was the most important predictor for development of diabetes at 24 weeks. Logistic regression analysis of HbA1c tertiles revealed that patients in the second and third tertiles had increased odds (OR 5.5 and OR 55.0, respectively) of development of diabetes at 24 weeks compared with patients in the lowest HbA1c tertile (table 4 and 5 supplementary data).

After 24 weeks, nearly a third (32.9%) of patients required no change or initiation of antidiabetic medication, while the majority of patients required a combination of metformin and a DPP4 inhibitor. Seven patients required at least one antidiabetic drug. Three patients had to start insulin treatment, of whom two patients developed serious AEs involving severe hyperglycemia after the first PAS-LAR injection. These patients had pre-existing diabetes which was managed with either maximum oral antidiabetic drugs, a GLP-I analogue, or insulin treatment. After initiation of insulin treatment, PAS-LAR treatment was discontinued and they were all switched back to their previous LA-SSA and PEGV treatment, after which their diabetes recovered completely (supplementary data).

Table 3. Antidiabetic medications initiated after pasireotide LAR initiation.

Antidiabetic medication	Baseline	24 weeks
None	46 (75.4%)	19 (31.1%)
Metformin	15 (24.6%)	19 (31.1%)
DPP4 inhibitors	0	14 (23.0%)
Sulfonylureas	3 (4.9%)	12 (19.7%)
Insulin	3 (4.9%)	6 (9.8%)
GLP-I analogues	2 (3.3%)	3 (4.9%)
Thiazolidinediones	1 (1.6%)	1 (1.6%)

Patients may have taken more than one antidiabetic medication.

Non-diabetes related adverse events

In the run-in phase during reduction of the PEGV dose, (28 of 61) 46% of patients developed symptoms of active acromegaly such as fatigue, increased sweating, arthralgia, and headache. We observed no transient elevated transaminases during the study. Two thirds (12 of 18) of patients with diarrhea experienced symptoms of steatorrhoea. These symptoms were generally mild and transient. Although no diagnostic evaluation was performed to confirm exocrine pancreatic insufficiency, patients generally experienced symptomatic improvement with temporary pancreatic enzyme supplementation.

DISCUSSION

The PAPE study is the first study to assess the efficacy and safety of PAS-LAR in acromegaly patients previously using combination treatment of first generation LA-SSAs and PEGV. Our results show that 12 weeks of PAS-LAR treatment results in a 66.1% reduction in PEGV dose, while 73.8% of the patients attain IGF-I within the reference range (≤1.2 ULN). Moreover, 67.8% of patients could discontinue PEGV at 24 weeks. These findings indicate that PAS-LAR has an important PEGV sparing effect. However, this came at the expense of a higher frequency of diabetes which doubled from 32.8% at baseline to 68.9% after 24 weeks treatment.

The patients who had normal IGF-I levels at 12 weeks (PAS-LAR monotherapy group) had a significantly lower baseline PEGV dose than those with elevated IGF-I levels at 12 weeks (combination treatment group) 64 mg/week (CI; 50–78) versus 157 mg/week (CI; 118–197) (P = 0.008). Furthermore, patients in the PAS-LAR group also had significantly lower baseline IGF-I levels than those in the combination treatment group; 0.83 x ULN (CI; 0.71 – 0.95) versus 1.01 x ULN (CI; 0.96 – 1.07) (P = 0.002). The observed trend of a higher frequency of patients after radiotherapy in the PAS-LAR monotherapy group (20.0%) compared to the combination treatment group (6.5%) was not statistically significant (P = 0.15). We found no differences in the frequency of previous surgery between both groups. Apparently, patients in the PAS-LAR group have a lower disease activity at baseline, which is reflected by their lower baseline PEGV dose, IGF-I level, and GH level, or that they are more sensitive to LA-SSA, possibly due to a higher SST_2 expression.

An additional interesting observation is the rapid decrease in IGF-I levels after the first injection of PAS-LAR. This observation could be related to a decrease in insulin secretion, which reduces IGF-I production (28). After two PAS-LAR 60 mg injections,

mean IGF-I levels decreased from 1.44 x ULN (CI; 1.44 – 1.73) to 1.08 x ULN (CI; 0.95 – 1.22). We cannot rule out a carry-over effect of LA-SSAs, but these rapid reductions are usually not observed during treatment with first generation LA-SSA.

After the reduction in the PEGV dose during the first 12 weeks, mean GH levels decreased, while IGF-I levels increased. This finding is compatible with reports of increased GH levels during PEGV treatment (6). At 24 weeks, GH levels were further significantly decreased in all subjects, both in the PAS-LAR monotherapy as in the PAS-LAR and PEGV group. Probably, the lower GH levels can be explained by the discontinuation of, or further dose reduction in PEGV, plus the additional suppressive effect of PAS-LAR on GH secretion.

In our study the frequency of hyperglycemia-related adverse events was higher (88.5%) than in previous studies (19, 20, 29-31), but the increase in frequency of diabetes (32.9% at baseline to 68.9% at 24 weeks) is in line with the PAOLA study (22% to 70%) (20). The higher frequency of hyperglycemia related adverse events could be explained by the inclusion of older subjects while we included more patients with pre-existing diabetes at baseline compared to previous studies. We used an HbA1c cut-off of <9.0% as an inclusion criterion, whereas previous studies used a cut-off of <8.0%. The reduction of the PEGV dose during the first 12 weeks worsened glucose metabolism, due to an increase in GH actions.

The degree of hyperglycemia after PAS-LAR treatment is dependent on the degree of glycemic control at baseline. The patients who required insulin treatment are the patients that developed rapid hyperglycemia after the first injection of PAS-LAR. The non-insulin group had a slower onset of hyperglycemia and the diabetes they developed was less severe. In general, their diabetes could be managed after the third injection with oral medication.

We observed an increased frequency of steatorrhoea after initiating PAS-LAR treatment which we ascribed to exocrine pancreatic insufficiency. This is a rare AE during treatment with LA-SSA (32). Because patients in our study had already been exposed for more than 6 months to LA-SSAs, we did not expect an increase in the incidence of steatorrhea. Although we did not formally assess exocrine insufficiency, the exogenous addition of exocrine pancreatic enzymes improved the symptoms and after a few months most patients could stop this replacement therapy.

Several limitations of our study warrant consideration. Firstly, the efficacy of PAS-LAR we observed at 24 weeks (ie. after 12-weeks of PAS-LAR treatment) could be

influenced by a carry-over effect of octreotide LAR or lanreotide ATG following their discontinuation at 12 weeks. After withdrawal of LA-SSA, patients may remain in biochemical remission for up to four months, and this appeared to be dependent on the IGF-I level at time of discontinuation (27, 33). The efficacy of 73% in our study is higher than reported previously (19, 20). This may be explained by the carry-over effect of first-generation LA-SSAs in our study, and the fact that patients in our study were controlled before study entry. Secondly, the diagnosis of diabetes mellitus was based on the ADA criteria (27). Although healthy volunteer data have recommended incretin-based therapies for management of PAS-LAR-induced hyperglycemia, in the Netherlands these drugs are only reimbursed if patients fail on maximum doses of metformin, sulfonylureas, and a BMI of ≥ 35 kg/m^2 (23). The patients who developed severe hyperglycemia after the first injection had pre-existing diabetes and they all required swift interventions with insulin treatment. Therefore, intensive blood glucose monitoring is required after the start of treatment and antidiabetic treatment should be promptly initiated, preferably DPP4 inhibitors and GLP-I analogues, because treatment with metformin and sulfonylureas appears to be not very effective. Baseline HbA1c levels are the most important predictors for development of diabetes during the study. GH levels significantly decreased during the study. This is partly caused by the discontinuation and reduction in dose of PEGV, but mainly by treatment with PAS-LAR.

Our data indicate that PEGV treatment can neither compensate nor offset PAS-LAR-induced hyperglycemia. Probably, because PEGV improves insulin sensitivity and the hyperglycemic effect of PAS-LAR is independent of insulin sensitivity and is considered to be more an insulin and incretin suppressive effect (12-15, 23). Additionally, reducing the PEGV dose that causes active acromegaly may lead to more insulin resistance, while treatment with PAS-LAR leads to a further deterioration of glycemic control, especially in patients with (pre)diabetes.

Although the efficacy of pasireotide LAR, with or without PEGV, is high, caution should be paid to the long-term sequelae of the pasireotide-induced diabetes. The PEGV sparing effect of PAS-LAR and thereby proposed cost reductions will very likely be consumed by the diabetes mellitus related medication and healthcare costs. It is unknown whether patients that initially do not develop pasireotide-induced hyperglycemia, have a higher lifetime risk of developing diabetes and could create additional costs. Therefore, more long-term follow-up studies are needed.

In conclusion, pasireotide LAR is a viable treatment option in patients previously controlled with combination treatment of first-generation LA-SSA and PEGV.

Switching to pasireotide LAR enables a reduction in the necessary PEGV dose and/or in a lower number of injections. However, switching to PAS-LAR coincides with a higher frequency of diabetes.

Author contributions

AM, AVDL, and SN conceived and designed the study. AM was responsible for data management and statistical analyses after discussion with all authors. All authors participated in data interpretation and in writing of the report.

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Appendix

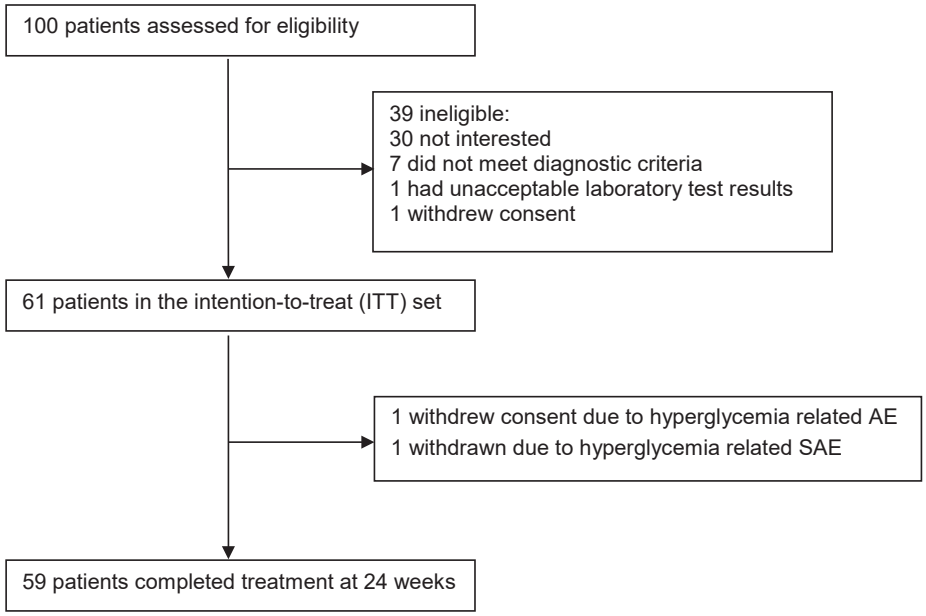

Figure 1. Trial profile.
AE = Adverse Event, SAE = Serious Adverse Events

Serious adverse events

The first patient was a 68-years old male diagnosed with acromegaly in 1995, for which has underwent transnasal adenectomy. His acromegaly was controlled with octreotide LAR 30 mg and PEGV 80 mg/week, and his diabetes was controlled with maximum doses of metformin and gliclazide. After the first injection he developed severe hyperglycemia with glucose levels of 33 mmol/l and his HbA1c rose from 6.0 to 9.8%. He was admitted for treatment with long-acting insulin. After the second pasireotide LAR injection he was switched back to his previous combination treatment, and his diabetes recovered completely within two months. The second patient was a 65-years old male diagnosed with acromegaly in 2005. His acromegaly was controlled with lanreotide 120 mg and PEGV 120 mg/week, and he his diabetes was managed with twice daily insulin treatment. After reduction of the PEGV dose his glycemic control worsened (HbA1c increased from 6.7 to 9.4%), and after the first injection he also developed severe hyperglycemia with glucose levels of 31 mmol/l and his HbA1c

increased further to 12.6%. He was admitted and he was switched to once daily long-acting and three times daily short-acting insulin treatment. After the fourth injection of pasireotide LAR he was switched back to his previous combination treatment, and his glycemic control improved. The third patient was a 54-years old male with acromegaly well controlled with lanreotide ATG and PEGV 100 mg/week. Although his diabetes was well controlled with metformin and weekly exenatide treatment. After the first injection he developed hyperglycemia (glucose increased from 6.9 to 23 mmol/l, and HbA1c from 6.3 to 10.6%), requiring insulin treatment. Although his IGF-I normalized from 2.19 x ULN at week 12 weeks to 0.84 x ULN at 20 weeks, and he could discontinue PEGV, he decided to withdraw from the study because he was declined to use daily insulin injections.

Binomial regression statistics

Binomial logistic regression analysis was carried out to identify independent predictors for normalization of IGF-I at 24 weeks, and the development of diabetes at 24 weeks. First, we assessed linearity of the continuous variables with respect to the logit of the dependent variable via the Box-Tidwell procedure. Secondly, we analysed the presence of multicollinearity using the Variance Inflation Factor (VIF) and tolerance values. If the assumption of linearity of the logit was met, and no significant multicollinearity (VIF <5 and tolerance >0.2) was present, we assessed the independent variables using univariate logistic regression analysis. Each independent variable was also analysed for interaction, and if the interaction term was significant (P <0.05), it was included in the final model. Only independent variables with P-values <0.20 were included in the final multivariate logistic regression model.

We first assessed the influence of age, sex, BMI, baseline PEGV dose, IGF-I, GH, previous surgery, radiotherapy and diabetes on the likelihood that participants normalised their IGF-I levels to within the reference range at 24 weeks. Univariate regression analysis revealed that of the nine included variables only baseline PEGV dose, bodyweight and age had a P-value < 0.20, and these three variables were included in the final multivariate logistic regression analysis (table 1 supplementary data). The model explained 38.5% (Nagelkerke R^2) of the variance in IGF-I levels.

Table 1. Differences between controlled and non-controlled patients at 24 weeks

	IGF-I normal at 24 weeks	IGF-I elevated at 24 weeks	*P*-values
N	45	16	
Age (years, mean 95% CI)	55.0 (11.8)	49.3 (11.2)	0.10
Females (n, %)	23 (51%)	6 (37.5%)	0.35
PEGV dose baseline (mg/week, mean 95% CI)	98 (80 – 116)	236 (137 – 335)	< 0.0005
Weight baseline (kg, mean 95% CI)	90.6 (85.6 – 95.7)	101.9 (92.4 – 111.4)	0.022
BMI baseline (kg/m^2, mean 95% CI)	28.7 (27.5 – 29.9)	31.1 (28.2 – 34.0)	0.27
Presence of diabetes at 24 weeks (n, %)	27 (62.8%)	8 (50.0%)	0.37
HbA1c baseline (%, mean 95% CI)	6.0 (5.6 – 6.4)	6.0 (5.8 – 6.1)	0.96

IGF-I normal defined as IGF-I ≤ 1.2 x ULN. IGF-I elevated defined as IGF-I > 1.2 x ULN. Data are expressed as mean (95% confidence interval), median (interquartile range), or n (%).

We analyzed the impact of baseline HbA1c, fasting plasma glucose levels, age, sex, BMI, baseline PEGV dose, IGF-I, GH, previous surgery, and radiotherapy on the likelihood that participants develop diabetes after 24 weeks. Univariate regression analysis revealed that of the 10 variables only baseline HbA1c, fasting plasma glucose and age had a *P*-value <0.20, and these variables were included in the final multivariate logistic regression model (table 2 supplementary data). This model explained 58.4% (Nagelkerke R^2) of the variance in diabetes at 24 weeks.

Table 2. Multivariate regression analysis of predictors associated with IGF1 normalization at 24 weeks (n = 61

Predictor	*B*	SE	Wald	*df*	*P*	OR	95% CI for OR	
							Lower	Upper
Baseline PEGV dose	−0.014	0.005	7.495	1	0.006	0.986	0.976	0.996
Baseline bodyweight	−0.026	0.20	1.587	1	0.208	0.975	0.937	1.014
Age	0.013	0.031	0.165	1	0.685	1.013	0.953	1.076
Constant	4.796	2.822	2.889	1	0.089	121.033		

Table 3. Differences between diabetic and non-diabetic patients at 24 weeks

	Diabetic patients at 24 weeks	Non-diabetic at 24 weeks	*P*-values
N	41	20	
Age (years, mean 95% CI)	55.5 (52.2 – 58.8)	49.3 (43.0 – 55.6)	0.053
Females (n, %)	17 (41.5%)	12 (60.0%)	0.17
HbA1c baseline (%, mean 95% CI)	6.2 (6.0 – 6.4)	5.6 (5.5 – 5.7)	< 0.0001
PEGV dose baseline (mg/week, mean 95% CI)	137 (90–176)	133 (94–179)	0.45
Weight baseline (kg, mean 95% CI)	94.0 (88.2 – 99.8)	92.5 (85.8 – 99.3)	0.76
BMI baseline (kg/m^2, mean 95% CI)	29.4 (28.0 – 30.8)	29.3 (27.3 – 31.3)	0.92

Data are expressed as mean (95% confidence interval), median (interquartile range), or n (%).

Table 4. Multivariate regression analysis of predictors associated with development of diabetes at 24 weeks (n = 61).

Predictor	*B*	SE	Wald	*df*	*P*	OR	95% CI for OR	
							Lower	Upper
Baseline HbA1c	6.125	1.731	12.528	1	0.001	457.60	13.374	15657.722
Baseline glucose	1.254	0.779	2.589	1	0.108	3.504	0.761	16.136
Age	−0.11	0.036	0.091	1	0.763	0.989	0.922	1.061
Constant	−41.480	11.985	11.979	1	0.001	0.000		

Table 5. Logistic regression analysis of HbA1c tertiles associated with development of diabetes at 24 weeks (n = 61). Tertile 1 = HbA1c lowest to 5.7% Tertile 2 = HbA1c 5.7% – 5.9% Tertile 3 = HbA1c 5.9% and higher.

Predictor	*B*	SE	Wald	*df*	*P*	OR	95% CI for OR	
							Lower	Upper
Tertile 1 (reference)			16.780	2	< 0.0005			
Tertile 2	1,705	0.838	4.140	1	0.042	5.5	1.065	28.416
Tertile 3	4.007	0.979	16.770	1	<0.00005	55.0	8.080	374.380
Constant	−1.299	0.651	3.979	1	0.046	0.273		

Chapter 4

Efficacy and safety of switching to pasireotide in acromegaly patients controlled with pegvisomant and somatostatin analogues: PAPE extension study.

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ABSTRACT

Objective: to assess the efficacy and safety after 48 weeks of treatment with pasireotide long-acting-release (PAS-LAR) alone or in combination with pegvisomant in patients with acromegaly. In addition, we assessed the relation between insulin secretion and pasireotide-induced hyperglycemia.

Design: The PAPE extension study is a prospective follow-up study until 48 weeks after the core study of 24 weeks.

Methods: 59 out of 61 patients entered the extension study. Efficacy was defined as the percentage of patients achieving IGF-I normalization (≤ 1.2 x the Upper Limit of Normal (ULN)) at 48-weeks through protocol-based adjustment of pegvisomant and PAS-LAR doses. At baseline, insulin secretion was assessed by an oral glucose tolerance test (OGTT).

Results: At the end of the study median IGF-I was 0.98 x ULN, and 77% of patients achieved normal IGF-I levels with a mean pegvisomant dose of 64 mg/week, and an overall cumulative pegvisomant dose reduction of 52%. Frequency of diabetes mellitus increased from 68% at 24 weeks to 77% at 48 weeks, and 9 patients discontinued PAS-LAR treatment, mainly because of severe hyperglycemia. Pasireotide-induced hyperglycemia was inversely correlated with baseline insulin secretion ($r = -0.37$, $P < 0.005$).

Conclusions: PAS-LAR normalizes IGF-I levels in most acromegaly patients, with a fifty percent pegvisomant-sparing effect. However, PAS-LAR treatment coincided with a high incidence of diabetes mellitus. The risk for developing diabetes during PAS-LAR treatment seems inversely related to insulin secretion at baseline.

INTRODUCTION

Acromegaly is a systemic condition most commonly caused by pituitary adenomas secreting excess growth hormone (GH) and insulin-like growth factor I (IGF-I) levels, leading to increased mortality and morbidity (1). The main goals of the present treatment for acromegaly are to normalize GH and IGF-I levels, reduce or control tumor size, and to improve quality of life (QoL) and multisystem comorbidities (2, 3).

First-generation long-acting somatostatin analogues (LA-SSAs) are considered the mainstay medical treatment of acromegaly. LA-SSAs suppress GH secretion by preferential binding to somatostatin receptor subtype 2a (SST_{2a}). LA-SSAs have favorable safety profiles and a clinically neutral impact on glucose homeostasis (4, 5).
In clinical practice only about 40% of patients treated with monotherapy LA-SSAs achieve biochemical normalization of GH and IGF-I. Therefore, most patients are refractory to treatment with LA-SSAs, and require additional therapies (6-8).

The competitive GH receptor antagonist pegvisomant (PEGV) is currently the most effective treatment to normalize circulating IGF-I levels in acromegaly, as monotherapy or in combination with LA-SSA (9-14). PEGV has as advantage that it improves insulin sensitivity (15-18). In combination with LA-SSA a lower necessary PEGV dose is required to normalize IGF-I levels in acromegaly than compared with PEGV monotherapy (19, 20).

Pasireotide long-acting release (PAS-LAR) is a second-generation multi-receptor somatostatin analogue designed with a broader binding somatostatin receptor profile than first-generation LA-SSAs (21). PAS-LAR has been shown to provide superior clinical efficacy over LA-SSA in treatment-naïve acromegaly patients and in patients inadequately controlled with LA-SSAs (22, 23). PAS-LAR has a similar safety profile to LA-SSAs, with the exception of a higher incidence of hyperglycemia, and this incidence has been reported to occur in about 60-88% of patients during treatment with PAS-LAR (22-24). Recently, we have reported the efficacy and safety of PAS-LAR alone or in combination with PEGV in acromegaly patients up to 24 weeks treatment (PAPE core study) (24). Switching to PAS-LAR treatment resulted in a 66% reduction in PEGV dose, but was simultaneously associated with a higher incidence of diabetes mellitus. Here, we present the long-term 48 weeks results of the efficacy and safety of PAS-LAR alone or in combination with PEGV treatment in acromegaly.

SUBJECTS AND METHODS

Study design

The PAPE study is a prospective, open-label, single-centre study in acromegaly patients, designed to assess the efficacy and safety of PAS-LAR alone, or in combination with PEGV (24) (ClinicalTrials.gov, number NCT02668172). The primary endpoint was to assess efficacy at 24 weeks. Here, we report the secondary endpoints: efficacy and safety from 24 up to 48 weeks. During this follow-up, the goal was to achieve IGF-I normalization (IGF-I ≤ 1.2 x ULN) through protocol-based dose titration of PEGV and PAS-LAR (Figure 1). In the core study two groups of patients continued in the extension phase: the PAS-LAR 60 mg monotherapy group and the PAS-LAR/PEGV combination group (24).

Patients in the PAS-LAR 60 mg monotherapy group continued with the 60 mg dose until 48 weeks, unless their IGF-I levels decreased below the median sex- and age adjusted IGF-I normal limits. In that case, the PAS-LAR dose was decreased to 40 mg every 4 weeks. Subsequently, if after at least two PAS-LAR 40 mg injections, IGF-I levels remained suppressed (below the median IGF-I), the PAS-LAR dose was further

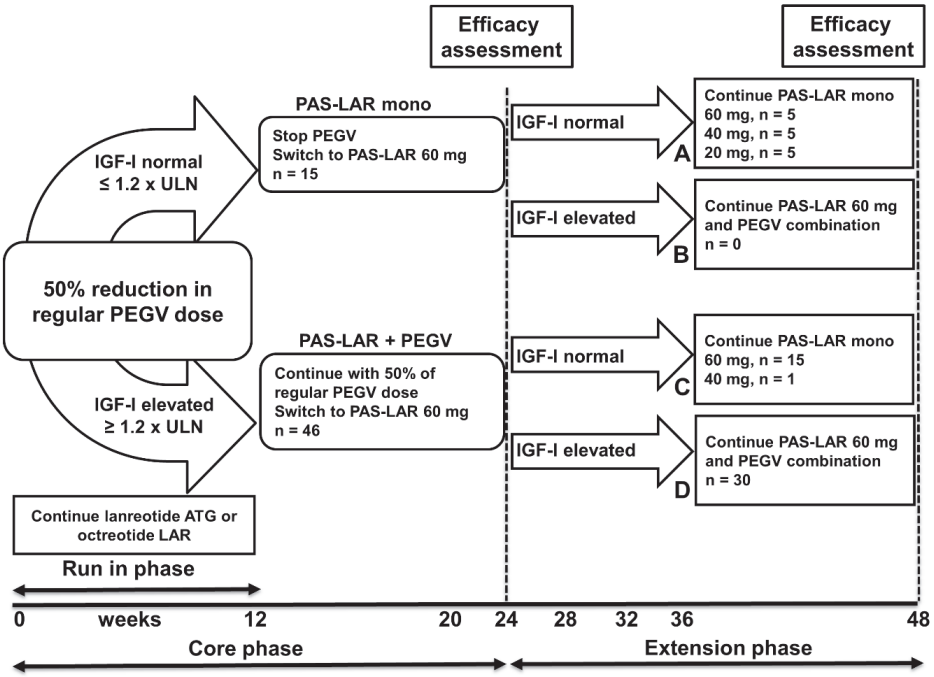

Figure 1. PAPE study design. The number of participants in each study arm were based on the intention-to-treat population (n = 61).

decreased to 20 mg (ARM A). However, if IGF-I became elevated (≥ 1.2 x ULN) during PAS-LAR monotherapy, PEGV treatment was restarted in the same dose of the run-in phase (ARM B).

Patients in the PAS-LAR/PEGV combination group who achieved IGF-I normalization at 24 weeks using PEGV doses ≤ 80 mg/week, were after 24 weeks instructed to discontinue PEGV treatment and to continue with PAS-LAR 60 mg monotherapy during follow-up (ARM C) (Figure 1). In patients using PEGV doses higher than 80 mg/week, the dose was reduced by 50% every 4 weeks until their IGF-I was normalized (≤ 1.2 x ULN). If IGF-I levels became elevated above 1.2 x ULN during combination treatment, the PEGV dose was further increased every 4 weeks depending on the measured IGF-I levels after adjusting treatment (ARM D):

- IGF-I between 1.2–1.5× ULN, the PEGV dose was increased by 20%.
- IGF-I between 1.5–1.7× ULN, the PEGV dose was increased by 30%.
- IGF-I between 1.7–2.0× ULN, the PEGV dose was increased by 40%.
- IGF-I ≥ 2.0 x ULN, the PEGV dose was increased by 50%.

If monitoring revealed glucose levels in the diabetic range according to the American Diabetes Association (ADA) criteria (25), metformin was initiated as first-line treatment option. If glycemic control was not achieved after metformin treatment, a DPP-4 inhibitor was added as second choice. When patients still did not achieve normoglycemia they were switched to a treatment with sulfonylureas or GLP-1 receptor analogues. Finally, insulin was started in patients intolerant to GLP-I analogues or when glycemic control was not achievable with GLP-1 analogues. All patients received intensive blood glucose monitoring after start of treatment. Patients who developed rapid hyperglycemia received insulin treatment. The PAPE study was approved by the medical ethics committee of the Erasmus University Medical Centre, and all patients provided written informed consent.

Patients

After 24 weeks, 59 out of 61 patients entered the extension phase (supplemental figure 1). The in- and exclusion criteria have been reported previously (24). Briefly, key inclusion criteria were patients with good metabolic control of acromegaly (IGF-I ≤ 1.2 x ULN) for at least 6 months combination treatment of weekly PEGV and maximum doses of first-generation LA-SSAs. Key exclusion criteria were pituitary surgery or radiotherapy within the 6 months prior to study entry and poorly controlled diabetes mellitus, defined as HbA1c ≥ 9.0%.

Study assessments

Laboratory measurements were described previously in the core study (24). Safety assessments included: assessment of heart function by electrocardiogram at baseline, 24 weeks, and 48 weeks. The severity of adverse events (AEs) was evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) (26). At baseline after an overnight fast, a standardized oral glucose tolerance test (OGTT) was performed with 1.75 g glucose per kg body weight (maximum 75 g while simultaneously glucose and insulin levels were measured at t = −15 minutes, t = 0 min., t = 30 min., t = 60 min., t = 90 min., and t = 120 min. (27). We used published indexes of ß-cell function to estimate the first phase and second phase insulin secretion during the OGTT (28).

Outcomes

The secondary efficacy endpoints were descriptive in nature and were based on the intention-to-treat population. For patients who discontinued the study before 48 weeks imputation based on the principle of last observation carried forward (LOCF) was used. Main efficacy endpoint was defined as the percentage of responders at 48 weeks in the intention-to-treat population, and in each treatment arm (PAS-LAR monotherapy and PAS-LAR/PEGV combination treatment), with an exact 95% confidence interval (95% CI). Response was defined as IGF-I ≤ 1.2 x ULN. Other descriptive endpoints for efficacy included the percentage of patients who could stop PEGV treatment after 48 weeks, and the percentage cumulative PEGV dose reduction, which was calculated as the sum of all administered PEGV doses of all patients at 48 weeks compared with baseline. As reported previously (24), secondary endpoints for safety included the incidence of hyperglycemia and diabetes mellitus, vital signs, and electrocardiogram.

Statistical analyses were performed using SPSS software (version 24 for Windows; SPSS Inc., Chicago, Illinois) and GraphPad Prism® Version 6.04 (GraphPad Software, San Diego, USA). Categorical data were represented as observed frequencies and percentages. Continuous data were represented as mean and 95% CI or median and range. The Kolmogorov-Smirnov and the Shapiro-Wilk test were used to test normality of variables. If assumption of normality was met, the paired t-test was used. For non-normally distributed variables the Wilcoxon signed-rank test was used. For data that did not meet the criteria for normality, logarithmic transformation was applied. Correlation analyses were performed using the Pearson correlation test. *P*-values of < 0.05 were considered statistically significant.

RESULTS

Efficacy

The baseline characteristics of the study population have been published before in the core study (24). The percentage of patients achieving IGF-I and GH normalization are shown in table 1.

Table 1. Percentage of patients achieving normal IGF-I and GH levels during the study

	Baseline	12 weeks	24 weeks	48 weeks
IGF-I ≤ 1.2 x ULN				
Overall	53/61 (86.9%)	15/61 (24.6%)	45/61 (73.8%)*	47/61 (77.0%)*
PAS-LAR monotherapy	14/15 (93.3%)	15/15 (100%)	14/15 (93.3%)	14/15 (93.3%)*
PAS-LAR/PEGV combination	39/46 (84.8%)	0/46 (0%)	31/46 (67.4%)	33/46 (71.7%)*
GH ≤ 2.5 µg/l				
Overall	23/61 (37.7%)	29/61 (47.5%)	41/61 (67.2%)*	41/61 (67.2%)*
PAS-LAR monotherapy	10/15 (66.7%)	11/15 (73.3%)	15/15 (100%)*	14/15 (93.3%)*

*Data based on last observation carried forward (LOCF).

At 48 weeks, 77.0% of patients had IGF-I levels within the reference range with a mean IGF-I of 0.98 x ULN (95% CI 0.90 – 1.06) (table 1 and figure 2A). Stratified by treatment group; 93.3% of patients using PAS-LAR monotherapy achieved IGF-I normalization at 24 weeks, which was sustained at 48 weeks. While 67.4% of patients using PAS-LAR and PEGV combination therapy achieved IGF-I normalization at 24 weeks, which increased to 71.7% at 48 weeks. Overall after 48 weeks, 12 of the 14 non-controlled patients had IGF-I levels between 1.2–1.4× ULN. During the extension phase the mean PEGV dose had to be increased from 47 mg/week (95% CI 21–73) to 64 mg/week (95% CI 33–95). At 48 weeks the cumulative reduction in PEGV dose decreased to 52.0% after 48 weeks compared with baseline, and 50.8% (31/61) of patients were off PEGV treatment at the end of study.

In figure 2B GH serum levels are shown stratified by treatment group. Patients in the PAS-LAR monotherapy group had significantly lower baseline GH levels (GH 2.5 µg/l (95% CI 0.8 – 4.2)) than patients in the PAS-LAR/PEGV combination group (GH 11.5 µg/l (95% CI 5.5 – 17.4)). Both groups showed a significant decrease in GH serum levels after initiation of PAS-LAR treatment, with subsequently stable suppressed GH levels in the PAS-LAR monotherapy group until 48 weeks.

The 15 patients in the PAS-LAR monotherapy group remained controlled throughout the study (Figure 1). In fact, in 10 patients (66.7%) a dose reduction was possible to PAS-LAR 40 mg and 20 mg (ARM A). No patients had to restart PEGV treatment

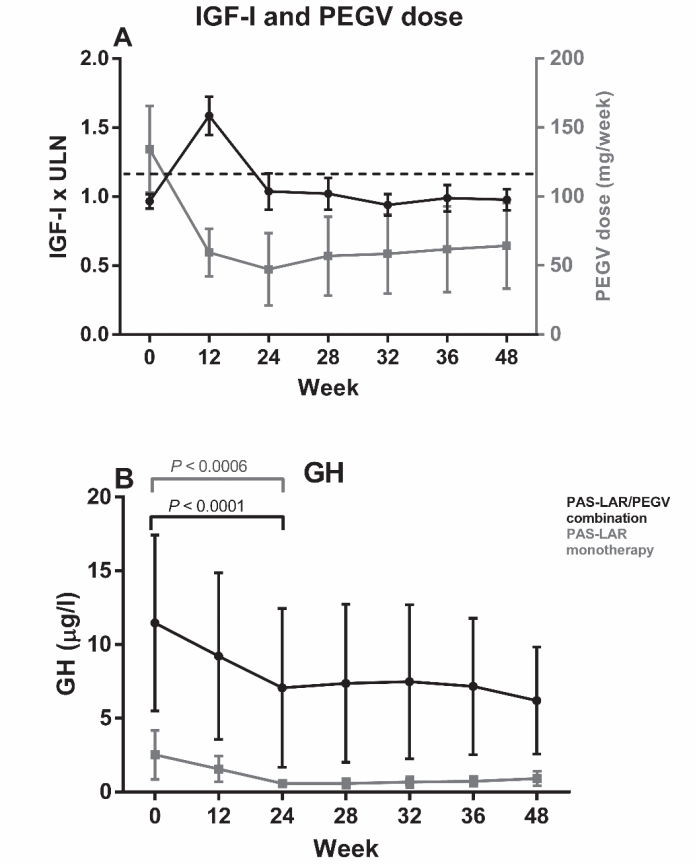

Figure 2. IGF-I serum levels and the weekly PEGV dose (2A). Dotted straight black line reflects the IGF-I 1.2 x ULN cut-off. GH serum levels (2B) are depicted in the PAS-LAR monotherapy group in grey and in the combination PAS-LAR/PEGV group in black. Data are expressed as mean (95% CI).

in this group (ARM B). In patients using PAS-LAR and PEGV combination therapy, 16 (34.8%) patients switched to PAS-LAR monotherapy during the extension phase, and in one patient a further dose reduction was possible to PAS-LAR 40 mg (ARM C). The remaining 30 patients required PAS-LAR and PEGV combination treatment, of which 13 patients (21.3%) achieved less than 25% PEGV dose reduction (ARM D). In 12 patients (19.7%) between 25 to 50% PEGV dose reduction was achieved, and in 5 patients (8.2%) a reduction of more than 50% could be attained.

Pasireotide LAR monotherapy

Among the 15 patients that were switched to PAS-LAR 60 mg monotherapy at 12 weeks, 10 patients had a progressive decline in IGF-I during follow-up, with levels dropping below the median IGF-I reference range. In these patients, the PAS-LAR dose

was therefore reduced to 40 mg every 4 weeks. Subsequently, in five of those patients the dose was further decreased to 20 mg. At baseline, these patients used a relatively low median PEGV dose of 60 mg/week. In one additional patient who could stop PEGV after 24 weeks, the PAS-LAR could be further reduced to 40 mg during the follow-up.

Partial and non-responders

We observed that 10 patients on combination treatment who achieved IGF-I normalization after 24 weeks and therefore could stop PEGV, had to restart PEGV treatment during follow-up because their IGF-I levels again increased above 1.2 x ULN (arm D). One patient required a higher dose of PEGV after 48 weeks (100 mg/week) than at baseline (80 mg/week). In three patients using very high doses of PEGV (at baseline (mean baseline PEGV dose 580 mg/week), we could not attain a significant PEGV dose reduction (mean PEGV dose was remained 560 mg/week after 48 weeks).

Safety

Hyperglycemia and diabetes mellitus

The most common AE related to PAS-LAR treatment was hyperglycemia, and mainly recorded during the core study (24). In total 60 out of 61 patients had a hyperglycemia-related AE, of which 47 (77.0%) were grade 1 and 2 (table 2). During the extension study grade 3 hyperglycemia was recorded in two patients, while no patients had a grade 4 hyperglycemia-related AE.

Table 2. Adverse events regardless of study-drug relationship (>5%) until 48 weeks

Adverse events	Grade 1/2	Grades 3/4
Hyperglycemia	48 (78.7%)	14 (23.0%)
Diarrhoea	21 (34.4%)	0
Fatigue	19 (31.1%)	0
Arthralgia	16 (26.2%)	0
Myalgia	16 (26.2%)	0
Headache	13 (21.3%)	0
Pain injection site	12 (19.7%)	0
Dizziness	11 (18.0%)	0
Hypoglycemia	9 (14.8%)	0
Arthralgia	16 (26.2%)	0
Nausea	7 (11.5%)	0
Alopecia	5 (8.2%)	0
Abdominal pain	4 (6.6%)	0

At 48 weeks, diabetes of most patients was managed with a combination of metformin and a DPP-4 inhibitor. 73.8% patients required at least one antidiabetic medication. Between 24 and 48 weeks, five patients developed mild diabetes which required additional treatment with metformin alone or combined with a DPP-4 inhibitor. Due to hypoglycemic symptoms related to sulfonylurea treatment, four patients were switched to a GLP-I analogue (supplementary table 1).

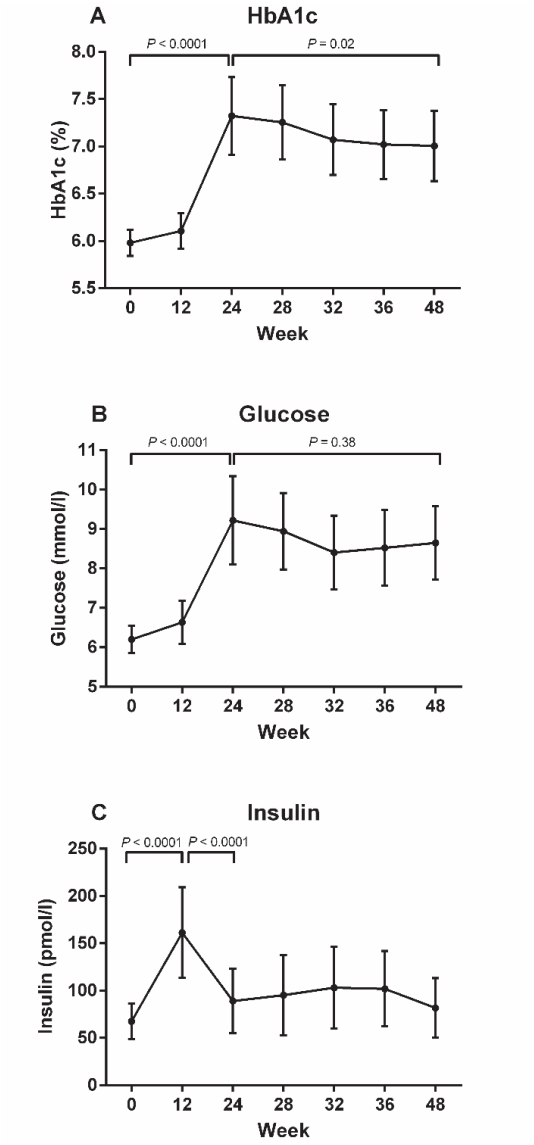

Figure 3. HbA1c (3A), fasting plasma glucose (3B), fasting plasma insulin (3C) concentrations are expressed as mean (95% CI).

During the extension phase the incidence of diabetes mellitus increased slightly from 68.9% to 77.0%. Mean HbA1c and fasting plasma glucose levels increased significantly after PAS-LAR treatment, but decreased slightly after initiation of antidiabetic treatment during follow-up (Figure 3A and 3B).

Mean HbA1c concentrations increased from 6.0% (5.8 – 6.1) at baseline to 7.3% (6.9 – 7.7) after 24 weeks, but decreased slightly to 7.0% (6.6 – 7.4) after 48 weeks. Stratified by treatment group; in the PAS-LAR monotherapy group, mean HbA1c increased from 6.0% (95% CI 5.5– 6.5%) at baseline to 7.1% (95% CI 6.3 – 7.9%) at 24 weeks, and decreased to 6.8% (95% CI 6.0 – 7.7%) at 48 weeks. In the combination treatment group, HbA1c levels showed a similar pattern, increasing from 6.0% (95% CI 5.9 – 6.1%) at baseline to 7.4% (95% CI 6.9 – 7.9%) at 24 weeks, and decreasing to 7.1% (95% CI 6.6 – 7.5%) after 48 weeks. No significant difference was observed in HbA1c levels between patients using PAS-LAR monotherapy and combination treatment at baseline (P = 0.36), 24 weeks (P = 0.72), and after 48 weeks (P = 0.26).

Fasting plasma glucose levels followed a similar profile with 6.2 mmol/l (5.8 – 6.5) at baseline to 9.2 mmol/l (8.1 – 10.3) at 24 weeks, and 8.7 mmol/l (95% CI 7.7 – 9.6) after 48 weeks.

Excluding patients that were receiving insulin therapy at baseline (n = 3), fasting insulin levels rose from 67.5 mmol/l (95% CI 48.8 – 86.2) at baseline to 161.4 mmol/l (95% CI 113.5 – 209.3) after 12 weeks. Insulin levels dropped significantly after initiation of PAS-LAR treatment. After 24 weeks, fasting insulin levels decreased significantly to 89.0 mmol/l (95% CI 54.9 – 123.1). We observed a significant inverse relationship between the insulin area under the curve (AUC) during OGTT at baseline and the increase in HbA1c levels between baseline and 24 weeks (r = −0.30, P = 0.03). The estimates for ß-cell function showed a stronger correlation with the increase in HbA1c; first phase insulin secretion (Stumvoll index, r = −0.37, P = 0.005) and second phase insulin secretion (r = −0.38, P = 0.004).

Non-hyperglycemia related adverse events

After hyperglycemia, diarrhea (34.4%), and fatigue (31.1%) were the most common AEs (table 2). Headache was reported by 13 (21.3%) patients and most frequently experienced in the first week after initiation of PAS-LAR treatment, but resolved later after consecutive injections. One patient was diagnosed with sick sinus syndrome during PAS-LAR treatment. After discontinuation of PAS-LAR this AE resolved and she was successfully switched to PEGV monotherapy. Slightly more patients developed diarrhea during the extension phase (n = 21) compared to core phase of the study (n = 18).

DISCUSSION

The results of this clinical study suggest that the efficacy of PAS-LAR was long-term sustained, as after 48 weeks, 77% of patients achieved IGF-I normalization while a cumulative PEGV dose reduction of 54.2% could be achieved, reconfirming the PEGV sparing effect of PAS-LAR. Hyperglycemia was the most important and most common adverse event during the follow-up until 48 weeks. The incidence of diabetes mellitus increased from 68.9% at 24 weeks to 77% at 48 weeks.

It is important to note that the extension phase of our study was not designed to detect a statistically significant difference between the treatment groups after 48 weeks, but it was undertaken to optimally titrate the PEGV and PAS-LAR dose to achieve control of IGF-I within the reference ranges. To achieve IGF-I normalization, we used a systematic PEGV and PAS-LAR dose titration protocol.

We observed that PAS-LAR elicited a wide range of clinical response in this study, ranging from patients that were very sensitive to very resistant. On one hand of the spectrum, patients could stop PEGV treatment and switch to a lower PAS-LAR dose of 40 mg and even 20 mg/month. This IGF-I over-suppression to below median range during PAS-LAR treatment has also recently been described in two patients with acromegaly that were uncontrolled with octreotide LAR 20-30 mg/month and, after crossover to PAS-LAR 40 mg/month, achieved suppressed IGF-I levels below the normal range (29). Conversely, on the other end of the spectrum, a number of patients achieved less than 25% PEGV dose reduction. This heterogeneous clinical response to PAS-LAR may be explained by the inclusion of a heterogeneous acromegaly population with a large variation in baseline PEGV dose.

It is unlikely that a carry-over effect of first-generation LA-SSAs had a large impact in our study on the ultimately achieved PEGV dose reduction after 48 weeks. Nevertheless, it is possible that the initial response in IGF-I normalization of the 10 patients who had to restart PEGV treatment during the extension phase was related to a disappearing carry-over effect of the first-generation LA-SSAs.

It is not clear why the three patients using the highest doses of PEGV could not reduce their PEGV dose after switching to PAS-LAR. Theoretically, one could hypothesize that patients using combination treatment of LA-SSA and high doses PEGV have relatively low SST_2 and high SST_5 expression compared to patients using LA-SSA monotherapy, and that therefore these patients would be good responders to PAS-LAR treatment. It remains to be determined whether SST_2 or SST_5 protein expression is responsible

for driving the response to PAS-LAR treatment in our study. Furthermore, it is still unknown how PEGV is metabolized in humans, and a wide inter-individual variation in PEGV serum levels have been observed when the same doses were administered (17, 30-32). PEGV serum levels are increased during combination treatment of LA-SSA and PEGV, but it is unknown whether this also occurs during combination treatment with PAS-LAR. Therefore, theoretically patients using high doses of PEGV may benefit less from switching to PAS-LAR.

The insulin-suppressive effect of PAS-LAR may be related to the degree of pasireotide-induced hyperglycemia, as insulin AUC and (more pronounced) baseline ß-cell function were inversely correlated with the observed increase in HbA1c between baseline and 24 weeks. These findings indicate that the lower the insulin secretion is at baseline, the greater will be the risk of pasireotide-induced hyperglycemia during follow-up, even in patients with well-controlled diabetes at baseline. Therefore, besides pre-treatment baseline glucose and HbA1c levels (33), pancreatic ß-cell function is probably an additional and independent risk factor of pasireotide-induced hyperglycemia. Patients using PAS-LAR and PEGV combination treatment did not have a lower HBA1c level than patients using PAS-LAR monotherapy. This observation suggests that the PEGV insulin sensitizing effect does not work in pasireotide-induced hyperglycemia (33).

Our results show that PAS-LAR treatment reduces IGF-I and GH levels after two injections which parallels the reduction in insulin levels which also occurs after two injections, suggesting that the early onset effect of PAS-LAR on IGF-I reduction may be (partly) mediated by suppressing insulin secretion. This is further supported by the observation that although the effects of PAS-LAR on GH suppression were superimposable compared with octreotide LAR, PAS-LAR treatment induced a greater suppression of IGF-I (22).

CONCLUSIONS

PAS-LAR monotherapy or in combination with PEGV normalizes IGF-I levels in most acromegaly patients despite an about fifty percent reduction in cumulative PEGV doses. However PAS-LAR therapy coincides with a high incidence of diabetes mellitus, and the risk for developing diabetes during PAS-LAR therapy seems inversely related to insulin secretion at baseline.

Declaration of interests

AM received a speaker fee from Novartis Pharma. AVDL is a consultant for Novartis Pharma, Pfizer International, and received grants from Novartis Pharma, Ipsen Pharma International and Pfizer International. SN received research and speakers' fee grants from Ipsen Pharma International, Novartis Pharma, Pfizer International and consulting fee from Ipsen Pharma International. The other authors have nothing to declare.

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Author contributions

AM, AVDL, and SN conceived and designed the study. AM was responsible for data management and statistical analyses after discussion with all authors. All authors participated in data interpretation and in writing of the report.

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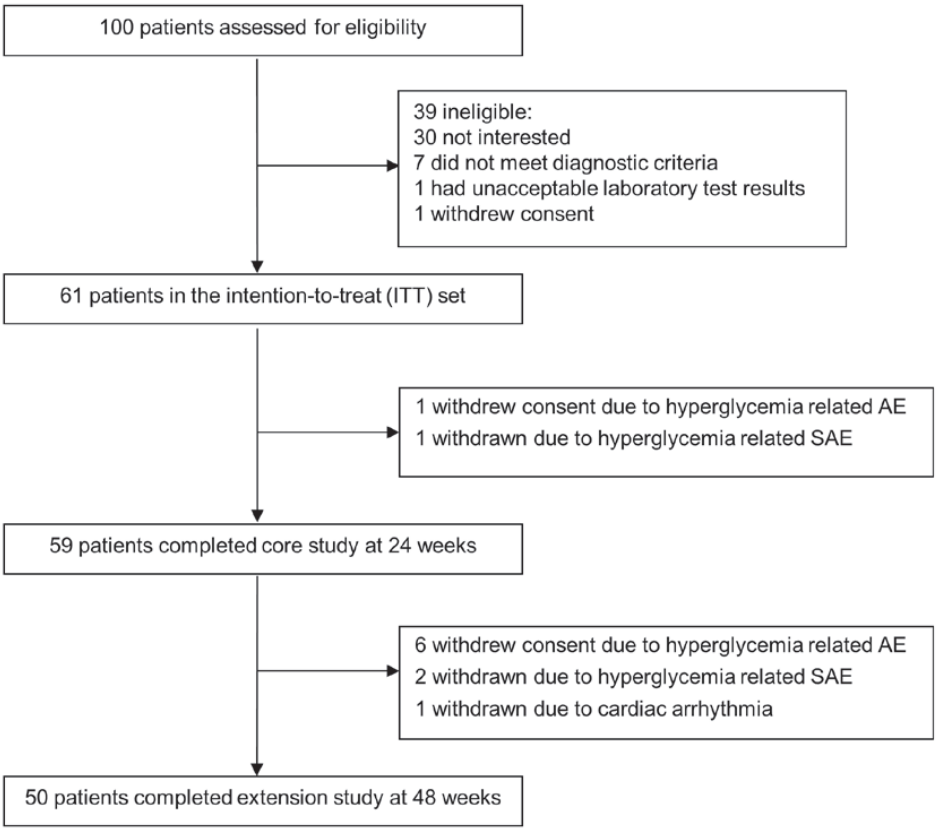
APPENDIX



Supplemental figure 1. Trial profile. AE = Adverse Event, SAE = Serious Adverse Events

Supplemental table 1. Antidiabetic medications initiated after pasireotide LAR initiation.

Antidiabetic medication	Baseline	24 weeks	48 weeks
None	46 (75.4%)	19 (31.1%)	16 (26.3%)
Metformin	15 (24.6%)	19 (31.1%)	25 (41.0%)
DPP4 inhibitors	0	14 (23.0%)	17 (27.9%)
Sulfonylureas	3 (4.9%)	12 (19.7%)	8 (13.1%)
Insulin	3 (4.9%)	6 (9.8%)	6 (9.8%)
GLP-I analogues	2 (3.3%)	3 (4.9%)	7 (11.5%)
Thiazolidinediones	1 (1.6%)	1 (1.6%)	1 (1.6%)

Patients may have taken more than one antidiabetic medication. Data based on last observation carried forward (LOCF).

Chapter 5

Pasireotide responsiveness in acromegaly is mainly driven by somatostatin receptor subtype 2 expression

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ABSTRACT

Background: The response to first-generation somatostatin receptor ligands (SRLs) treatment in acromegaly correlates with expression of somatostatin receptor subtype 2 (SST_2). However, pasireotide shows the highest binding affinity for SST_5. It has been suggested that in acromegaly SST_5 expression is better at predicting the response to pasireotide LAR (PAS-LAR) treatment than SST_2 expression.

Aim: To investigate in active acromegaly patients whether response to SRL treatment correlates to PAS-LAR treatment, and to what extent SST_2 and SST_5 expression are correlated to response to PAS-LAR treatment.

Methods: We included 52 patients from a cohort that initially received SRL treatment, followed by SRL and pegvisomant combination treatment, and finally PAS-LAR treatment. The long-term response to PAS-LAR was evaluated using a PAS-LAR score. In 14 out of 52 patients, somatotroph adenoma tissue samples were available to evaluate SST_2 and SST_5 expression using a previously validated immunoreactivity score (IRS).

Results: The percentage IGF-I (x ULN) reduction which was observed after SRL treatment correlated with PAS-LAR response score during follow-up ($r = 0.40$, $P = 0.003$, $n = 52$). After exclusion of SRL pretreated patients, SST_2 IRS was positively correlated to PAS-LAR score ($r = 0.58$, $P = 0.039$, $n = 9$), while SST_5 IRS showed no relation ($r = 0.35$, $P = 0.36$, $n = 9$).

Conclusions: In a cohort of patients partially responsive to SRLs, the IGF-I lowering effects of PAS-LAR treatment correlated with the effect of SRLs treatment and seemed to be mainly driven by SST_2 expression instead of SST_5.

INTRODUCTION

Acromegaly is a severe systemic condition most commonly caused by a somatotroph adenoma that secretes excessive levels of growth hormone (GH) and insulin-like growth factor I (IGF-I), leading to increased mortality and morbidity (1). Treatment modalities that normalize GH and IGF-I levels restore normal life expectancy (2). This goal can be achieved pharmacologically, both by inhibiting pituitary GH secretion and blocking peripheral GH action.

First-generation long-acting somatostatin receptor ligands (SRLs, octreotide and lanreotide) represent the cornerstone for medical treatment of acromegaly. The biochemical response to SRL treatment has been consistently shown to be positively correlated to somatostatin receptor subtype 2 (SST_2) protein expression on the adenoma (3-7).These compounds inhibit pituitary GH secretion by preferential binding with high affinity to SST_2. However, biochemical normalization of GH and IGF-I can only be achieved in about 40% of patients. Therefore, the majority of patients are partially or even completely resistant to SRLs. An effective treatment option to normalize IGF-I levels in partially resistant patients is the addition of the GH receptor antagonist PEGV to SRLs. A recent study from our group showed that patients using SRLs and PEGV combination treatment had a lower SST_2 expression at time of surgery compared with medically naïve patients (8). The required PEGV dose to achieve IGF-I normalization was inversely correlated to SST_2 expression, but not to SST_5 expression (8).

Pasireotide long-acting release (PAS-LAR) is a novel multireceptor somatostatin analogue, which binds with high affinity to all SSTR subtypes but SST_4. In contrast to octreotide, pasireotide shows high subnanomolar affinity to SST_5 (9). In vitro studies have shown that pasireotide modulates somatostatin receptor trafficking and phosphorylation in a distinct manner from octreotide (10, 11), inducing less SST_2 internalization, phosphorylation and ß-arrestin recruitment than octreotide. In medically naïve acromegaly patients PAS-LAR has demonstrated superior efficacy in reducing IGF-I levels over octreotide LAR, while the effect on GH reduction was superimposable (12). This latter observation was also recently confirmed *in vitro* (13). We recently reported the 24-weeks results of the Pasireotide LAR and Pegvisomant (PAPE) study (14). This prospective open-label conversion study assessed the efficacy and safety of PAS-LAR alone or in combination with PEGV in acromegaly patients controlled with SRLs and PEGV combination treatment (14). Switching to PAS-LAR resulted in a significant PEGV dose reduction, but also a higher incidence of diabetes mellitus (14).

It is assumed that the efficacy of a given somatostatin receptor ligand is directly correlated to the SSTR subtype binding profile and the pattern of SSTR expression in the somatotroph adenoma (5, 6). However, although guidelines do not report specific recommendations so far, it is generally assumed that octreotide and lanreotide are more effective when SST_2 is highly and predominantly expressed, while pasireotide is more effective when SST_5 is the predominant subtype and SST_2 is absent or poorly expressed (15). The aims of the present study were therefore: 1) to investigate whether the IGF-I response after SRL treatment correlates to the IGF-I response after PAS-LAR treatment; 2) to investigate to what extent SST_2 and SST_5 immunoreactivity are correlated to responsiveness to PAS-LAR treatment in somatotroph adenomas.

MATERIALS AND METHODS

Patients and somatotroph adenoma tissue selection

Data collection of acromegaly patients was performed at the Erasmus MC Pituitary Center in Rotterdam. We initially started with a cohort of 61 acromegaly patients who received PAS-LAR treatment during their participation in the PAPE study (Figure 1) (14).

All these patients have previously been treated with SRLs, followed by SRL and PEGV combination therapy. Cabergoline was used in 7 patients in combination with SRLs, and in two patients during the PAPE study (14). After exclusion of patients that received postoperative radiotherapy (n = 7), and patients that received SRL treatment less than 4 months (n = 2), 52 patients remained and were finally included in the study cohort. In total 19 out of these 52 patients previously underwent neurosurgery. Reasons for surgery included adenomas with reasonable chance for cure such as (intrasellar) microadenomas, or macroadenomas with risk of visual impairment.

We selected only those patients with sufficient adenoma tissue available to perform immunohistochemistry (IHC). One patient underwent a second surgery during follow-up. For clarity, in this latter case we analyzed only the tissue sample of the first surgery. From the 14 remaining somatotroph adenoma tissue samples included for IHC analysis (SSTR subcohort), 10 tissue samples have been stained previously (8), while 4 cases were newly stained (figure 1). We retrospectively collected data on medical history and clinical response to first-generation SRLs. Prospective data on the PEGV dose and IGF-I levels were used from the PAPE study (14). The PAPE study was registered with ClinicalTrials.gov, number NCT02668172. All patients were included after written informed consent.

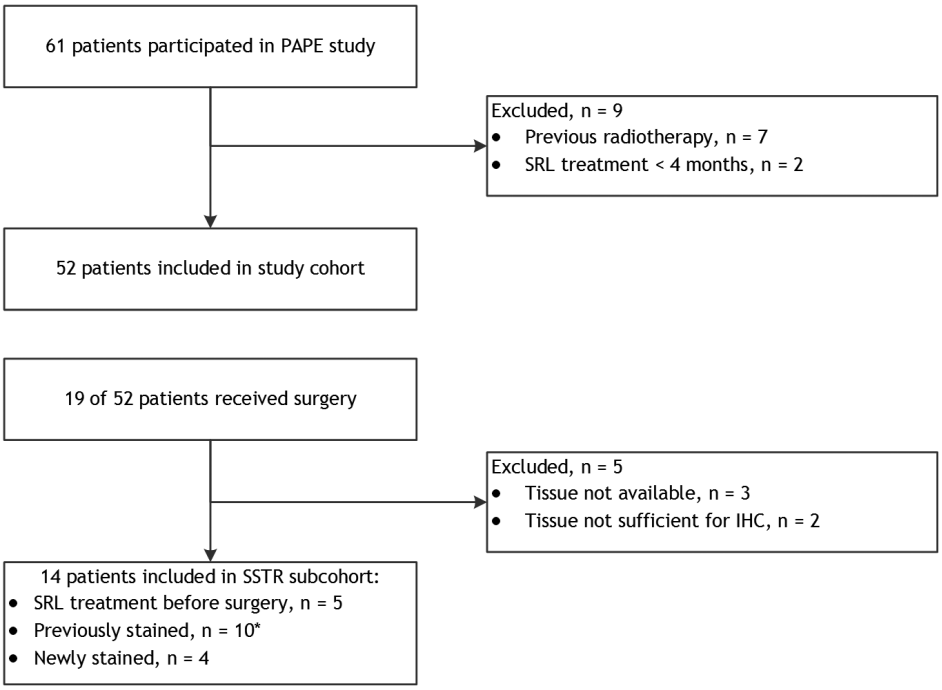

Figure 1. Flowchart of the selection procedure for the study cohort and the somatotroph adenoma tissue samples included in the SSTR subcohort.
All patients eventually received SRL and PEGV combination treatment, and were switched to pasireotide LAR treatment during the PAPE study (14). SRL = first-generation long-acting somatostatin receptor ligands; PEGV = pegvisomant; PAPE = pasireotide LAR and pegvisomant study; SSTR = somatostatin receptor subtype; IHC = immunohistochemistry.

Outcomes

Response to SRL treatment was defined as IGF-I x age-adjusted upper limit of normal (IGF-I x ULN), and as percentage of IGF-I suppression after at least 4 months SRL treatment. In patients that underwent surgery, post-operative IGF-I levels after at least 3 months were considered. Response to PAS-LAR during the PAPE study was divided into short-term and long-term response. Short-term treatment response was defined as IGF-I levels (x ULN) at 24 weeks (i.e. after 3 injections of PAS-LAR 60 mg). During the extension phase from 24 until 48 weeks, both the PAS-LAR dose and PEGV dose were titrated according to a protocol to achieve IGF-I levels within the normal range. Therefore, the long-term response to PAS-LAR was based on a composite "PAS-LAR treatment response score" (PAS-LAR score) in order to fully capture the effect of PAS-LAR taking into account PEGV dose reduction, discontinuation and eventually PAS-LAR dose reduction. The PAS-LAR score comprised five categories representing the difference in PEGV dose and PAS-LAR dose at week 48 versus baseline (week number 0): 0 = PEGV dose reduction 0-33% (in combination with PAS-LAR 60 mg), 1 = PEGV dose

reduction 33-66% (+ PAS-LAR 60 mg), 2 = PEGV dose reduction 66-100% (+ PAS-LAR 60 mg), 3 = PEGV treatment discontinued and PAS-LAR dose reduced to 40 mg, 4 = PEGV treatment discontinued and PAS-LAR dose reduced to 20 mg every 4 weeks. 100% PEGV dose reduction corresponds to PAS-LAR 60 mg monotherapy. Higher PAS-LAR score corresponds to a better response to PAS-LAR treatment.

IGF-I assays

Total IGF-I serum concentrations during the PAPE study were measured by the immunometric IDS-iSYS assay (Immunodiagnostic Systems Limited; Boldon, United Kingdom; intraassay coefficient of variation (CV) 8.1%, interassay CV 2.1%) (16). Total IGF-I serum concentrations before and after SRL treatment were measured using different assays: Immulite 2000 assay, a solid-phase, validated enzyme-labelled chemiluminescent immunometric assay (DPC Biermann GmbH/Siemens, Fernwald, Germany; intraassay variability of 2–5%, interassay variability of 3–7%), the immunometric IDS-iSYS assay (Immunodiagnostic Systems Limited; Boldon, United Kingdom; intraassay coefficient of variation (CV) 8.1%, interassay CV 2.1%), and two different radioimmunoassays (Diagnostic Systems Laboratories, Webster, Tex., USA, intraassay CV 3.9%, interassay CV 4.2%), and Medgenix Diagnostics, Fleurus, Belgium; intraassay coefficient of variation (CV) 6.1%, interassay CV 9.9%). Total IGF-I was interpreted according to the sex and age-dependent ranges used in accordance with previous reports (17, 18). Because different IGF-I assays were used over time, IGF-I levels were expressed as upper limits of normal (ULN), and not as the absolute values.

Immunohistochemistry

Somatotroph adenoma tissues were stained for hematoxylin and immunostained for SST_2 and SST_5. Formalin-fixed paraffin-embedded tumor samples were cut into sequential 4-µm-thick sections and deparaffinized and stained using a fully automated Ventana BenchMark ULTRA stainer (ref: 790-2208, Ventana, Tucson, Ariz., USA) according to the manufacturers' instructions at the Pathology Department. Binding of peroxidase-coupled antibodies was detected using 3,39-diaminobenzidine as a substrate, and the sections were counterstained with hematoxylin. The rabbit monoclonal anti-SSTR2 antibody clone UMB-1 (SS-8000, BioTrend, Köln, Germany) was used at a dilution of 1:50, and the rabbit monoclonal anti-SSTR5 antibody clone UMB-4 at a dilution of 1:400 (ab109495, Abcam, Cambridge, UK). Normal pancreatic tissue served as a positive control for both SST_2 and SST_5 staining. For negative controls, the primary antibody was omitted. Immunostaining of the adenomas was scored semiquantitatively based on an immunoreactivity scoring system (IRS). The IRS is calculated by the product of the percentage of positive stained cells (0: 0%; 1: <10%; 2: 10–50%; 3: 51–80%; 4: 80%) and the staining intensity (0: no staining; 1: weak staining;

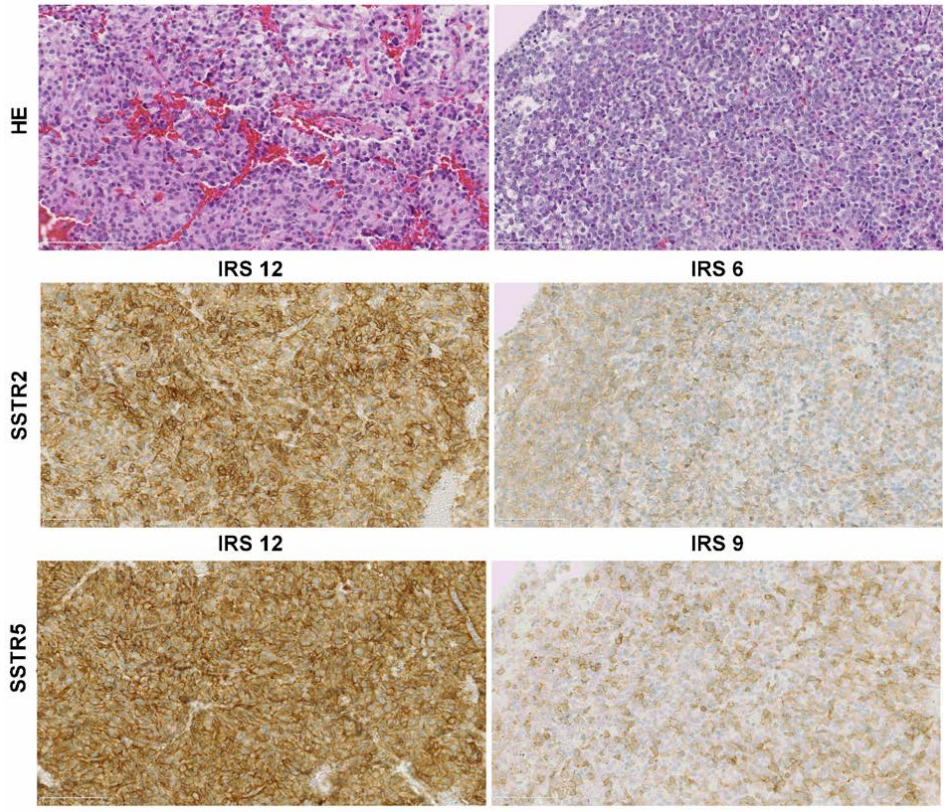

Figure 2. SST_2 and SST_5 protein expression of somatotroph adenomas scored by the immunoreactivity score (IRS).
HE = Haematoxylin and eosin; SSTR2 = somatostatin receptor subtype 2; SSTR5 = somatostatin receptor subtype 5, IRS = immunoreactivity score.

2: moderate staining; 3: strong staining) (19). The IRS ranges between 0 and 12. The newly stained somatotroph adenoma tissue samples were scored by two independent investigators (A.M. and E.C.) based on the histopathological description of the sample provided by the pathologist. Both investigators were blinded to each other's findings and the patients' characteristics. Figure 2 shows two representative cases.

Statistical methods

Categorical data were represented as observed frequencies and percentages. Continuous data were represented as mean and 95% confidence interval (CI) or median and range. The Kolmogorov-Smirnov and the Shapiro-Wilk test were used to test normality of variables. If assumption of normal data distribution was met, the paired t-test was used. For non-normally distributed variables the Wilcoxon signed-rank test was used. Results of correlation analyses were represented as Spearman's Rank correlation

coefficients (r). We considered *P*-values < 0.05 (two-tailed) to be statistically significant. Statistical analyses were performed with SPSS version 24 (IBM SPSS Statistics for Windows, Armonk, N.Y., USA) and graphs were drawn using GraphPad Prism version 6 for Windows (GraphPad Software, San Diego, California, USA).

RESULTS

Patient characteristics and treatment modalities

Characteristics of the 52 patients included in the study cohort are presented in Table 1. After initial treatment with SRLs, most patients (84.6%) had IGF-I levels above 1.2 x the ULN. All patients continued to receive SRL and PEGV combination treatment with a median PEGV dose of 100 mg/week (IQR 60–160).

Table 1. Patient characteristics entire cohort

Characteristics	Patients (n = 52)
Age (median, range)	53.5 (26 – 80)
Female patients (n, %)	22 (42.3%)
Macroadenomas (n, %)	44 (84.6%)
Previous surgery (n, %)	19 (34.6%)
SRL treatment prior to surgery (n, %)	5 (9.6%)
SRL treatment duration (months, mean, 95% CI)	11.2 (8.2 – 14.2)
IGF-I before SRL therapy (x ULN, mean, 95% CI)	3.14 (2.80 – 3.49)
IGF-I after SRL therapy (x ULN, mean, 95% CI)	2.13 (1.82 – 2.45)
IGF-I ≤ 1.2 x ULN after SRL monotherapy (n, %)	13 (27.1%)
Weekly PEGV dose (mg, mean, 95% CI)	137 (101–172)

IGF-I levels are shown before initiation of SRL monotherapy and after ≥ 4 months SRL monotherapy. PEGV dose during SRL and PEGV combination therapy at baseline of PAPE study.

Table 2 reports the characteristics of patients that were included in the SSTR subcohort (n = 14). All these patients harboured a macroadenoma. 5 out of 14 patients had received SRL treatment prior to surgery, [4 achieved initial IGF-I normalization (< 1.2 x ULN)] and the remaining nine patients were drug-naïve before surgery. During follow-up, all 14 patients received SRL and PEGV combination therapy (median PEGV dose 100 mg/week (IQR 80–145)). After 24 weeks, the median PEGV dose was 45 mg/week (IQR 23–75) and decreased to 0 mg/week (IQR 0–70) after 48 weeks. Three out of 14 (21.4%) patients were on PAS-LAR monotherapy after 24 weeks, increasing to 8 patients (57.1%) after 48 weeks. More in detail, in two patients PAS-LAR dose was reduced to 40 mg and in two other patients to 20 mg monotherapy every 4 weeks.

Table 2. Patients' characteristics of the SSTR subcohort

Patient number	Sex, Age (y)	SRL pre-treat-ment	IGF-I (ULN) after SRL	Percentage of IGF-I reduction after SRL	IGF-I (ULN) after PAS-LAR 24 weeks	Baseline PEGV dose (mg/week)	48 weeks PEGV dose (mg/week)	48 weeks percentage PEGV dose reduction	48 weeks PAS-LAR dose (mg)	48 weeks PAS-LAR score	SST_2 (IRS)	SST_5 (IRS)
1	M, 80	No	0,72	65,9	,90	80	0	100	40	3	6	12
2	F, 37	No	1,67	48,5	1,05	80	0	100	60	2	12	12
3	M, 57	No	1,48	42,7	1,28	80	50	37.5	60	1	8	1
4	M, 38	No	1,77	18,2	1,78	700	540	22.9	60	0	1	12
5	F, 36	Yes	3,83	15,5	1,11	100	50	50	60	1	1	0
6	F, 36	No	3,17	7,1	2,91	400	400	0	60	0	6	9
7	M, 61	Yes	1,51	54,2	,79	80	0	100	60	2	12	12
8	F, 71	No	2,21	5,5	1,24	120	60	50	60	1	2	12
9	M, 51	No	3,14	21,2	1,16	120	0	100	60	2	9	12
10	M, 53	Yes	1,89	44,9	1,14	100	100	0	60	0	12	12
11	F, 56	No	0,76	74,6	,39	20	0	100	20	4	12	8
12	F, 46	Yes	1,00	62,6	,49	100	0	100	20	4	9	4
13	M, 46	Yes	1,85	19,4	,49	70	0	100	60	2	12	6
14	F, 69	No	0,61	60,0	,72	220	0	100	40	3	9	12

Detailed description of patients' general characteristics, IGF-I levels after SRL and PAS-LAR treatment, and the SST_2 and SST_5 immunoreactivity score (IRS). In addition, PEGV doses are shown during SRL and PEGV combination treatment at baseline and after switching to PAS-LAR treatment at 48 weeks. The PAS-LAR score takes into account both the achieved PEGV dose reduction and PAS-LAR dose reduction at 48 weeks compared with baseline.

In the study cohort (n = 52), at 24 weeks, the median PEGV dose was 45 mg/week (IQR 30–80) and 10 of 52 (19.2%) patients were on PAS-LAR monotherapy. At 48 weeks, the median PEGV dose decreased to 40 mg/week (IQR 0 – 90) and 25 of 52 (48.1%) patients were on PAS-LAR monotherapy.

Protein expression of SST_2 and SST_5

The median SST_2 IRS was 9 (IQR 5–12), the median SST_5 IRS was 12 (IQR 5.5–12), and the median SST_2/SST_5 ratio was 1.0 (IQR 0.6 – 1.8). We did not find a statistically significant difference in SST_2 and SST_5 expression between medically naïve (n = 9) and SRL pre-treated (n = 5) patients (*P* = 0.31 and *P* = 0.25, respectively). More in detail, median SST_2 IRS was 12 (IQR 5 – 12) in pre-treated patients and 8 (4 – 10.5) in the naïve ones, while median SST_5 IRS was respectively 6 (2– 12) and 12 (8.5 – 12) in SRL pre-treated and naïve patients.

The relation between SSTR immunoreactivity and response to SRL treatment is shown in the supplemental data (page 118) (20). In line with previous findings, the percentage IGF-I reduction after SRL treatment was positively correlated to SST_2 IRS, while an inverse trend was observed between SST_2 IRS and IGF-I (x ULN) levels after SRL treatment. The PEGV dose at baseline during the PAPE study was inversely correlated to the SST_2 IRS.

Relationship between response to SRL treatment and PAS-LAR treatment in study cohort

We observed a significant positive correlation between IGF-I (x ULN) levels after SRL treatment and IGF-I levels after 24 weeks PAS-LAR treatment (r = 0.50, *P* = 0.0002, n = 52, 3A). However, no relation was observed between the percentage IGF-I reduction after SRL treatment and after 24 weeks PAS-LAR treatment (r = 0.026 *P* = 0.85, n = 52) (Supplemental Figure 1A) (20). With respect to response to PAS-LAR after 48 weeks, IGF-I (x ULN) levels after SRL treatment showed a strong inverse correlation with the PAS-LAR score (r = −0.53, *P* = 0.0006, n = 52, Figure 3B). Moreover, the percentage IGF-I (x ULN) reduction after SRL treatment was positively correlated to the PAS-LAR score (r = 0.40, *P* = 0.003, n = 52, Figure 3C) as well. We also observed a significant relationship between IGF-I (x ULN) levels after SRL treatment and after 48 weeks PAS-LAR treatment (r = 0.30, *P* = 0.028, n = 52) (Supplemental Figure 1B) (20).

Relationship between response to SRL treatment and PAS-LAR treatment in SSTR subcohort

In the SSTR subcohort, we observed a positive correlation between IGF-I (x ULN) levels after SRL treatment and after 24 weeks PAS-LAR treatment (r = 0.58, *P* = 0.029,

n = 14, Figure 4A), while no corelation was observed with percentage IGF-I reduction (Supplemental Figure 1C) (20). Higher SST_2 IRS was correlated with lower IGF-I levels (x ULN) after 24 weeks PAS-LAR treatment (r = −0.61, *P* = 0.020, n = 14, Figure 4B), while SST_5 IRS did not show any relation to IGF-I levels (r = 0.16, *P* = 0.58, n = 14, Figure 4C).

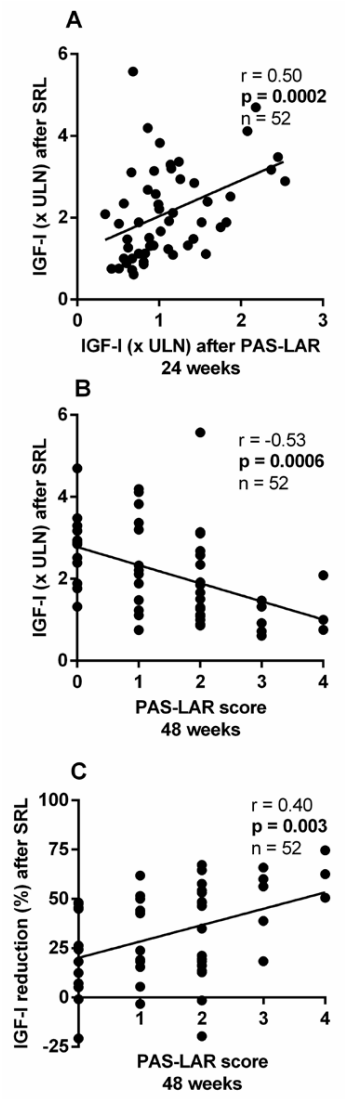

Figure 3. Relation between response to SRL treatment and response to PAS-LAR treatment in the study cohort. IGF-I (x ULN) levels after SRL treatment were correlated to IGF-I (x ULN) levels after PAS-LAR treatment at 24 weeks (3A), and inversely correlated to the PAS-LAR score at 48 weeks (3B). The percentage IGF-I (x ULN) reduction after SRL treatment was positively correlated to the PAS-LAR score at 48 weeks (3C).

With respect to the 48 weeks PAS-LAR response, the PAS-LAR score at 48 weeks was inversely correlated to IGF-I (x ULN) levels after SRL treatment (r = −0.71, *P* = 0.005, n = 14, Figure 5A), and positively correlated to the percentage IGF-I reduction after SRL treatment (r = 0.80, *P* = 0.001, n = 14, Figure 5B). Furthermore, IGF-I (x ULN) levels

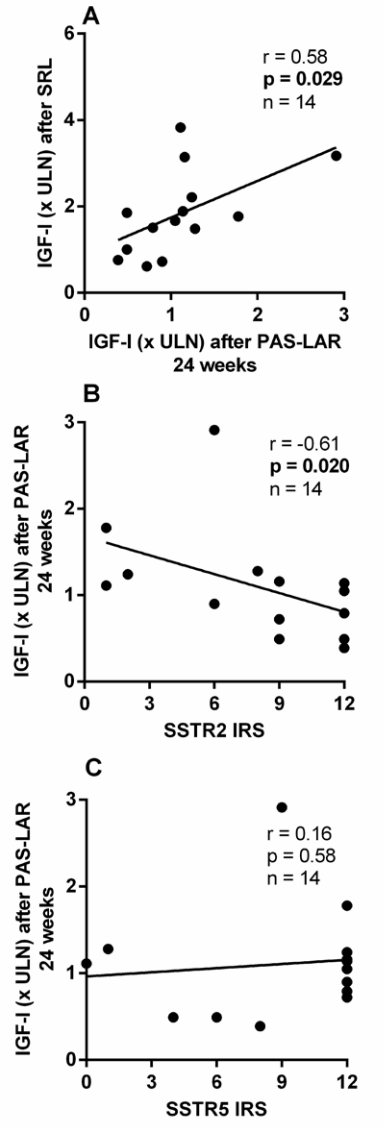

Figure 4. Relation between response to SRL treatment and the response to PAS-LAR treatment at 24 weeks in the SSTR subcohort. IGF-I (x ULN) levels after PAS-LAR treatment at 24 weeks were correlated to IGF-I levels after SRL treatment (4A), and inversely correlated to SST_2 expression (4B), but not to SST_5 expression (4C).

after SRL treatment were correlated to IGF-I (x ULN) levels after PAS-LAR treatment at 48 weeks (r = 0.58, P = 0.031, n = 14, Supplemental Figure 1D) (20).

We observed a trend, although not statistically relevant, for a direct correlation between SST_2 IRS and the PAS-LAR score (r = 0.41, *P* = 0.14, n = 14, Figure 5C), and no relation was found between SST_5 IRS and PAS-LAR score (r = −0.073, *P* = 0.80, n = 14, Figure 5D). Interestingly, considering only those patients naïve to SRL treatment before surgery (n = 9), the correlation between SST_2 IRS and the PAS-LAR score (r = 0.69, *P* = 0.039, n = 9) was statistically significant, while this was not the case for SST_5 IRS.

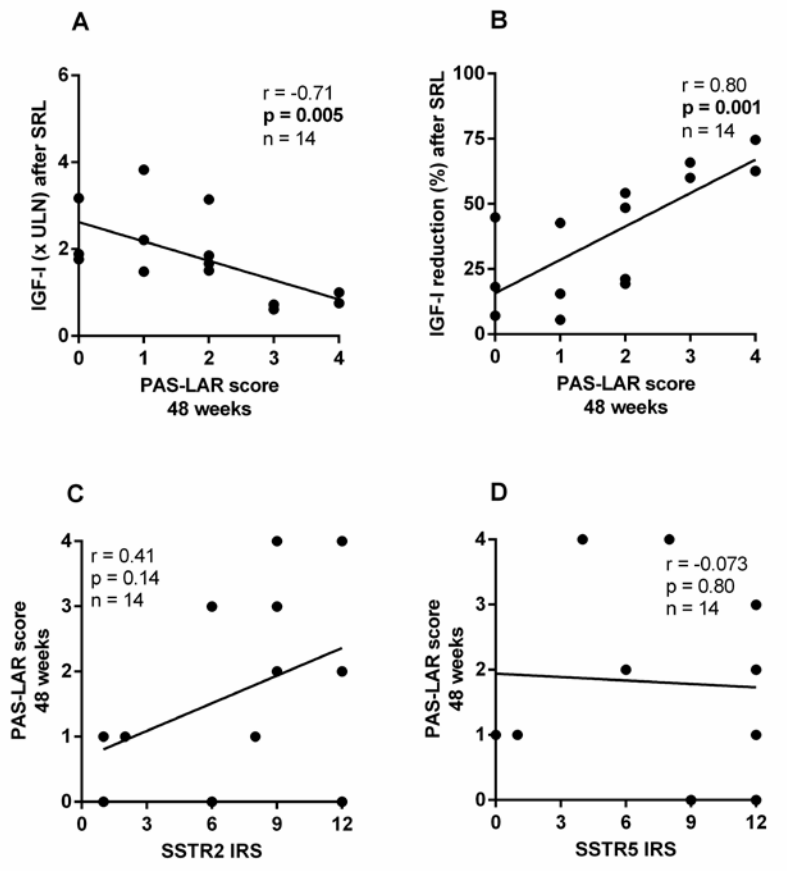

Figure 5. Relation between response to SRL treatment and the response to PAS-LAR treatment at 48 weeks in the SSTR subcohort. The PAS-LAR score at 48 weeks was inversely correlated to IGF-I (x ULN) levels after SRL treatment (5A), and positively correlated to the percentage IGF-I reduction after SRL treatment (5B). The PAS-LAR score showed a trend for a relation with SST_2 expression (5C), but SST_5 expression did not show any relation (5D).

DISCUSSION

Our results suggest that in acromegaly patients the responsiveness to PAS-LAR treatment is mainly correlated to SST_2 expression, and not to SST_5. This observation is further supported by the finding that after 48 weeks treatment the percentage IGF-I reduction after SRL treatment was correlated to the PAS-LAR treatment response score. Our study provides the novel finding that the *in vivo* response to PAS-LAR is directly correlated to both the clinical response to first-generation SRL treatment and SST_2 expression on adenoma tissue.

However, these results are not unexpected. Indeed, our data confirm previous *in vitro* studies, carried out in primary cultures of GH-secreting adenomas, showing that the efficacy of naïve somatostatin (SRIF-14), octreotide and pasireotide in the reduction of GH secretion was positively correlated with SST_2 mRNA expression, but not with SST_5 (13, 21, 22). According to these findings, our group and other authors have reported that the effect of octreotide and pasireotide on GH suppression is almost superimposable both *in vitro* and *in vivo* (12, 13, 23).

The observed positive relation between SST_2 protein expression and the percentage IGF-I reduction after SRL treatment, is in line with previous studies (5, 6). Furthermore, an inverse relation between the PEGV dose and SST_2 expression was recently reported, suggesting that the required PEGV dose to normalize IGF-I levels in patients with partial response to SRLs is a surrogate marker for the degree of SRL resistance (8). In our cohort, the SRL pretreated patients had a trend for a higher SST_2 expression than medically naïve patients. This finding is in contrast to previous studies (5, 6), and it is probably correlated to a lack of statistical power in our study (5 vs 9 adenoma samples).

Our results show that IGF-I levels after PAS-LAR treatment were directly correlated to SST_2 expression, and not to SST_5 expression. After exclusion of SRL pretreated patients, SST_2 IRS was also significantly correlated to the PAS-LAR score, a tool designed to uncover the impact of switching to PAS-LAR treatment in patients using SRLs and PEGV combination treatment. While there is no evidence that SST_5 expression is affected by SRL presurgical treatment, it has been widely demonstrated that patients receiving SRL treatment prior to neurosurgery show significantly lower SST_2 protein expression compared to medically naïve patients (4-6, 13). Although this is not evident in our cohort, a pooled analysis of SRL pretreated and medically naïve patients can introduce bias. Exclusion of the SRL pretreated patients from our analysis

(n = 5) resulted in a stronger relationship between SST_2 IRS and the response to PAS-LAR treatment.

A strength of our study lies in the relatively large number of patients in which the clinical efficacy of SRLs, SRL/PEGV combination treatment and PAS-LAR treatment were systematically investigated in combination with data on SSTR expression of somatotroph adenomas in a well characterized subgroup of patients. The main limitation of our study lies in the retrospective collection of data on IGF-I levels during SRL treatment and the use of different IGF-I assays during follow-up. The IGF-I levels measured after 24 weeks PAS-LAR treatment may be partly influenced by the carry-over effect of withdrawal of SRLs after 12 weeks. While the carry-over effect of SRLs may have influenced the short-term response to PAS-LAR after 24 weeks, the response to PAS-LAR treatment after 48 weeks is probably not affected. In our cohort, the PAS-LAR score at 48 weeks might therefore be the most informative marker for responsiveness to PAS-LAR treatment.

Two studies have previously investigated the relationship between the immunohistochemical expression of SST_2 and SST_5 in somatotroph adenomas and the clinical response to first-generation SRL and PAS-LAR treatment in acromegaly (24). *Iacovazzo* et al suggested that SST_5 expression drives the responsiveness to PAS-LAR treatment in patients resistant to first-generation SRLs. These authors investigated a cohort of 39 acromegaly patients requiring post-operative SRL treatment, of which 11 patients were resistant to SRL and were switched to PAS-LAR treatment. They observed that none of the patients lacking SST_5 expression was responsive to PAS-LAR, whereas 5 out of 7 patients with membranous expression of SST_5 were responsive to PAS-LAR. Furthermore, patients with a higher SST_5 score had a greater reduction in IGF-I levels. However, they found no difference in SST_2 expression between pasireotide responders and non-responders.

These results are in contrast with our findings, which suggest that SST_2 expression, and not SST_5 expression, is more important for the clinical response to PAS-LAR. The main difference between our study and the study from *Iacovazzo* et al. (24), is that we included mainly patients who were partially responsive to SRLs, while Iacovazzo et al included only SRL resistant patients. Secondly, the patients in our cohort all received pegvisomant treatment before switching to pasireotide LAR during the PAPE study, whereas the patients in the Iacovazzo study did not receive PEGV treatment, and were directly switched to pasireotide LAR (Table 2). In addition, these differences are unlikely to be explained by the use of a different SSTR expression scoring system. Although the other authors used a scoring method proposed by *Volante* et al

which takes into account both subcellular localization and extent of staining (25), the method we used from *Remmele* et al is a semiquantitative score which takes into account both intensity and percentage of positive cells (19). Interestingly, both scoring systems have been recently found to show high inter-laboratory and inter-observer agreement for SSTRs expression in neuroendocrine tumors (26).

Furthermore, in our cohort we cannot rule out a direct effect of PEGV treatment on SSTR expression (8). PEGV is known to increase serum GH levels (27), which could result in reduced hypothalamic GHRH secretion, which in turn may lead to a down-regulation of SSTR expression. Although the impact of PEGV on SSTR expression is plausible (28), there is no evidence that PEGV treatment plays a major role in the modulation of SSTR expression via the activation of GH-IGF-I axis.

While our study suggests that pasireotide acts mainly via SST_2 in somatotroph adenomas, in corticotroph adenomas SST_5 seems to be more important. Several *in vitro* studies have demonstrated that pasireotide is more effective than octreotide in reducing ACTH secretion and/or intracellular cAMP levels in AtT20 cells or corticotroph adenomas primary cultures (29-32). On the other hand, preclinical studies have indicated that pasireotide and octreotide are equally effective (in vitro) in lowering GH levels (21, 23, 33, 34). This suggests a predominant role of SST_2 in mediating the inhibitory effect of pasireotide on GH secretion in somatotroph adenomas.

In conclusion, our results suggest that SST_2 expression of somatotroph adenomas is more important than SST_5 in driving the responsiveness to PAS-LAR treatment in a peculiar subset of acromegaly patients (e.g. partial responders). It is plausible that the enhanced efficacy of PAS-LAR compared to first-generation SRLs is mediated by its stronger inhibition of insulin secretion, rendering the liver less sensitive to GH action (35). The enhanced efficacy of PAS-LAR could also be the consequence of a differential activation of SST_2 by the different compounds (e.g. reduced activation of SST_2 internalization and faster recycling on the cell membrane) (36, 37), rather than by the higher binding affinity of PAS-LAR for SST_5. Future studies should investigate whether this is indeed the case.

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Author contributions

AM, LH, and SN conceived and designed the study. AM was responsible for data management and statistical analyses after discussion with all authors. All authors participated in data interpretation and in writing of the report.

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SUPPLEMENTAL DATA

Relationship between SSTR immunoreactivity and response to SRL treatment

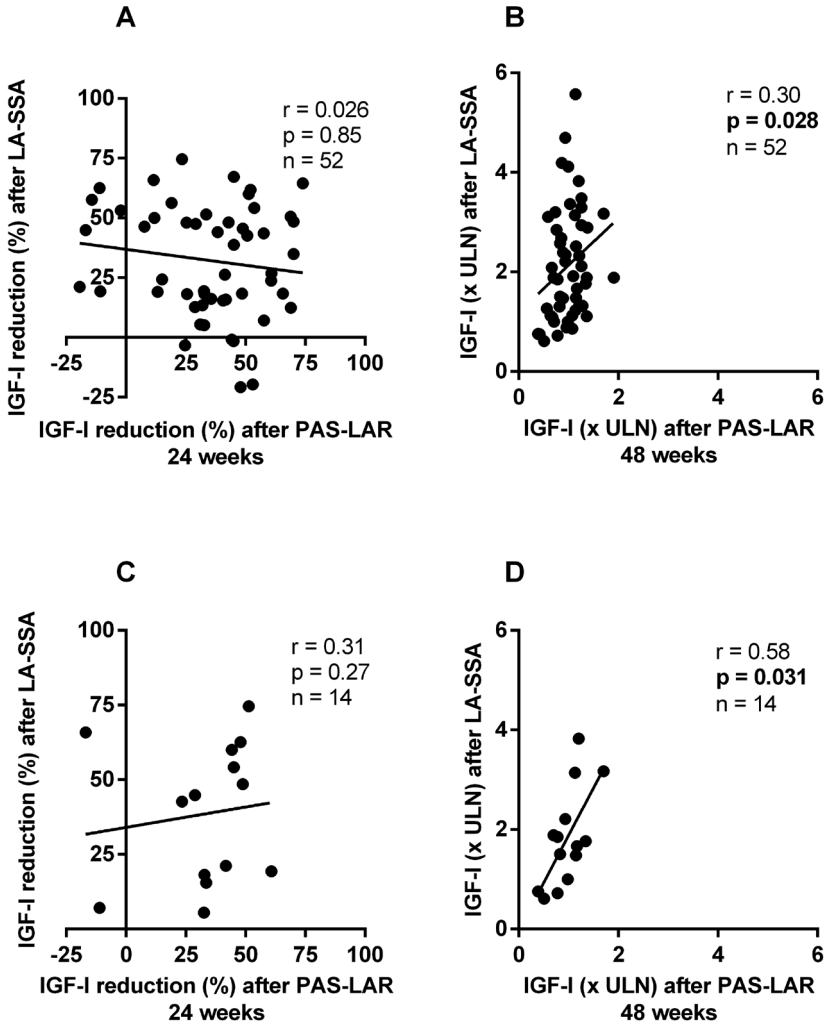
The percentage IGF-I reduction after SRL treatment was positively related to SST_2 IRS (r = 0.54, *P* = 0.046, n = 14), but no correlation was found with SST_5 IRS (r = 0.090, *P* = 0.76, n = 14).

IGF-I (x ULN) levels after SRL treatment showed an inverse trend with SST_2 IRS (r = −0.31. P = 0.28, n = 14), while no correlation was observed with SST_5 IRS (r = −0.12, *P* = 0.69, n = 14) (Figure 2A and 2B). Exclusion of SRL pretreated patients did not change the relation to SST_2 (r = −0.34, *P* = 0.37, n = 9) and SST_5 IRS (r = 0.10, *P* = 0.98, n = 9).

Relationship between baseline PEGV dose during combination treatment and SSTR immunoreactivity

We observed an inverse correlation between the PEGV dose at baseline and SST_2 IRS (r = −0.61, *P* = 0.020, n = 14), but SST_5 IRS was not correlated with the PEGV dose (r = 0.32, *P* = 0.27, n = 14).

SUPPLEMENTAL FIGURE 1



Chapter 6

The acylated/unacylated ghrelin ratio is similar in acromegaly patients during different treatment regimens

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ABSTRACT

Background: Data on plasma acylated ghrelin (AG) and unacylated ghrelin (UAG) levels in acromegaly are limited. High ratios of AG/UAG are linked with type 2 diabetes, obesity and hyperphagia (e.g. in Prader-Willi syndrome).

Objective: To assess fasting plasma AG and UAG levels, and the AG/UAG ratio in acromegaly patients on combination treatment of long-acting somatostatin analogues and pegvisomant. As a control, we used patients controlled with pegvisomant monotherapy, and medically naïve patients with active acromegaly.

Methods: Fasting venous blood samples (combination therapy N = 60; monotherapy N = 4; naïve N = 5) were collected and directly stabilized with AEBSF to inhibit deacylation of AG. Plasma AG and UAG levels were determined by double-antibody sandwich EIA and the AG/UAG ratio was calculated.

Results: Plasma AG and UAG levels were significantly lower in acromegaly patients on combination treatment (AG: 8.5 pg/ml, 2.9-21.1 (median, interquartile range)) (UAG: 26.9 pg/ml, 11.2-42.1) compared to patients using pegvisomant alone (AG: 60.5 pg/ml, 58.8-77.4) (UAG: 153.7 pg/ml, 127.3-196.0) and medically naïve acromegaly patients (AG: 24.0 pg/ml, 12.6-49.7) (UAG: 56.3 pg/ml, 43.4-61.5). However, AG/UAG ratios were similar in all groups.

Conclusions: Although plasma AG and UAG are suppressed during combination treatment of LA-SSA and PEGV, the AG/UAG ratio remained similar. This shows that somatostatin analogues decrease both AG and UAG, which suggest that they do not alter metabolism significantly in acromegaly patients.

INTRODUCTION

Ghrelin is a small peptide hormone secreted mainly by neuroendocrine X/A cells in the stomach (1, 2). In the circulation it consists of two isoforms: acylated ghrelin (AG) and unacylated ghrelin (UAG). Both isoforms are detectable in equal amounts in the circulation (3). AG differs from UAG in being acylated by attachment of a medium-chain fatty acid at its serine-3 residue. AG is acylated by the intracellular enzyme ghrelin O-acyl transferase (GOAT) and is responsible for the distinct metabolic and non-metabolic effects of ghrelin in vivo (4-11). AG acts on the hypothalamus through the growth hormone secretagogue receptor (GHSR1a) and is known to be diabetogenic, orexigenic and obesogenic. UAG does not bind to the GHSR1a receptor at physiological concentrations and, therefore, was considered to be inactive. However, recent studies have shown that UAG is able to counteract the metabolic effects of AG (9, 12).

Because AG and UAG have distinct biological effects and can affect each other, the AG/UAG ratio may be a more important parameter than individual levels of AG and UAG (13-19). For example, elevated AG/UAG ratios have been associated with diabetes, obesity and hyperphagia (13, 20-25). Hyperphagia is a hallmark of Prader-Willi syndrome, a rare cause of genetic early onset obesity, which is characterized by elevated total ghrelin levels, but changing AG/UAG ratios throughout life (20, 22, 26). Recently, in patients with Azlheimer's disease it was shown that rivastigmine, an acetylcholinesterase and butyrylcholinesterase inhibitor, improved appetite by increasing the AG/UAG ratio (27)

Regarding the relation of ghrelin with growth hormone (GH), it is known that ghrelin stimulates GH secretion, while ghrelin itself is reduced after GH infusion. However, the exact physiological role of ghrelin in the regulation of GH release is not entirely established.

In acromegaly, a prototype disease characterized by excessive GH levels, the emerging picture from previous studies is that in medically naïve patients during active disease total ghrelin levels are lowered compared with controls (28-30). Ghrelin levels are elevated after surgery, while they are reduced during treatment with long-acting somatostatin analogues (LA-SSAs) (29-32). Acromegaly patients treated with the competitive GH receptor blocker pegvisomant (PEGV) have higher total ghrelin levels than patients with active disease (33).

However, to our knowledge, the effect of combination treatment with LA-SSAs and PEGV on plasma AG and UAG levels in acromegaly remains unknown. Similarly, AG and UAG levels have not been assessed together in patients with acromegaly.

Therefore, the aim of this study was to assess fasting plasma AG and UAG levels and to determine the AG/UAG ratio between acromegaly patients under combination treatment with somatostatin analogs and pegvisomant (N = 60), and compare them to pegvisomant monotherapy (N = 4) and medically naïve acromegaly patients (N = 5).

PATIENTS AND METHODS

Study design

We prospectively recruited 69 acromegaly patients at our outpatient clinic at Erasmus University MC, Rotterdam between August 2015 and June 2016. The majority these patients were long-term biochemically controlled with combination treatment of LA-SSAs and PEGV (N = 60), 4 patients were on pegvisomant monotherapy and 5 patients were medically naïve with active acromegaly. We excluded patients with eating disorders, active malignancies, active inflammatory or infectious diseases, epilepsy and psychiatric disorders. Acromegaly patients were considered diabetic either if they were taking antidiabetic medication, or had a prior history of diabetes mellitus, or had glycated hemoglobin levels ≥ 6.5%.

In addition to measurement of plasma AG and UAG we assessed in the fasting state: glucose, insulin, HbA1c, IGF1 and GH. Also body weight and height. Serum glucose, insulin and HbA1c were determined with standard laboratory methods. The updated homeostasis model assessment (HOMA-2) was used to assess insulin resistance (HOMA-IR) and beta cell function (HOMA-IB) from pairs of fasting glucose and insulin levels.

All patients gave their written informed consent, and the study was approved by the Medical Ethics Committee of Erasmus MC, Rotterdam.

Materials

Vacutainers were obtained from Becton Dickinson (Breda, Netherlands; cat# 367899; 6 ml K2 EDTA), 4-(2-Aminoethyl) benzenesulphonyl fluoride hydrochloride (Pefabloc, SC AEBSF) was purchased from Roche Applied Science (cat# 11429876001; Almere, Netherlands). Stock solutions of 200 mg/ml AEBSF were prepared in distilled water (34, 35).

Human AG and UAG were determined by a double-antibody sandwich enzyme immunoassay (EIA) kits obtained from Bertin Pharma (Montigny-le-Bretonneux, France; A05106 and A05119, respectively) (34).

Total IGF1 concentrations were measured by chemiluminescent immunometric assay (IDS-iSYS; Immunodiagnostic Systems Limited; Boldon, United Kingdom), and were interpreted according to the sex-dependent and age-dependent ranges. GH levels were measured using the IDS-iSYS assay, this assay is free of interference from PEGV (36).

Blood collection, AEBSF treatment and storage

Overnight fasting venous blood samples for the measurement of plasma AG and plasma UAG were withdrawn and collected in EDTA tubes. One 4 ml EDTA tube per patient was collected. AEBSF was immediately added to all blood samples (dilution 1:100; final 2mg/ml) to prevent deacylation of AG to UAG (34, 37). Whole blood was mixed gently by inversion (3x) and stored on water ice (4°C) until centrifugation at 2500 g at 4°C for 5 minutes. Plasma of these venous blood samples was then rapidly aliquoted in four 1.5 ml Eppendorf tubes (300 µl each). All plasma samples were stored at –80°C until the assay was performed. AEBSF was stored for a maximum of one month after dilution.

Acylated and unacylated ghrelin ELISAs

After thawing on ice, plasma samples were centrifuged for 1 min at 1500 g 4°C, and kept on ice before transferring to the assay plates. All samples were measured in duplicate (50 µl/well) according to the manufacturer's protocol (34).

A sigmoidal third order cubic polynomial fitting was used to determine concentrations from the calibration curves. This resulted in r^2 values >0.99 in the majority of the assays. For the Bertin Pharma EIAs, the average intra-assay percent coefficient of variation (%CVs) for AG was 2.1 and for UAG 4.6. The average inter-assay %CVs for AG was 9.5 and for UAG 12.8. Their %CVs were assessed over six assays. The lower limit of detection was 4 pg/ml.

Statistical analysis

Analyses were performed using SPSS software (version 24 for Windows; SPSS Inc., Chicago, Illinois) and GraphPad Prism® Version 6.04 (GraphPad Software, San Diego, USA). The Kolmogorov-Smirnov test was used to test normality of variables (data were considered to be normally distributed when $p > 0.05$). Comparisons across all groups were analysed with Kruskall-Wallis test. Comparisons between patient groups were analysed by Wilcoxon signed-ranks tests and Mann-Whitney U tests. Correlation analyses were done using Spearman's rank correlation test. Data were expressed as median [interquartile range (IQR)] as they were not normally distributed. P-values of < 0.05 were considered statistically significant.

RESULTS

Clinical characteristics

Table 1 shows the patients demographics, characteristics and disease history. Plasma AG and UAG were measured in a total of 69 acromegaly patients. One third of patients (20/60) on combination treatment had previously received surgery, while 7 of 60 patients had received surgery and radiotherapy in the past. In patients on PEGV monotherapy 2 of 4 patients had received both surgery and radiotherapy. In medically naïve patients with active disease 2 of 5 patients had previously received surgery. Clinical characteristics were comparable among the groups with respect to age, sex, BMI and previous therapy. Patients on combination treatment with LA-SSAs and PEGV and patients on PEGV monotherapy had IGF1 levels within the age- and sex adjusted normal limits.

Figure 1 shows the median fasting levels of AG, UAG and the AG/UAG ratio. Levels of AG and UAG were significantly different between the different groups (AG P = 0.004 and UAG P = 0.005 Kruskal-Wallis test).

Table 1. Characteristics of all patients in the three study groups.

Parameters	LA-SSA + PEGV	PEGV	Medically naïve acromegaly
No. of patients	60	4	5
Sex – male (%)	32 (53)	2 (50)	2 (40)
Age, years, mean (range)	54 (27 – 81)	62 (44 – 82)	44 (29 – 62)
BMI (kg/m^2)	28.8 (26.0 – 31.7)	30.9 (24.1 – 34.9)	30.3 (26.1 – 35.4)
Previous therapy			
Surgery (%)	20 (33)	0	2 (40)
Radiotherapy (%)	0	0	0
Surgery and radiotherapy (%)	7 (11.7)	2 (50)	0
IGF1 (nmol/l)	25.7 (22.2 – 32.3)	27.0 (22.4 – 36.0)	96.1 (64.0 – 166.0)
IGF1 x ULN	0.99 (0.85 – 1.12)	1.09 (0.90 – 1.28)	3.63 (2.20 – 4.50)
GH (µg/L)	4.6 (1.7 – 8.6)	3.1 (0.7 – 17.6)	15.4 (11.7 – 112.0)
Fasting glucose (mmol/l)	6.1 (5.6 – 6.8)	4.6 (4.3 – 4.9)	5.5 (5.1 – 6.2)
HbA1c (%)	5.9 (5.7 – 6.2)	5.6 (5.5 – 5.9)	5.6 (5.3 – 6.4)
Diabetes mellitus (%)	14 (23)	0 (0)	1 (20)
HOMA-IR score	1.1 (0.7 – 1.5)	NA	NA
HOMA-β score	67.2 (49.3 – 90.5)	NA	NA
PEGV dose (mg/week)	100 (60 – 160)	175 (95 – 289)	-

Data are expressed as median and interquartile range (IQR), unless specified otherwise. NA = not available

Median (IQR) AG levels were significantly lower in patients using combination treatment compared with patients using PEGV monotherapy and with medically naïve patients, 8.5 (2.9 – 21.1) pg/ml versus 60.5 (58.8 – 77.4) pg/ml (P = 0.0002 Mann-Whitney U test) versus 24.0 (12.6 – 49.7) pg/ml (P = 0.03 Mann-Whitney U test).

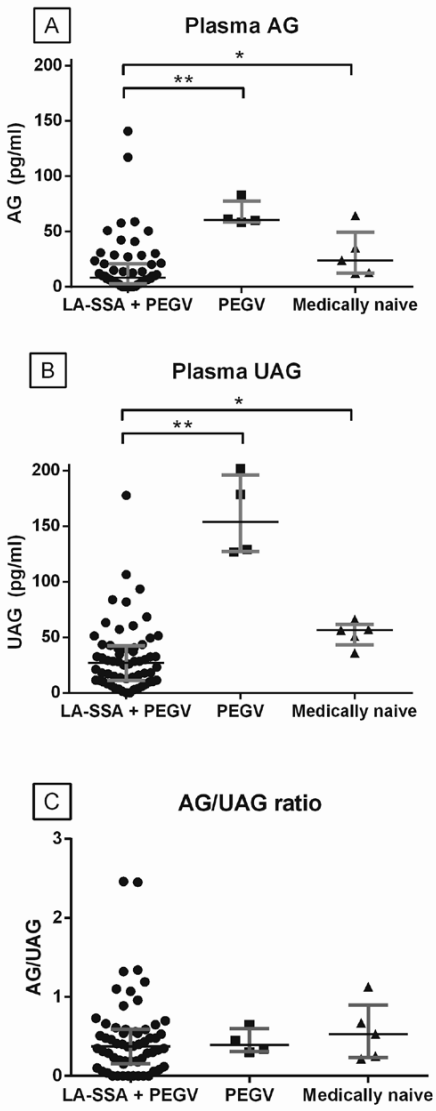

Figure 1. Plasma acylated ghrelin (AG), plasma unacylated ghrelin (UAG) and acylated ghrelin/unacylated ghrelin (AG/UAG) ratio in acromegaly patients on combination treatment of LA-SSAs and PEGV (N=60), acromegaly patients on PEGV monotherapy (N=5), and medically naïve acromegaly patients (N=4). Data are expressed as median ± interquartile range (IQR).

There was no significant difference in AG between patients using PEGV and medically naïve patients (*P* = 0.25 Wilcoxon Signed Rank test).

Although UAG levels were higher than AG levels in all groups, they showed a similar pattern. Median (IQR) UAG levels were significantly lower during combination treatment compared with the other groups, 26.9 (11.2 – 42.1) pg/ml versus 127.3 (153.7 – 196.0) pg/ml (P = 0.0002 Mann-Whitney U test), versus 56.3 (43.3 – 61.5) pg/ml (*P* = 0.03 Mann-Whitney U test). Similar to AG, UAG levels were not different between patients using PEGV and medically naïve patients (*P* = 0.13 Wilcoxon Signed Rank test).

AG/UAG ratios were not significantly different between the groups (P = 0.65). Median (IQR) AG/UAG ratio was 0.38 (0.16 – 0.59) in the combination group versus 0.40 (0.31 – 0.60) in the PEGV monotherapy group versus 0.53 (0.24 – 0.90) in medically naïve patients.

The highest AG level of 140.6 pg/ml was observed in a 55-year-old male patient with long-term controlled acromegaly with lanreotide Autogel 120 mg every 4 weeks and PEGV 100 mg per week. At the time of the blood withdrawal this patient had an IGF1 level of 1.17 times the ULN. His type 2 diabetes (HbA1c 6.9%) was well regulated with metformin and he received testosterone replacement due to hypogonadism.

In another patient, we observed the second highest AG level of 117.1 pg/ml and the highest observed UAG level of 177.5 pg/ml. This was a 27-year-old acromegaly patient who received a transsphenoidal hypophysectomy twice in the past. At the time of the blood withdrawal she was controlled with octreotide LAR every 4 weeks and PEGV 80 mg per week.

Concentrations of plasma AG (figure 1A) and UAG (figure 1B) were very low in the LA-SSA and PEGV combination treatment group. In 17 (28%) patients on combination treatment AG levels were undetectable, while in 4 (6.7%) patients UAG levels were undetectable.

We found no statistically significant relationship in acromegaly patients between plasma AG, UAG and AG/UAG ratios versus biochemical (GH, IGF1, HbA1c levels, HOMA-IR score, HOMA-B score and clinical parameters (age, sex, BMI, diabetes, previous surgery, previous radiotherapy, PEGV dose) among the groups. Although not significant, a negative correlation (r = −0.25, *P* = 0.06) was observed between previous surgery and UAG levels in patients on combination treatment.

DISCUSSION

Our main finding was that the AG/UAG ratio was not altered in acromegaly patients during different treatment regimens. AG and UAG levels were suppressed during combination treatment with LA-SSAs and PEGV, compared with patients using PEGV alone and with medically naïve active acromegaly. This is in line with literature on total ghrelin levels, although they lack information on the ratios between AG/UAG. It is questionable whether higher single AG and UAG levels have a physiological role during PEGV treatment and active disease if the ratios remain the same. Therefore, assessment of total ghrelin assessment is probably not clinically useful.

The AG/UAG ratio is probably more clinically relevant than measurement of total ghrelin. The main problem with previous studies examining ghrelin levels in acromegaly patients is the small number of samples assessed and the use of ghrelin assays that do not distinguish between acylated and unacylated ghrelin. Commercial radioimmunoassays (RIA) and one-site competitive ELISAs measure total human serum ghrelin by using labelled unacylated ghrelin as a tracer and a polyclonal antibody against the C-terminal end of human ghrelin. These assays overestimate ghrelin levels because they measure peptide fragments as well as full-length peptide. These fragments exist naturally in the circulation and lack the N-terminal region, but can also be artificially produced during the assay procedure. Two-site ELISAs use antibodies directed against both ends of the peptide, and are therefore highly specific and will only measure unfragmented ghrelin. Using their two-site sandwich ELISA as a comparison, Akamizu *et al* have demonstrated that about 40-60% of the total ghrelin measured by RIA is likely fragmented (38). This was further confirmed by Prudom *et al* who showed that their two-site sandwich-ELISAs for AG and UAG provided greater specificity (39). They found that dynamic changes in acyl-ghrelin were dampened and less visible in the RIAs. AG has a short half-life and in circulation it is rapidly degraded into UAG. For this reason, blocking deacylation is crucial for reliable measurements of AG and UAG (34, 37).

The observation of low AG and UAG levels during combination treatment of LA-SSA and PEGV suggests that this effect is caused by the somatostatin analogues. This finding is in line with previous studies showing that LA-SSAs suppress ghrelin levels in acromegaly patients (29, 31). Freda *et al* evaluated fasting- and serum ghrelin levels after an oral glucose tolerance test in patients with active acromegaly at baseline and after either surgery or administration of LA-SSAs. They observed that fasting total ghrelin levels were higher in patients after surgery, but fell significantly after treatment with LA-SSAs. They suggested that the postoperative lowering of insulin levels

and improved insulin sensitivity may have contributed to the postoperative rise of ghrelin levels (29). Another report has also suggested that acromegaly patients with greater insulin resistance have lower total ghrelin levels (28). Several studies have indicated that hyperinsulinemia inhibits AG and UAG secretion, conversely AG itself inhibits insulin secretion (14, 40-42). However, we could only assess insulin levels in the combination group and, therefore, cannot draw any conclusions on the difference in insulin sensitivity between the groups.

The observation of higher AG and UAG levels in patients using PEGV suggests that PEGV itself can stimulate AG and UAG. Roemmler *et al* showed that acromegaly patients using PEGV treatment had higher total ghrelin levels compared with healthy controls, patients with active acromegaly and inactive acromegaly (33). This finding suggests that treatment with PEGV might disrupt the feedback loop of ghrelin and GH, leading to elevated ghrelin levels. The ghrelin receptor (GHSR1a) is expressed in normal pituitary and somatotroph adenomas (43). GH administration has been shown to suppress total ghrelin levels in GH deficient patients (44). In rodents, GH administration in cultured stomach tissue reduced total ghrelin secretion, whereas hypophysectomy increased ghrelin levels (45-47). These results support the notion that GH exerts a negative feedback action on ghrelin secretion. Although there are no studies evaluating the direct effect of pegvisomant on ghrelin secretion, these data indirectly suggest that blockade of the GH receptor with pegvisomant leads to a positive feedback action on ghrelin secretion.

In patients using combination treatment median plasma AG levels were 8.5 pg/ml (range 0-140.6 pg/ml) and median UAG levels 26.9 pg/ml (0-177.5 pg/ml). This is considerably lower than levels that have been observed in healthy controls, measured using equivalent two-site sandwich assays and stabilized with AEBSF. Adrichem *et al* found median plasma AG levels of 57.2 pg/ml (range 10–273 pg/ml) and median plasma UAG levels of 64.9 pg/ml (range 8–331pg/ml) in 28 healthy controls, while Liu *et al* reported AG levels ranging from 43-366 pg/ml in four healthy volunteers (35, 48).

AG and UAG exert distinct effects on glucose homeostasis and insulin sensitivity. AG has diabetogenic actions, it induces insulin resistance and reduces insulin secretion. However, UAG displays antidiabetogenic actions (13-19). UAG alone or in combination with AG improves insulin sensitivity through the suppression of AG levels in obese subjects with type 2 diabetes (9). Studies have shown that insulin resistant obese subjects have an elevated AG/UAG ratio compared to insulin sensitive obese subjects (13, 24, 25), which can be explained by a relative UAG deficiency in obese subjects (23). Conversely, low AG/UAG ratios are associated with an improved metabolic state (21).

Prader-Willi syndrome is characterized by distinct nutritional phenotypes, from anorexia in infancy to hyperphagia and obesity in childhood (26). Recently, it was revealed that although total hyperghrelinemia was observed at all ages throughout life in PWS, the AG/UAG ratio changed over time driving opposite phenotypes. While the AG/UAG ratio was low during infancy it switched to a high AG/UAG ratio at later life (20, 22).

These data illustrate that AG and UAG have opposing actions, and that the AG/UAG ratio yields more physiological importance than measurement of absolute levels of AG and UAG. Although our patients on combination therapy had lower AG and UAG levels, the AG/UAG ratio was similar between all groups, this suggests that treatment with LA-SSAs and PEGV does not alter the relation of AG with respect to UAG.

In summary, the plasma AG/UAG ratio is not altered in acromegaly patients during medical treatment. Absolute levels of individual assessments of AG and UAG, however, were lower than observed during the assessment of total ghrelin levels. Assessment of the AG/UAG ratio is more clinically relevant, because it is a better reflection of the physiological bioactive state of ghrelin than measurement of total ghrelin. Therefore, we recommend assessment of AG and UAG separately and calculation of the AG/UAG ratio.

Declaration of interest

A. Muhammad received a research grant and a speaker fee from Novartis Pharma. A.J. van der Lely is a consultant for Novartis Pharma, Pfizer International, Alizé Pharma and received grants from Novartis Pharma, Ipsen Pharma International and Pfizer International. S. Neggers received research and speakers' fee grants from Ipsen Pharma International, Novartis Pharma, and Pfizer International. P.J. Delhanty, M. Huisman and J. A. Visser have nothing to declare.

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Author contributions

Conception and design: All authors
Collection and assembly of data: Ammar Muhammad
Data analysis and interpretation: All authors
Manuscript writing: All authors
Final approval of manuscript: All authors

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Chapter 7

Kupffer cells clear pegvisomant: observations in two patients with acromegaly

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Chapter 8

General discussion and future perspectives

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INTRODUCTION

The main goal of this thesis was to study the position of pasireotide LAR in the medical management of acromegaly in relation to pegvisomant. To meet this goal we conducted the pasireotide LAR and pegvisomant (PAPE) study with the aim to assess the efficacy and safety of switching to pasireotide LAR with and without PEGV treatment in acromegaly patients controlled with SRL and PEGV combination treatment. The results of the PAPE study were discussed in *Chapters 3 and 4*. Based on the PAPE study we formulated our recommendations on the optimal position of pasireotide LAR in the medical treatment algorithm of acromegaly. In *Chapter 5* we assessed whether the responsiveness to pasireotide LAR treatment was correlated with the responsiveness to first-generation SRL treatment and whether this also correlated with the somatostatin receptor subtype protein expression of the somatotroph adenoma. In *Chapter 6* we investigated acylated and unacylated ghrelin levels in acromegaly patients during combination treatment. Finally, in *Chapter 7* we undertook a pilot study to examine whether Kupffer cells are involved in the clearance of pegvisomant in vivo. This chapter will review the strengths and weakness of the studies presented in this thesis. The section ends with discussion on future developments and perspectives on new medical treatments of acromegaly.

Pegvisomant monotherapy

In **Chapter 2** we aimed to assess the efficacy and safety of withdrawing SRL treatment and switching to weekly PEGV monotherapy in 15 acromegaly patients controlled with combination treatment. We observed that switching to weekly PEGV monotherapy is a feasible treatment strategy without compromising biochemical control. After 12 months of PEGV monotherapy 73% of patients had IGF-I levels within the normal range. The efficacy of switching to PEGV monotherapy depends on the PEGV dose at baseline and the duration of SRL treatment. In this study we deliberately included patients that used low doses of PEGV of ≤ 80 mg/week, as we expected that these patients could maintain control of disease activity in the short term after withdrawal SRL treatment. We observed that IGF-I levels started to increase 4 months after SRL treatment discontinuation. This so-called carry-over effect of SRLs confirms observations in previous studies (1-3). We observed a large variation in PEGV serum levels between controlled and non-controlled subjects which was remains not well understood (4). The major drawback of this study is the small sample size.

Pasireotide and pegvisomant study

The aim of the PAPE study was to assess the real life efficacy and safety of switching to PAS-LAR in acromegaly patients biochemically controlled with SRL and PEGV combination treatment. The patients included in this study all received SRL and PEGV

combination treatment because they were partially to completely resistant to SRL monotherapy. To assess the efficacy of PAS-LAR on IGF-I normalization patients had to be 'uncontrolled' which was achieved by reduction of the PEGV dose by 50% for 3 months. We found that PAS-LAR treatment normalized IGF-I levels in 77% of patients and the PEGV dose could be reduced by 50% at 48 weeks. While the efficacy of PAS-LAR at 24 weeks is partly influenced by the carry-over effect of first-generation SRLs which can take up to 4 months, at 48 weeks this was not the case. We observed a heterogeneous and wide range of clinical response to PAS-LAR treatment. This ranged from patients who were overresponsive to PAS-LAR and could reduce PAS-LAR to the lowest available dose of 20 mg, and patients who were completely unresponsive and could not reduce their high PEGV dose. This heterogeneity is illustrated by the observation that the patients in the PAS-LAR monotherapy group had a lower disease activity at baseline, with lower PEGV dose, IGF-I levels and GH levels compared with patient in the PAS-LAR and PEGV combination group.

An important observation in our study was the potent reduction of IGF-I after the first PAS-LAR injection. Although this early onset of effect of PAS-LAR is to some extent influenced by the carry-over effect of SRL treatment, this rapid reduction is probably caused by suppression of insulin secretion. The concomitant suppression of IGF-I secretion and insulin secretion after the first two PAS-LAR injections suggests that suppression of insulin is an important mechanism which explains the efficacy of PAS-LAR. Another explanation is that unlike octreotide, pasireotide induces less SST_2 internalization which could imply that there is a lower likelihood desensitization to pasireotide treatment.

Safety

Hyperglycemia and diabetes mellitus

The most common and clinically relevant adverse event during the PAPE study was the development of hyperglycemia and diabetes mellitus. At baseline 32.8% of patients had diabetes in our cohort, which increased to 77% at the end of the study. The majority of patients developed mild to moderate hyperglycemia which occurred mainly during the core study and was manageable with a combination of metformin and or vildagliptin. Several reasons account for the higher frequency of hyperglycemia in our study compared with previous clinical studies (5-8). First we included more patients with diabetes, as we used a more liberal HbA1c cut-off (<9%) as inclusion criterion. Secondly, we included older patients and more patients with pre-existent diabetes who were already on antidiabetic medication. Third, the reduction of the PEGV dose during the study progressively worsened glucose metabolism.

As expected, and in line with previous studies (7, 9), the development of severe hyperglycaemia after PAS-LAR treatment was correlated with baseline HbA1c and use of antidiabetic medication (*Chapter 3*). In order to find better predictors for pasireotide-induced hyperglycaemia we performed an oral glucose tolerance test (OGTT) at baseline. We estimated ß-cell function as a measure for insulin secretion based on a model by *Stumvoll et al* (10, 11). An important caveat for the interpretation of insulin secretion during the OGTT is the use of antidiabetic medication. While we excluded the use of insulin as an obvious confounder, the results should be interpreted with caution in patients using sulfonylureas and GLP-I analogues as both antidiabetic drugs increase insulin secretion (12, 13). It was not feasible to discontinue all antidiabetic drugs before the OGTT. A novel finding is that we observed an inverse correlation between both the insulin area under the curve and the (residual) ß-cell function at baseline with the increase in HbA1c between baseline and at 24 weeks (*Chapter 4*). This entails that patients with attenuated (first-phase) insulin secretion had a greater risk of pasireotide-induced hyperglycaemia during the study. These data demonstrate that the baseline ß-cell function is an important independent determinant for hyperglycaemia during PAS-LAR treatment and probably a better marker than single measurements of HbA1c or fasting plasma glucose level. We do not know to what extent GLP-I secretion accounts for the increase in HbA1c levels during the study, as we did not measure GLP-I levels. Of note, the involvement of incretin response was previously suggested by van der Hoek et al (14). They observed that a single injection of short acting pasireotide elicited an acute increase in glucose levels after pasireotide injection in acromegaly patients, which could not be exclusively explained by concomitant insulin suppression, suggesting the involvement of the incretin response (14).

The patients using PAS-LAR and PEGV combination treatment did not have a lower HbA1c level than patients using PAS-LAR monotherapy. This observation suggests that the insulin sensitizing effect of PEGV cannot compensate for the insulin and incretin suppressive effect of PAS-LAR.

Our study was not designed to evaluate which antidiabetic treatment was most effective for pasireotide-induced hyperglycaemia. Previous studies have recommended DPP-4 inhibitors and GLP-I analogues for treatment of pasireotide-induced hyperglycemia. Following the Dutch reimbursement regulations we could only start DPP-4 inhibitors and GLP-I analogues after failure to achieve glycaemic control with maximum doses of metformin and sulfonylurea. However, ideally initiation of incretin-based antidiabetic drugs is the first-line treatment of choice.

Exocrine pancreatic insufficiency

We observed a higher frequency of steatorrhea during PAS-LAR treatment in acromegaly patients than reported previously in clinical trials (5, 6). This adverse event was in general transient and improved with pancreatic enzyme supplementation (*Chapter 3*). Inhibition of exocrine pancreatic function is a well-known (side) effect of SRLs (15). To our knowledge, there are no studies that directly compared the effect of octreotide versus pasireotide on exocrine pancreatic function. But, because of its broader SSTR binding profile, pasireotide has been suggested to be more effective than octreotide in suppressing exocrine pancreatic function (16). For example, pancreatic surgeons have used pasireotide to suppress leakage of exocrine secretions following pancreatectomy (17), with the aim to reduce the risk of pancreatic fistula formation, a major cause of mortality and morbidity for these patients (18).

Costs

We hypothesized that the PEGV sparing effect of PAS-LAR would result in a reduction in treatment costs. Although we have not performed a cost-effectiveness analysis, it is likely that switching to PAS-LAR will not lead to a long-term reduction of overall treatment costs. As mentioned in the discussion of **Chapter 3**, the PEGV sparing effect of PAS-LAR would likely be offset by the diabetes mellitus related medication and healthcare costs.

Current consensus guideline second line medical treatment

In 2018, the Acromegaly Consensus Group published updated consensus guidelines with recommendations on the medical management of acromegaly (19). The consensus statement recommended medical treatment for patients with persistent disease activity despite surgical resection of the adenoma and patients in whom surgery is not advisable (19). In line with the previous consensus statement published in 2014 (20), first-generation SRLs remain the first-line treatment for patients with persistent disease activity after surgery. The new consensus guideline recommends either PAS-LAR or PEGV treatment based on the presence or absence of clinically relevant residual tumour and impaired glucose tolerance. PEGV was recommended for patients with impaired glucose metabolism and/or those who experience worsening hyperglycemia on SRL treatment. PAS-LAR was recommended for patients with a clinically relevant residual tumour unsuitable for resection and without impaired glucose tolerance. However, in patients with a clinically relevant residual tumour and impaired glucose tolerance SRL and PEGV was recommended (19). In the next paragraphs we will outline our expert-opinion based clinical recommendations for the medical management of acromegaly with a focus on the position of PAS-LAR and the differences with the current consensus guideline.

Recommendations for the medical management of acromegaly

In figure 1 we present our recommendations for the second line medical management of acromegaly based on our clinical experience with PEGV and PAS-LAR (*Chapter 3 and 4*) and clinical evidence from previous studies. In accordance with the consensus guideline (19), first-generation SRLs such as lanreotide autogel (ATG) and octreotide LAR are considered the first-line medical treatment of acromegaly (figure 1).

Second-line and alternative treatments

In line with the consensus guideline (19), we recommend PEGV combined with or substituted for first-generation SRL treatment as the second-line treatment of choice in patients with <20% IGF-I reduction (no significant response) during first-generation SRL monotherapy (figure 1).

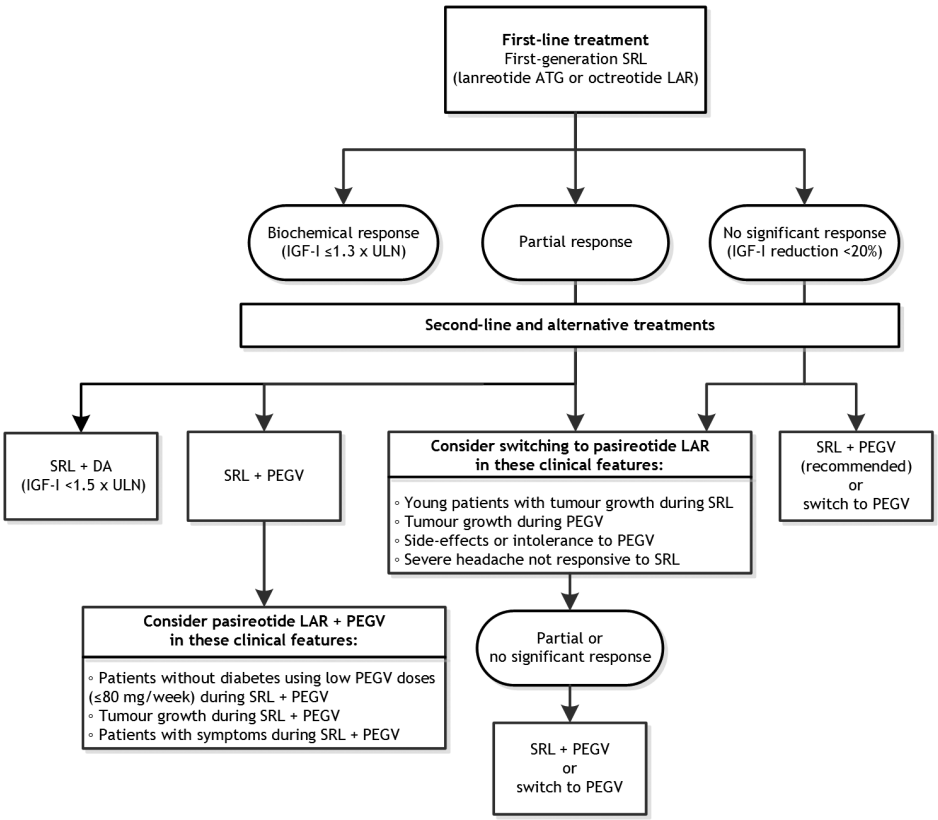

Figure 1. Proposed algorithm for the medical management of acromegaly. Radiation therapy is not mentioned in this algorithm, but it should be considered in patients with biochemically persistent disease and/or tumour growth despite surgery or medical therapy. Abbreviations: ATG, Autogel; DA, dopamine agonist; IGF-I, insulin-like growth factor 1; LAR, long-acting release; SRL, somatostatin receptor ligand; PEGV, pegvisomant; ULN, upper limit of normal.

PEGV, alone or in combination with first-generation SRL, can normalize IGF-I levels in the majority of patients, provided that the PEGV dose is sufficiently increased. In combination treatment the recommended starting dose with either lanreotide ATG or octreotide LAR every 4 weeks is the highest approved dose of respectively 120 mg and 30 mg. To determine the optimal PEGV starting dose to achieve IGF-I normalization, the prediction formulas of *Franck et al* can be used (21).

In contrast to the current consensus guideline (19), we recommend first-generation SRLs and PEGV combination treatment as the second-line treatment of choice in all non-responders (figure 1). Combination treatment has the advantage of a lower required PEGV dose to normalize IGF-I levels compared with PEGV monotherapy (23-25). Due to PEGV dose reduction it may reduce the injection frequency for patients. In a subset of patients, combination treatment might improve QoL compared to SRL monotherapy, including those who are biochemically controlled (26). PEGV monotherapy does not increase tumour size, and combined with SRLs it does control tumour size and may induce tumour shrinkage in a vast majority of patients (25). Another advantage of SRL and PEGV combination treatment is that SRLs are generally effective in resolving headaches through a proposed mechanism of inhibition of nociceptive peptides, which makes it the preferable treatment for patients with headache (27, 28). In general, if patients report improvements in clinical symptoms during SRL monotherapy, we recommend imitating SRL/PEGV combination treatment, except in those patients with poorly controlled or brittle diabetes during SRL treatment. These patients are good candidates for PEGV monotherapy, because PEGV improves glucose metabolism by reducing insulin resistance (29-34). This is in accordance with the current consensus guideline (19), which also recommends PEGV monotherapy in patients without biochemical response to first-generation SRL treatment, but with pre-existing impaired glucose metabolism.

In patients with partial response to first-generation SRLs, defined as a significant reduction in IGF-I levels without normalization, we recommend combination treatment with cabergoline (if IGF-I ≤1.5 x ULN) (35) (figure 1). In contrast to the current consensus guideline, who propose considering co-treatment with cabergoline (if IGF-I <2.5 x ULN) in patients with inadequate control on first-generation SRL treatment, we recommend co-treatment with cabergoline if IGF-I levels remains modestly elevated (IGF-I ≤1.5 x ULN), since IGF-I normalization was observed only in those patients (35). Besides, cabergoline is recommended in patients with no access to PEGV. Although the use of PEGV, combined with or substituted for first-generation SRLs in partial responders, is not covered by the current guidelines (19), we recommend SRL and PEGV combination treatment as the second-line treatment of choice (Figure 1).

Moreover, due to the marginal additional efficacy of dose escalating and shortening of the dosing intervals of SRLs in partial SRL responders (2, 36, 37), we recommend switching these patients to SRL and PEGV combination treatment or to consider pasireotide LAR monotherapy according to the proposed algorithm (figure 1).

Switching to pasireotide LAR monotherapy can be considered as an alternative to PEGV monotherapy or combination therapies in patients with the following baseline clinical characteristics (Figure 1):

- Macroadenomas in young patients (aged <40 years) that show tumour growth during first-generation SRL monotherapy (i.e., clinically aggressive tumours) (38). Pasireotide LAR monotherapy should be considered early in the management of resistant tumours in young patients, and as a treatment step before starting radiotherapy. This is in line with the current consensus guideline (19), which recommends switching from first-generation SRL treatment to pasireotide LAR monotherapy in patients with a clinically relevant residual tumour that is insuitable for resection.
- The same argument can be applied for patients previously not controlled by first-generation SRLs with tumour growth during PEGV monotherapy. Tumour growth may reflect the presence of an aggressive tumour, for that reason pasireotide LAR monotherapy can be considered as a next treatment step before starting radiotherapy.
- Patients previously not controlled by first-generation SRLs who experience side-effects or who are intolerant to PEGV monotherapy, may benefit from switching to pasireotide LAR monotherapy.
- Patients with headache not responsive to first-generation SRL treatment. These patients with severe incapacitating headache have high a likelihood to experience improvement in headache symptoms with pasireotide LAR treatment.

Instead of PEGV, either as monotherapy, or in combination with first-generation SRL, switching to pasireotide LAR therapy combined with PEGV can be considered as third-line treatment option in patients with the following baseline clinical characteristics (Figure 1):

- Patients without diabetes that use low PEGV doses (≤80 mg/week) during combination therapy with first-generation SRL and PEGV. Due to the PEGV sparing effect of pasireotide LAR, the PEGV doses can be reduced or some cases even discontinued.
- Patients with tumour growth during first-generation SRL and PEGV combination therapy. We postulate that pasireotide LAR monotherapy may improve tumour size control or even tumour shrinkage. However, at present there are no data on tumour response to pasireotide LAR and PEGV combination therapy available.
- Patients biochemically controlled during first-generation SRL and PEGV combination therapy, who use first-generation SRL every 3 weeks or have symptoms of active

acromegaly in the fourth week after first-generation SRL administration, may experience symptomatic improvement after switching to pasireotide LAR and PEGV combination therapy.

The package insert recommends starting pasireotide LAR 40 mg every four weeks (39), but we recommend to start with either 40 or 60 mg pasireotide LAR every four weeks. We expect that patients with SRL partial responders will require lower doses of pasireotide LAR, and then we recommend starting with pasireotide LAR 40 mg. For combination treatment with PEGV we recommend initiation of pasireotide LAR at a dose of 60 mg every four weeks while at the same time tapering down the PEGV dose by 33%.

In our experience, IGF-I levels decrease more rapidly with pasireotide LAR than with octreotide LAR or lanreotide ATG after treatment initiation. While it can take up to 6 months for IGF-I levels to normalize with first-generation SRLs therapy, pasireotide LAR induces a significant reduction in IGF-I levels within 2 months (40), and the early response is associated with the more long-lasting response at 48 weeks (41). If biochemical control cannot be achieved by pasireotide LAR monotherapy, then either switching to PEGV monotherapy or combination treatment with first-generation SRLs should be considered (figure 1). The same applies for pasireotide LAR and PEGV combination treatment, but in this case the patients are switched back to their previous PEGV dose, alone or combined with SRL treatment.

Management of pasireotide-induced hyperglycemia

Optimal management of hyperglycemia during pasireotide LAR therapy is essential because of several reasons. First, optimal management is required to initially control diabetes as a long-term risk factor for cardiovascular disease in acromegaly. Secondly, early onset proactive management of hyperglycemia after pasireotide LAR initiation is likely to improve patient long-term compliance with pasireotide LAR. A wider use of pasireotide LAR in the medical management of acromegaly depends on the physician's willingness to try pasireotide LAR and to applicate it in the appropriate patients. The challenge for the endocrinologist is to evaluate in each individual patient whether advantages of pasireotide LAR such as higher likelihood of biochemical control, tumour size control and quality of life outweigh side effects of hyperglycemia and diabetes. We provide recommendations to achieve optimal management of pasireotide-induced hyperglycemia in figure 2. In contrast to previous published recommendations for management of pasireotide-induced hyperglycemia (42-45), we use a more liberal strategy in frequency of monitoring our patients.

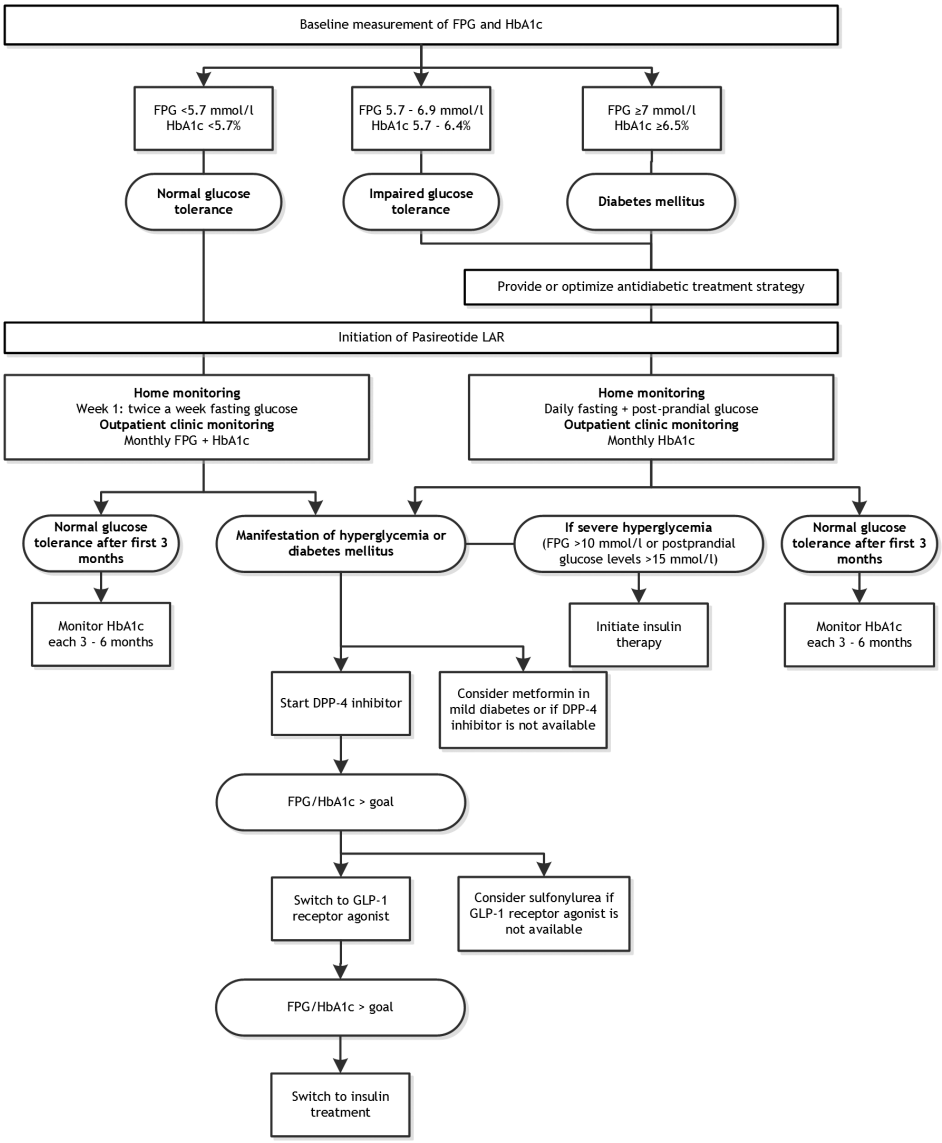

Figure 2. Proposed algorithm for management of pasireotide-induced hyperglycemia. Abbreviations: DPP-4, dipeptidyl peptidase-4; FPG, fasting plasma glucose; GLP-1, glucagon-like peptide 1; HbA1c, glycated haemoglobin, LAR, long-acting release.

Baseline HbA1c is the most important predictor for development of diabetes after pasireotide LAR administration (7, 9, 40, 41). We recommend that baseline FPG and HbA1c levels should be assessed prior to initiating pasireotide LAR treatment. In case of impaired fasting glucose and/or diabetes at baseline, lifestyle management and/or adequate antidiabetic treatment should be started or optimized before initiating pasireotide LAR. Clinical studies have consistently shown that after pasireotide LAR

initiation FPG and HbA1c levels rise the first 3 months, and remain stable without further antidiabetic therapy (7, 9, 39, 46, 47). Therefore, proactive blood glucose monitoring is important, especially in the first 3 months after initiation. After the first 3 months of pasireotide LAR treatment, the intensity of blood glucose monitoring can be decreased. In contrast to *Wildemberg* (44) and *Samson* (43), who propose home blood glucose monitoring twice a week for 3 months for patients with normoglycemia, and we recommend monitoring at the outpatient clinic with monthly FPG and HbA1c the first 3 months. Because of the low likelihood of deterioration of glucose tolerance in young patients (aged <40 years) with normal glucose tolerance at baseline, this latter group can be monitored less intensively and with a lower frequency (figure 2). However, patients with pre-existing impaired glucose tolerance and diabetes, which make up the majority of acromegaly patients, require more extensive monitoring. These patients should receive home blood glucose monitoring the first 3 months by measurement of daily fasting and 2-hours postprandial glucose levels, and monthly HbA1c monitoring at the outpatient clinic. For diabetic patients using insulin, we advise continued home blood glucose monitoring with additional HbA1c monitoring especially at the start of pasireotide LAR treatment, and thereafter every 3 months in accordance with the current American Diabetes Association (ADA) guidelines (48).

If glucose monitoring reveals glucose in the diabetic range per ADA criteria, we recommend first-line treatment with a dipeptidyl peptidase-4 (DPP-4) inhibitor and to consider metformin in patients with mild diabetes or if DPP-4 inhibitors are not available (figure 2). If patients do not achieve their ADA criteria based FPG/HbA1c goal with these interventions, we recommend switching to a GLP-1 receptor analogue once daily. If a GLP-1 receptor agonist is not available, we recommend treatment with sulfonylureas. Treatment with insulin is required in patients intolerant to GLP-1 analogues or if GLP-1 analogues fail to provide glycemic control. In case of severe hyperglycemia (i.e., FPG >10 mmol/l or postprandial glucose levels >15 mmol/l), which may occur even after the first injection, it is important to promptly initiate insulin treatment. In our experience, diabetic patients using insulin treatment or high doses of oral antidiabetic agents have a high risk of severe hyperglycemia after the first injection of pasireotide LAR. On the other hand, in nondiabetic patients the onset of hyperglycemia occurs later during treatment and is less severe. Therefore, it is paramount to be aware of early-onset severe hyperglycemia and promptly initiate antidiabetic therapy, but also to be vigilant about the development of late-onset hyperglycemia and diabetes.

Pasireotide-induced hyperglycemia is reversible after discontinuation of pasireotide LAR (44). Our data indicate that within 8 weeks after discontinuation of pasireotide

LAR and re-initiation of first-generation SRL or PEGV, FPG and HbA1c levels return to baseline (40). To prevent hypoglycemia in patients using insulin or sulfonylureas, it is recommended to gradually taper down these antidiabetic agents with more frequent blood glucose monitoring. Sulfonylureas should be rapidly tapered down within the first 2 weeks to avoid hypoglycemia, whereas GLP-I receptor analogues can be safely continued with a lower dose for a longer period.

Prediction of pasireotide LAR treatment response in acromegaly

We hypothesized that SST_5 protein expression of the somatotroph adenoma was correlated with clinical responsiveness to PAS-LAR in acromegaly. However, in our cohort we observed a positive correlation between the efficacy of PAS-LAR and the efficacy of first-generation SRLs. This was confirmed by the observation that SST_2 protein expression of the somatotroph adenoma, and not of SST_5, correlated with clinical responsiveness to PAS-LAR. Moreover, in the same patients we found a strong correlation between the percentage of IGF-I reduction after first-generation SRL treatment and response to PAS-LAR treatment during the PAPE study. This does not mean that pasireotide and octreotide/lanreotide are equally effective in reducing IGF-I levels. The effect of pasireotide and octreotide on GH suppression is superimposable both in vitro and in vivo, but pasireotide is more effective in reducing IGF-I levels in vivo (5, 49). In other words, overall, patients who responded to first-generation SRLs also seemed to respond to PAS-LAR treatment and vice-versa. The latter is illustrated by our observation that the three patients using the highest doses of PEGV could not reduce their PEGV dose after switching to PAS-LAR (*Chapter 4*). Our observation that SST_2 is the main receptor driving the biochemical responsiveness to PAS-LAR treatment builds on previous preclinical studies in primary cultures of somatotroph adenomas that pasireotide exerts its anti-secretory effects mainly via activation of SST_2 (49-52). Besides its broader binding affinity for the different SST_2 subtypes, pasireotide activates intracellular second messenger pathways and modulates SST internalization and trafficking in a manner different from SRIF and octreotide (53, 54). For example, compared with octreotide, pasireotide induces less SST_2 internalization and quicker recycling of SST_2, which counteracts SST_2-desensitization. These findings indicate that the mere concept of binding affinity is too limited to explain the complex biological activity of pasireotide. It is important to clarify that our results contrasted findings of *Iacovazzo et al* who found that SST_5 expression on somatotroph adenomas correlated with responsiveness to PAS-LAR (55). As discussed in *Chapter 5*, the opposing results between the Iacovazzo study and our study is related to a different selection of patients. In our study we included patients who were mainly partially responsive to SRLs who received PEGV treatment, while *Iacovazzo et al* used only SRL resistant patients.

Our results suggest that SST_2 is the main driver of GH suppression to pasireotide in somatotroph adenomas, but we cannot exclude the involvement of SST_5 in GH suppression (49, 55). In general, in patients that are partial responders to SRLs, pasireotide acts mainly via SST_2. In presence of low SST_2 expression or in adenomas resistant to SST_2 targeting, pasireotide may also act via SST_5, while this is not the case for first-generation SRLs. Therefore, we can understand that in completely resistant patients (55) we observe an effect mainly driven via SST_5. As recently carefully reviewed by *Gatto et al* (56), using the classification of acromegaly proposed by *Cuevas-Ramos et al*, pasireotide mainly acts via SST_2 in type 1 disease (good response to first-generation SRLs, high SST_2 expression), while its effects could also be mediated via SST_5 in type 2 and type 3 acromegaly (unsatisfactory response to first-generation SRLs, low SST_2 expression).

Ghrelin acromegaly

In **Chapter 6** we investigated levels of plasma acylated ghrelin (AG), unacylated ghrelin (UAG), and the AG/UAG ratio in three groups of patients with acromegaly; in patients using SRL and PEGV combination treatment, patients using PEGV monotherapy, and medically naïve patients. We observed that both AG and UAG were suppressed during PEGV and SRL combination treatment and in some patients even undetectable. The underlying mechanism is probably a direct inhibitory effect of somatostatin on ghrelin secretion (57). Interestingly, the positive feedback action of PEGV on ghrelin secretion as observed in our study and by *Roemmler et al* (58) cannot offset the ghrelin suppressive effects of somatostatin. This observation mirrors our observation in the PAPE study that the insulin suppressive effect of pasireotide LAR cannot be compensated by the improvement in insulin sensitivity by treatment with pegvisomant.

We observed that the AG/UAG ratio was similar irrespective of medical treatment. Secondly is that the assessment of the AG/UAG ratio is clinically more relevant than measurement of total ghrelin, because the total ghrelin assay is unspecific as it measures a combination of inactive AG/UAG fragments and the full-length peptide. An advantage of the AG/UAG ratio is that it takes into account the different and sometimes opposing biological actions of AG and UAG. An important limitation of this study was the low number of patients included in the control groups. Also, in general there is a lack of the healthy volunteer data on AG and UAG levels measured with the two-site sandwich ELISA. Future studies should account for this.

Pegvisomant clearance

In the 1970s *Davis et al* described PEGylation as a means to decrease the immunogenicity of non-human proteins (59-61). Since then at least 15 registered PEGylated

drugs are in clinical use, and more are under clinical investigation (62). Although PEGV has been used in clinical practice for over a decade and is one of the most PEGylated drugs available, it is not well understood how PEGV is metabolized and cleared from the body. Understanding PEGV clearance could be important to understand the differences in PEGV serum levels between patients. It is known that the presence of several polyethylene glycol (PEG) molecules in the drug reduce the immunogenicity and improve the clearance of PEGV (63). Because PEG is a non-biodegradable molecule it is assumed that PEG molecules are cleared from the circulation by cells from the mononuclear phagocyte system, such as Kupffer cells (64, 65). Animal studies have shown that repeated administration of PEGV was associated with the presence of vacuolated macrophages (66). However, no studies have demonstrated this in patients with acromegaly treated with PEGV. As reported in *Chapter 7* we undertook this study to examine whether PEGV is expressed in the liver, and if so, whether it co-localizes with Kupffer cells. Using hepatic tissue material obtained from two acromegaly patients that were treated with PEGV, we observed immunohistochemical expression of PEG in the sinusoids where Kupffer cells are located. Immunofluorescence studies confirmed that PEG co-localized with Kupffer cells, but not with hepatocytes. The observation that PEG was not observed in hepatocytes is supported by a fundamental study by Tsoi et al (67). They examined the blood clearance mechanism of PEGylated nanomaterials in relation to hepatic cells of rats. They observed that after injection of PEG-coated quantum dots (PQD), PQD were internalized by cell mononuclear phagocyte system (MPS). The highest uptake was observed in Kupffer cells, but no uptake in hepatocytes. However, these data should be interpreted with caution as quantum dots have a substantially smaller molecular size compared to large molecules like PEGV. Kupffer cell activation in both cases can also be caused by liver diseases, which can impact hepatic metabolism of PEGV. Despite the possible confounding effect of liver diseases which could activate Kupffer cells and change PEG metabolism, both patients showed internalization of PEG in Kupffer cells. This suggests that the uptake of PEGV by Kupffer cells is independent of the underlying liver disease. Unfortunately, it is very difficult to obtain additional liver tissue samples of acromegaly patients using PEGV that do not have liver disease. We could also not study the relation between hypertransaminasemia and PEGV treatment (i.e. PEGV-induced hepatotoxicity), because both patients had normal transaminase levels during PEGV treatment. In this study we highlighted the possible role of Kupffer cells in the clearance of PEGV.

GENERAL CONCLUSION OF THESIS

The findings presented in this thesis represent a step forward in our understanding of the position of PAS-LAR in the modern medical treatment of acromegaly. The results of the PAPE study demonstrated that PAS-LAR has a high efficacy, illustrated by a 50% PEGV sparing effect, and substantial number of patients who could switch to PAS-LAR monotherapy. While PAS-LAR was in general well tolerated, the high efficacy came at the expense of a high frequency of hyperglycemia-related adverse events. With respect to the long-term treatment of acromegaly with PAS-LAR, the benefits of biochemical control and the improvement in quality of life should be weighed against the disadvantages of potential (short- and long-term) complications of diabetes. The main conclusion of our recommendations is that, in general, SRL and PEGV combination treatment remains the second line medical treatment option of choice, and PAS-LAR should be reserved as a third line medical treatment option. However, in specific subgroups of patients PAS-LAR is a viable and preferred treatment option for patients with tumour growth during SRL and/or PEGV treatment, patients with specific symptoms not responsive to SRLs, and patients using low doses of PEGV without diabetes. Besides baseline glycemia and use of antidiabetic drugs, residual beta cell function, as measured with the OGTT, is an important predictor for pasireotide-induced hyperglycemia. In our cohort previous response to SRL treatment and SST_2 protein expression of the somatotroph adenoma was strongly correlated with response to PAS-LAR treatment. In patients well-controlled on SRL and low-dose PEGV combination treatment switching to weekly PEGV monotherapy is a viable treatment strateqy without significant compromise of biochemical control.

FUTURE PERSPECTIVES

In this thesis the results of the PAPE study are presented until 48 weeks. In order to observe whether PAS-LAR provides long-term efficacy and safety longer follow-up studies are needed. The insulin and incretin suppressive effects of pasireotide suggest that it is plausible that pasireotide increases the life time risk of diabetes in patients who do not develop hyperglycemia initially after treatment initiation. Longer follow-up studies are required to evaluate the occurrence of late-onset hyperglycemia and whether diabetes indeed occurs earlier in patients using PAS-LAR. The availability of PAS-LAR has expanded the therapeutic options for patients to achieve biochemical control. However, whether PAS-LAR is indeed more effective van first-generation SRLs in reducing tumour volume has not extensively been studied. It is interesting to analyze whether PAS-LAR induces clinically relevant tumour size reduction in our

cohort, and whether this correlates with PAS-LAR treatment response score and SSTR protein expression of the somatotroph adenoma. Besides biochemical control and tumour size control, quality of life is an important treatment goal. Many acromegaly patients have impaired quality of life despite (long-term) biochemical control. In the PAPE study we systematically assessed quality of life using validated acromegaly health-related quality of life questionnaires. In the future we aim to publish our findings with respect to quality of life, with ultimate goal to predict which subset of patients is likely to experience an improvement in quality of life. Previous studies have indicated that patients with headache not responsive to SRL treatment might benefit from pasireotide treatment (68, 69). There is also a pressing need for robust biomarkers that reliably predict the response to PAS-LAR treatment. Beside SST_2 and SST_5 protein expression, other markers are needed, such as AIP protein expression, ß-arrestin expression, the granulation pattern viewed under an electron microscope, pituitary adenoma signal intensity with MRI. Preferably all these markers should be integrated and validated in multiple cohorts of centers of excellence that treat a high volume of patients with acromegaly. As increased PEGV serum levels are observed during SRL and PEGV combination treatment the question arises whether PAS-LAR elicits the same effect.

FUTURE DEVELOPMENTS IN MEDICAL TREATMENT

Many new drugs are currently under investigation for acromegaly. Given the lack of efficacy of conventional SRLs and the hyperglycemia-related adverse events of PAS-LAR, there is a need for new drugs which provide improved biochemical control with less side effects and less frequent injections. Because PEGV requires frequent high dose injections, efforts have been made to develop a **long-acting GHR antagonist** (GHRA). A novel concept to extend the half-life of a GHRA is by generating a chimeric fusion molecule comprised of a mutated GH linked to GHBP (70). The investigators have developed a technology to link a ligand to the extracellular domain of its receptor, effectively providing the ligand with its own native binding protein within a single molecular entity. This ligand-receptor fusion molecule exists in solution as both a monomer and dimer where the GH moiety of one molecule bound to the receptor portion of another molecule in a head-to-tail reciprocal dimer (70). The pharmacokinetic characteristics suggested this fusion molecule could requires injection every 21–28 days compared to daily GHRA administration. In a proof of concept study, three fusion GHRA molecules have demonstrated delayed clearance and biological activity in vivo (71). An advantage of this technology is that no PEGylation is required to generate the long-acting GHRA, but whether this drug is cheap enough to manufacture

remains to be determined. **Somatoprim** (DG3173) is an investigational SRL with high affinity for SST_2, SST_4, and SST_5 with a pharmacokinetic profile similar to octreotide (72, 73). Somatoprim showed selectivity for GH suppression with minimal inhibition of insulin secretion (72, 74). Recently, a phase II trial of somatoprim in treatment-naïve acromegaly patients was completed (73). **AP102** is a dual SST_2/SST_5-specific SRL that has been designed with the aim to reduce GH secretion without causing hyperglycemia. AP102 is a disulfide-bridged octapeptide SRL containing synthetic iodinated amino acids. Receptor binding studies have demonstrated that AP102 binds with similar subnanomolar affinity to SST_2 and SST_5 as to native somatostatin 14 (75). In contrast to pasireotide, AP102 does not bind to SST_1 and SST_3 (75, 76). In a recent study in rats, the effects of AP102 and pasireotide were investigated on hormonal secretion and glucose metabolism following acute (single dose) and chronic (28-day) administration (77). In contrast to pasireotide, acute and chronic administration of AP102 did not lead to significant hyperglycemia or impaired glucose tolerance. The authors suggested that the neutral effect of AP102 on glucose metabolism was caused by balanced inhibition of insulin and glucagon secretion, and uninhibited GLP-I secretion (77). Despite that both compounds have high binding affinities to SST_2 and SST_5, they produced different effects on glucose metabolism. Several possible mechanisms could account for this discrepancy. In addition to SST_2 and SST_5, pasireotide also binds with high affinity to SST_1 and SST_3, whereas AP102 shows selective binding to only SST_2 and SST_5. The broader SSTR binding profile could therefore also contribute to pasireotide-induced hyperglycemia. Besides SST_5, SST_1 is also expressed on pancreatic ß-cells (78). Recently, knocking down expression of SST_3 through the use of selective SST_3 antagonist mediated glucose stimulated insulin secretion by ß-cells (79, 80). The effects of AP102 on SST_2 and SST_5 phosphorylation patterns has not been studied yet, but may also account for the differences in glucose metabolism compared with pasireotide.

To what extent these upcoming agents will actually benefit acromegaly patients in real life remains to be seen. It is likely that there is no single agent that can be considered the “perfect agent” for all patients.

Besides medical treatment, lifestyle interventions such as diet and exercise may also contribute to biochemical and symptomatic control of acromegaly. Hypothetically, a carbohydrate restricted or ketogenic diet could improve insulin resistance, and therefore reduce the required dose of PEGV in acromegaly.

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SUMMARY

Acromegaly is a chronic systemic condition caused by excessive growth hormone (GH) secretion from a pituitary adenoma. Combination treatment with first-generation somatostatin receptor ligands (SRLs) and pegvisomant (PEGV) is an established effective and safe treatment to normalise IGF-I levels in acromegaly. However, this combination treatment requires chronic monthly SRL injections and daily to weekly PEGV injections which can be troublesome for patients and negatively impact patient adherence. We therefore hypothesized in **chapter 2** that switching patients from SRL and PEGV combination treatment to PEGV monotherapy can reduce the total number of injections, and therefore improve patient adherence. After 12 months treatment with PEGV monotherapy 73% of patients achieved IGF-I normalization, while the median weekly PEGV dose only minimally increased. No significant adverse events occurred during the study. Therefore we concluded that temporary switching from combination treatment to PEGV monotherapy is an effective and feasible treatment strategy. In line with previous studies we confirmed the carry-over effect of SRLs on IGF-I levels of over 4 months. The conclusion of this pilot study is that temporary switching from combination treatment tot PEGV monotherapy is an effective, safe and feasible treatment strategy.

Pasireotide (long-acting release) LAR is a second somatostatin receptor ligand approved for the treatment of patients with acromegaly for whom surgery is not option or not curative. Pasireotide LAR has shown superior efficacy over first-generation somatostatin analogues with respect to IGF-I reduction, but is associated with a higher frequency of hyperglycemia and diabetes mellitus. However, it is unknown what the optimal position is of pasireotide LAR in the modern management of acromegaly in relation to PEGV. We hypothesized that switching from combination treatment to pasireotide LAR could lead to a significant reduction in the required PEGV dose and therefore to a lower number of injections, and possibly lower costs. **Chapters 3 and 4** cover the results of the results of the PAsireotide LAR and PEgvisomant (PAPE) study. The PAPE study assessed the efficacy and safety of switching from combination treatment of first-generation somatostatin analogues and PEGV treatment to pasireotide LAR treatment. The main results show that pasireotide LAR is an effective treatment, as 77% of patients achieved normalisation of IGF-I levels after 48 weeks, and on average 50% of the PEGV dose could be reduced. An important observation is the heterogeneous response to pasireotide LAR in term of IGF-I normalization. This was related to several factors, including the large heterogeneity in the PEGV dose at baseline. An interesting finding was the potent reduction in IGF-I after the first pasireotide LAR injection. This early onset and strong suppression of IGF-I is probably

a direct effect of pasireotide on inhibition of insulin secretion. The concomitant suppression of both IGF-I and insulin is an important mechanism which can explain the efficacy of pasireotide LAR.

Safety

De most common and clinically relevant adverse event during the PAPE study was the occurrence of hyperglycemia and diabetes mellitus. The frequency of diabetes mellitus rose from 32.8% at baseline to 77% at 48 weeks. Most patients had a mild hyperglycemia occurring mainly after initiation of pasireotide LAR treatment and manageable with metformin and vildagliptin. The subgroup of patient with severe hyperglycemia had pre-existent diabetes which was well regulated with multiple antidiabetic drugs such as sulfonylureas, GLP-I analogues and insulin. The development of pasireotide-induced hyperglycemia was correlated with baseline HbA1c and use of antidiabetic drugs. An important novel independent predictor for pasireotide-induced hyperglycemia was the baseline ß-cell function as a measure for insulin secretion based on the oral glucose tolerance test. We observed an inverse correlation between baseline insulin secretion and the increase in HbA1c at 24 weeks.

Predictors of clinical response to pasireotide LAR

As pasireotide exhibits a higher binding affinity to somatostatin receptor subtype 5 (SST_5) compared with SST_2 we assumed that the efficacy of pasireotide LAR would correlate with the SST_5 protein expression of the pituitary adenoma (**chapter 5**). In our cohort we observed a positive correlation between the clinical response of pasireotide LAR on IGF-I reduction and the clinical response of first-generation somatostatin analogues on IGF-I reduction. These clinical results were supported by in vivo observations that SST_2, and not SST_5 protein expression of the pituitary adenoma correlated with clinical response to pasireotide LAR treatment during the PAPE study. Moreover, in the same patients we found a strong correlation between the percentage IGF-I reduction after treatment with pasireotide LAR and after previous treatment with first-generation somatostatin analogues. With other words, patients with good clinical response to first-generation somatostatin analogues also seem to respond well to pasireotide LAR, and vice-versa.

The main conclusion of this translational study is that in our cohort SST_2 is the most important predictor for IGF-I normalisation after treatment with pasireotide LAR.

Ghrelin in acromegaly

The metabolic hormone ghrelin exists in two different isoforms in the circulation, respectively Acylated Ghrelin (AG) and Unacylated Ghrelin (UAG). Both isoforms have

different and (sometimes) opposing biological effects. Earlier studies indicated that measurement of both these isoforms with a validated immunoassay provides more biological information than measurement of total ghrelin. Although ghrelin can stimulate growth hormone secretion, and ghrelin itself can be inhibited growth hormone, the exact physiological role of ghrelin on growth hormone regulation remains not fully elucidated. It is also not well understood what the influence is of different medical treatments on ghrelin levels in acromegaly. In **chapter 6** we therefore measured both AG and UAG in three different acromegaly patient groups. In this study patients biochemically controlled with combination treatment were compared with patients with medically naïve active acromegaly and patients using PEGV monotherapy. We found that both AG and UAG were suppressed during combination treatment compared with the other patient groups. This effect is likely caused by a direct inhibitory effect of somatostatin on ghrelin release. Novel however is the finding that the AG/UAG ratio between the different patient groups is not significantly different. This suggests that combination treatment of SRL and PEGV does not affect the relation between AG and UAG. Another message of this study is that adequate measurement of AG and UAG and calculation of the AG/UAG ratio can yield relevant information on the bioactive state of ghrelin.

Clearance of pegvisomant

Although PEGV is available for the treatment of pegvisomant for over 15 years, it remains unknown how pegvisomant is metabolized and cleared from the circulation. PEGV is a mutated GH molecule attached to multiple polyethylene glycol (PEG) molecules. These PEG molecules extend the half-life of the drug and reduce immunogenicity. We postulated that because PEG molecules are not biodegradable they are cleared by immune cells, such as kupffer cells (liver macrophages) of the mononuclear phagocytic system. Therefore, in the final chapter of this thesis, we performed this proof of concept study to investigate whether PEGV is expressed in the liver, and if so, whether PEGV can be taken up by kupffer cells. We observed that PEG is internalized by kupffer cells and not by hepatocytes using immunofluorescence techniques in hepatic tissue obtained from two patients with acromegaly that were treated with PEGV. This study suggests the involvement of the immune system in the clearance of pegvisomant.

SAMENVATTING

Acromegalie is een chronische systemische aandoening veroorzaakt door overmatige groeihormoonsecretie uit een hypofyseadenoom. Combinatiebehandeling met eerste generatie somatostatine analoga (SRL) en pegvisomant (PEGV) is een effectieve en veilige behandeling om IGF-I te normaliseren in acromegalie. Echter deze combinatiebehandeling vereist langdurige maandelijkse SRL injecties en dagelijks tot wekelijkse PEGV injecties, wat een negatief effect kan hebben op de therapietrouw. Onze hypothese in **hoofdstuk 2** van dit proefschrift was dat tijdelijk switchen van SRL en PEGV combinatiebehandeling naar PEGV monotherapie kan leiden tot een reductie van de totale aantal injecties wat mogelijk kan leiden tot een verbetering van de therapietrouw. Na 12 maanden behandeling met PEGV monotherapie hadden 73% van de patiënten IGF-I waarden binnen de normaalwaarden terwijl hierbij de mediane wekelijks PEGV dosis slechts minimaal steeg. Tijdens de studies traden geen klinisch significante bijwerkingen op. In lijn met voorgaande studies hebben we aangetoond dat somatostatine analoga minstens 4 maanden een doorwerkingseffect hebben op IGF-I waarden. De conclusie van deze pilot studies is dat tijdelijk switchen van combinatiebehandeling naar PEGV monotherapie een effectieve, veilige en praktische haalbare behandelstrategie is.

Pasireotide (langwerkende afgifte) LAR is een tweede generatie somatostatine analoog en is geregistreerd voor acromegaliepatiënten waarbij een operatie niet mogelijk of niet curatief is. Pasireotide LAR is effectiever dan eerste generatie somatostatine analoga op IGF-I reductie, maar is geassocieerd met een hogere frequentie van hyperglycemie en diabetes mellitus. Het is echter niet bekend wat de optimale positie is van pasireotide LAR in relatie tot pegvisomant in de hedendaagse behandeling van acromegalie. Wij postuleerden dat switchen van combinatiebehandeling naar pasireotide LAR kan leiden tot een significante reductie van de pegvisomant dosis en daarmee tot minder injecties en mogelijk minder kosten. **Hoofdstukken 3 en 4** gaan over de resultaten van de PAsireotide LAR and PEgvisomant (PAPE) studie. In de PAPE studie wordt de effectiviteit en veiligheid onderzocht van het switchen van SRL en PEGV combinatiebehandeling naar pasireotide LAR. Uit de resultaten van de PAPE studie blijkt dat pasireotide LAR een effectieve behandeling is, aangezien 77% van de patiënten normalisatie van IGF-I konden bereiken na 48 weken met hierbij een gemiddeld PEGV sparend effect van 50%. Een belangrijke observatie is de grote heterogeniteit in klinische respons op pasireoide LAR. Dit was gerelateerd aan verschillende factoren, waaronder de grote heterogeniteit van de gebruikte PEGV dosis op baseline. Een interessante bevinding was de sterke reductie van IGF-I na de eerste pasireotide LAR injectie. Deze vroegtijdige en sterke reductie van IGF-I is een

waarschijnlijk een direct effect van pasireotide op remming van de insuline secretie. De gelijktijdige suppressie van zowel IGF-I als insuline is een belangrijk mechanisme wat de effectiviteit van pasireotide LAR kan verklaren.

Veiligheid

De meest voorkomende en klinisch relevante ongewenste voorval tijdens de PAPE studie was het optreden van hyperglycemie en diabetes mellitus. De frequentie van diabetes mellitus steeg 32.8% bij aanvang van de studie naar 77% aan het einde. De meeste patiënten hadden hierbij een milde hyperglycemie voornamelijk optredend na het starten van behandeling met pasireotide LAR. Deze milde hyperglycemie kon worden gereguleerd met een combinatie van metformine en vildagliptine. De subgroep van patiënten met ernstige hyperglycemie hadden reeds diabetes mellitus die goed gereguleerd was met meerdere antidiabetische medicatie zoals sulfonylureumderivaten, GLP-I analoga en insuline. Het optreden van pasireotide-geinduceerde hyperglycemie werd voorspeld door de HbA1c waarde bij baseline en het gebruik van antidiabetische medicatie. Een belangrijke nieuwe onafhankelijke voorspeller voor hyperglycemie was de baseline ß-cel functie als een maat voor de insulinesecretie gebaseerd op de orale glucose tolerantie test. Wij vonden een omgekeerde correlatie tussen de baseline insulin secretie en de stijging van het HbA1c op 24 weken.

Voorspellers van klinische respons op pasireotide LAR

Aangezien pasireotide een verhoogde bindingsaffiniteit heeft voor somatostatine receptor subtype 5 (SST_5) vergeleken met SST_2 veronderstelden wij dat de effectiviteit van pasireotide LAR correleert met de mate van SST_5 eiwitexpressie op het hypofyseadenoom (**hoofdstuk 5**). In onze cohort vonden wij echter een positieve correlatie tussen de klinische respons van pasireotide LAR op IGF-I reductie en de klinische response van eerste generatie somatostatine analoga op IGF-I reductie. Deze klinische resultaten werden ondersteund door *in vivo* observaties dat SST_2 en niet SST_5 eiwitexpressie van de hypofyseadenoom correleerde met de klinische response op pasireotide LAR tijdens de PAPE studie. Bovendien vonden wij in dezelfde patiëntengroep een sterke correlatie tussen de percentage IGF-I reductie na behandeling met pasireotide LAR en na (eerdere) behandeling met eerste generatie somatostatine analoga. Met andere woorden, patiënten die eerder goed reageerden op eerste generatie somatostatine analoga leken ook goed te reageren op pasireotide LAR en vice-versa. De belangrijkste conclusie van deze translationele studie is dat in onze cohort SST_2 eiwitexpressie de belangrijkste voorspeller is voor IGF-I normalisatie na behandeling met pasireotide LAR.

Ghreline levels in acromegalie

Het stofwisselingshormoon ghreline bestaat uit twee verschillende verschijningsvormen in de circulatie, respectievelijk Geacyleerd Ghreline (AG) en Ongeacyleerde Ghreline (UAG). Beide verschijningsvormen hebben verschillende en (soms) tegengestelde biologische effecten. Uit eerdere studies is gebleken dat het meten van deze verschijningsvormen van ghreline in een gevalideerde immunoassay meer biologische informatie verschaft dan het louter meten van totale ghreline. Hoewel ghreline groeihormoonsecretie kan stimuleren en ghrelinesecretie geremd kan worden door groeihormoon is de fysiologische rol van ghreline in de regulatie van groeihormoon nog niet opgehelderd. Het is ook nog niet goed bekend wat de invloed is van de verschillende therapieën voor acromegalie op ghreline waarden. In **hoofdstuk 6** is daarom AG en UAG gemeten in drie verschillende acromegalie patiëntengroepen. In deze studie werd patiënten die biochemisch gecontroleerd waren met SRL en PEGV combinatiebehandeling vergeleken met patiënten met medisch naïeve acromegalie en patiënten met PEGV monotherapie. Uit deze studie blijkt dat zowel AG als UAG waarden onderdrukt werden tijdens combinatiebehandeling vergeleken met de andere patiëntengroepen. Dit effect wordt waarschijnlijk veroorzaakt door een direct remmend effect van somatostatine op ghrelineafgifte. Nieuw is echter de bevinding dat de AG/UAG ratio tussen de patiëntengroepen niet significant verschillend is. Dit suggereert dat combinatiebehandeling van SRL en PEGV de relatie tussen AG en UAG niet verandert. Een andere belangrijke boodschap van deze studie is dat adequate meting van AG en UAG en het bepalen van de AG/UAG ratio belangrijke informatie kan verschaffen over de bioactieve status van ghreline.

Klaring van pegvisomant

Hoewel PEGV meer dan 15 jaar beschikbaar is als behandeling van acromegalie is tot op heden nog niet bekend hoe PEGV gemetaboliseerd wordt en geklaard wordt uit de circulatie. Het unieke aan het PEGV is dat het een gemuteerde groeihoormoneiwit is die verbonden is met meerdere polyethylene glycol (PEG) moleculen die ervoor zorgen dat PEGV een verlengde halfwaardetijd heeft en minder immunogeen is. Wij postuleerden dat PEG moleculen, doordat zij niet biologisch afbreekbaar zijn, geklaard worden door immuuncellen, zoals kupffer cellen (lever macrofagen) van het mononucleair fagocytair systeem. Daarom is in dit laatste hoofdstuk van dit proefschrift een proof of concept studie uitgevoerd om te onderzoeken of PEGV tot expressie komt in de lever, en zo ja, of PEGV opgenomen kan worden door kupffer cellen. Door middel van immunofluoresecentietechnieken in leverweefsel van twee acromegaliepatiënten die behandeld werden met PEGV hebben wij geobserveerd dat kupffer cellen PEG moleculen opnemen, terwijl hepatocyten dit niet deden. Deze studie suggereert daarom betrokkenheid van het immuunsysteem in de klaring van pegvisomant.

LIST OF ABBREVIATIONS

AcroQoL	Acromegaly quality of life questionnaire
ACTH	adrenocorticotrophin hormone
ADA	American Diabetes Association
ADH	Antidiuretic Hormone
AE	Adverse event
AEBSF	4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride
AG	Acylated Ghrelin
AIP	Aryl hydrocarbon receptor-interacting protein
ALS	Acid labile unit
ALT	Alanine aminotransferase
AP102	Dual SST_2/SST_5 specific somatostatin analogue
AST	Aspartate aminotransferase
AUC	Area under curve
BMD	Bone mineral density
BMI	Body mass index
C2305	Pasireotide versus octreotide in acromegaly: head-to-head superiority study by *Colao et al*
CI	Confidence interval
CRT	Conventional radiotherapy
CTCAE	Common terminology criteria for adverse events
CV	Coefficient of variability
D2R	Dopamine subtype 2 receptor
DA	Dopamine agonist
DAPI	4',6-diamidino-2-fenylindool, fluorescent stain that binds strongly to adenine–thymine rich regions in DNA
DM	Diabetes mellitus type II
DPP4	Dipeptidyl peptidase 4
DSGA	Densely granulated somatotroph adenomas
DSMB	Data safety monitoring board
EDTA	Ethylenediaminetetraacetic acid
EIA	Enzyme immune assay
EKG	Electrocardiogram
ELISA	Enzyme-Linked Immuno Sorbent Assay
EMA	European Medicines Agency
ERK	Extracellular signal-regulated kinases
FDA	Food and Drug Administration
FFA	Free fatty acids

FFPE	Formalin-fixed, paraffin-embedded
FIPA	Familial isolated pituitary adenoma
FPG	fasting plasma glucose
GH	Growth hormone
GHBP	Growth hormone binding protein
GHR	Growth hormone receptor
GHRA	Growth hormone receptor antagonist
GHRH	Growth hormone releasing hormone
GHSR1a	Growth hormone secretagogue receptor 1 a
GIP	gastric inhibitory polypeptide
GLP-I	Glucagon-like peptide-I
GOAT	Ghrelin O-acyl transferase
GPCR	G protein-coupled receptor
GPOS	German pegvisomant observational study
HbA1c	Glycosylated hemoglobin, type A1C
HE	Haematoxylin eosin
HOMA-2	Homeostatic model assessment
HOMA-B	Homeostatic model assessment ß-cell function
HOMA-IR	Homeostatic model assessment insulin resistance
IGFBP1	Insulin-like growth factor-binding protein 1
IGFBP3	Insulin-like growth factor-binding protein 3
IGF-I	Insulin-like growth factor I
IHC	Immunohistochemistry
IQR	Interquartile range
IRB	Institutional review board
IRS	Immunoreactivity score
ITT	Intention-to-treat
JAK2	Janus kinase 2
kDa	kilodalton
LA-SSA	Long-acting somatostatin analogue
LC-MS/MS	Liquid chromatography–mass spectrometry
LFT	Liver function tests
LH	Luteinizing Hormone
LOCF	last observation carried forward
MAP	Mitogen-activated protein
MPS	Mononuclear phagocyte system
MRI	Magnetic resonance imaging
NYHA	New York Heart failure Association classification
OCT	Octreotide

OGTT	Oral glucose tolerance test
PAOLA	Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly study by *Gadelha et al*
PAPE	PAsireotide LAR and PEgvisomant study
PAS	Pasireotide
PAS-LAR score	Pasireotide LAR treatment response score
PAS-LAR	Pasireotide long acting release
PASQ	Patient-assessed acromegaly symptom questionnaire
PEG	Polyethylene glycol
PEGV	Pegvisomant
PI3K	Phosphoinositide 3-kinase
PQD	PEG-coated quantum dots
PRL	Prolactin
QoL	Quality of life
QTcF	QT interval corrected by the Fridericia correction formula
RAI	Radioactive iodine
RIA	Radioimmunossay
RT	Radiotherapy
SAE	Serious adverse event
SGSA	Sparsely granulated somatotroph adenomas
SRIF	Somatotropin releasing-inhibiting factor
SRL	somatostatin receptor ligand
SRS	Stereotactic radiosurgery
SSA	Somatostatin analogue
SST	Somatostatin
SST_1	Somatostatin receptor subtype 1
SST_2	Somatostatin receptor subtype 2
SST_5	Somatostatin receptor subtype 5
SSTR	Somatostatin receptor
STAT5	Signal transducer and activator of transcription 5
TET	Transient elevated transaminases
TG	Total ghrelin
TRH	Thyroid releasing hormone
TSH	Thyroid stimulating hormone
TSS	Transsphenoidal surgery
UAG	Unacylated ghrelin
ULN	Upper limit of normal
WAT	White adipose tissue

WHO	World health organization
X-LAG	X-linked acrogigantism
γ-GT	γ-glutamyl transpeptidase

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ABOUT THE AUTHOR

Ammar Muhammad was born on September 6th 1988 in Damascus, Syria. After graduating from high school in 2006 (Haarlemmermeerlyceum, Hoofddorp), he studied medicine at the Leiden University Medical Center (LUMC), from which he graduated in 2013. During medical school he attended a medical elective at the Department of Endocrinology and Metabolic Sciences, University of Cambridge, Addenbrookes Hospital. After medical school he performed laboratory and epidemiological research at the Department of Rheumatology. In 2014 he started his PhD entitled 'Insights in the modern second line medical management of acromegaly' at the Department of Medicine – Endocrinology/Pituitary Center Rotterdam under supervision of Professor van der Lelij and Doctor Neggers. During his PhD he managed the PAPE trial almost independently which resulted in several publications part of which are presented in this thesis. Besides running a clinical study, he performed several translational and basic science studies in collaboration with the department of pathology and the laboratory of experimental neuroendocrinology. He received the best clinical abstract award at the ENDO 2017 conference. Besides his thesis he treated acromegaly patients at the acromegaly outpatient clinic. After finishing his PhD training in 2018, he started his residency in internal medicine at the Sint Franciscus Gasthuis hospital in Rotterdam under supervision of Doctor Schrama.

PhD PORTFOLIO

Name PhD student:	Ammar Muhammad
Erasmus MC department:	Medicine, section Endocrinology, Pituitary Centre Rotterdam
Research school:	Netherlands Institute for Health Sciences (NIHES)
PhD period:	2014 – 2017
Promotor:	Prof. Dr. A.J. van der Lelij
Co-promotor:	Dr. S.J.C.M.M. Neggers

	Year	ECTS
General courses		
Basic Course for Clinical Investigators (BROK)	2014	2.0
Research Integrity Course	2015	0.3
Biostatistical Methods I: Basic Principles, part A (CC02a)	2014	2.0
Patient Oriented Research (CPO) course	2014	0.3
Design and Interpretation of Clinical trials Johns Hopkins course	2014	0.3
OpenClinica course	2015	0.3
English Biomedical Writing and Communication Course	2015	2.0
Research skills		
Weekly research meeting Endocrinology Laboratory	2014-2017	4.5
Seminars and workshops		
Seminars department of Internal Medicine/Erasmus MC lectures	2014-2017	4.0
5th Annual Acromegaly Expert Meeting (Lisbon, Portugal)	2014	1.0
12th Neuroendocrinology Symposium de Baar (Utrecht, the Netherlands)	2014	1.0
Erasmus Endocrinology Course (Noordwijkerhout, the Netherlands)	2014	1.0
Endocrine Retreat (Rotterdam, the Netherlands)	2015	1.0
Regional Endocrinology Case Meeting (Rotterdam, the Netherlands)	2015	1.0
Chiasma MPOWERED clinical study meeting (Athens, Greece)	2016	1.0
Diagnostic Error's in Medicine conference (Rotterdam, the Netherlands)	2016	1.0
Neuroendocrinology symposium (Rotterdam, the Netherlands)	2016	1.0
Dutch Pituitary Society patient day (Utrecht, the Netherlands)	2016	1.0

	Year	ECTS
Pituitary Society patient education day (Rotterdam, the Netherlands)	2017	1.0
Regional Endocrinology Case Meeting (Rotterdam, the Netherlands)	2017	1.0
(Inter)national conferences		
Internal Medicine Science Days (Antwerp, Belgium) – poster presentation Long-term Efficacy and Safety of Pegvisomant in combination with Long-Acting Somatostatin Analogues in Acromegaly	2014	1.0
4th Dutch Endocrine Meeting (Noordwijkerhout, the Netherlands)	2014	1.0
Internal Medicine Science Days (Antwerp, Belgium) – poster presentation What is the efficacy of switching to weekly pegvisomant in acromegaly patients well controlled on combination therapy?	2015	1.0
5th Dutch Endocrine Meeting (Noordwijkerhout, the Netherlands) – oral presentation What is the efficacy of switching to weekly pegvisomant in acromegaly patients well controlled on combination therapy?	2015	1.0
17th European Congress of Endocrinology (Dublin, Ireland)	2015	1.0
European Young Endocrine Scientists Conference (Modena, Italy) – oral presentation What is the efficacy of switching to weekly pegvisomant in acromegaly patients well controlled on combination therapy?	2015	1.0
6th Dutch Endocrine Meeting (Noordwijkerhout, the Netherlands) – oral presentation Impact of the GH-receptor antagonist pegvisomant on mammograpahic breast density in postmenopausal acromegalic women	2016	1.0
18th European Congress of Endocrinology (Munich, Germany) – oral presentation Case presentation Pasireotide LAR treatment in acromegaly	2016	1.0
17th Congress of the European NeuroEndocrine Association (ENEA) (Milan, Italy) – oral presentation Acylated and Unacylated Ghrelin levels in acromegaly patients during combination treatment	2016	1.0
3rd Junior Dutch Endocrine Conference (Leiden, the Netherlands) – oral presentation Acylated and Unacylated Ghrelin levels in acromegaly patients during combination treatment	2016	1.0
7th Dutch Endocrine Meeting (Noordwijkerhout, the Netherlands) – oral presentation Efficacy and Safety of Switching to Pasireotide LAR Alone or in Combination with Pegvisomant in Acromegaly (PAPE) study, 24 Weeks Results	2017	1.0

	Year	ECTS
99th Meeting of the Endocrine Society (Orlando, USA) – poster presentation Efficacy and Safety of Switching to Pasireotide LAR Alone or in Combination with Pegvisomant in Acromegaly (PAPE) study, 24 Weeks Results	2017	1.0
19th European Congress of Endocrinology (Lisbon, Portugal) – poster presentation Efficacy and Safety of Switching to Pasireotide LAR Alone or in Combination with Pegvisomant in Acromegaly (PAPE) study, 24 Weeks Results	2017	1.0
8th Dutch Endocrine Meeting (Noordwijkerhout, the Netherlands) – oral presentation Response to pasireotide LAR treatment in acromegaly is mainly driven by somatostatin receptor subtype 2 expression	2018	1.0
Erasmus Ohio collaborative meeting (Rotterdam, the Netherlands) – oral presentation Evidence of pegvisomant uptake by kupffer cells: observations in two patients with acromegaly	2018	1.0
Clinical activities		
Weekly grand round endocrinology department	2014-2017	4.5
Outpatient clinic for acromegaly patients (Clinical Research Unit)	2014-2017	10.0
Effect of Pegvisomant on Quality of Life and Insulin Sensitivity study Co-investigator	2014-2015	2.0
Phase 2A Pharmacokinetics and Pharmacodynamics of Lanreotide Prolonged Release Formulation (PRF) in Acromegaly Co-investigator	2015	0.3
Pasireotide LAR and Pegvisomant combination (PAPE) study in Acromegaly, a 48 weeks prospective open-label clinical trial Lead investigator	2015-2017	20.0
Maintenance of acromegaly Patients with Octreotide capsules compared With injections, Evaluation of REsponse Durability (MPOWERED) study Co-investigator	2016	0.3
European Pasireotide LAR Observational Study in Acromegaly (ACRONIS) Co-investigator	2016-2017	1.0
Teaching activities		
Skills training first year medical students Subject: Thyroid Erasmus University Medical Center, Rotterdam, the Netherlands	2014	1.0
Skills training first year medical students Subject: Thyroid Erasmus University Medical Center, Rotterdam, the Netherlands	2017	1.0

	Year	ECTS
Teaching basic course endocrinology for endocrine nurses Subject: hypothalamus and pituitary Radboud care academy, Nijmegen, the Netherlands	2017	1.0
Awards		
Endocrine Society Outstanding Abstract Award and Endo Early Career Forum Award	2017	
Other		
Peer-review for *Growth hormone & IGF-I research*	2017	0.3
Peer-review for *European Journal of Endocrinology*	2017	0.3
Peer-review for *Touch Endocrinology*	2018	0.3
Peer-review for *Endocrine Connections*	2019	0.3

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