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Evidence for better response to somatostatin analogue treatment in acromegalic patients treated with metformin

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ABBREVATIONS

ACTH - *Adrenocorticotropic hormone*

ADH - *Antidiuretic hormone*

AMP - *Adenosine monophosphat*

AMPK - *AMP-activated Proteinkinase*

ATP - *Adenosine triphosphate*

cAMP - *Cyclic adenosine monophosphat*

CBP - *CREB-Binding-Protein*

CREB - *cAMP response element-binding protein*

CT - *Computed tomography*

DA - *Dopamine agonist*

DNA - *Deoxyribonucleic acid*

GH - *Growth hormone*

GHRH - *Growth-hormone-releasing-hormone*

hGH - *Human Growth hormone*

IGF-1 - *Insuline-like Wachstumsfaktor*

L - Litre

LKB1 - *liver kinase B1*

MRI - *Magnetic resonance imaging*

oGTT - *orale Glucose tolerance test*

Pit1 - *Pituitary-specific positive transcription factor 1*

PKA - *Proteinkinase A*, *Proteinkinase A*

PRL - *Prolactin*

QoL - *Quality of Life*

Sirt1 - *Sirtuin-1*

SMS - *Somatostatin*

SPSS - *Statistical Package for Social Sciences*

SSA - *Somatostatinanalogues*

SSTR 5 - *Somatostatinreceptor 5*

SSTR2 - *Somatostainreceptor 2*

TSH - *Thyroid-stimulating hormone*

1. Introduction

1.1 Acromegaly

1.1.1 Definition and history

Acromegaly is a rare endocrine disorder resulting from overproduction of growth hormone (GH) (Ayuk & Sheppard, 2006). In general, somatotrophic pituitary adenomas are the cause for uncontrolled production of growth hormone. As a consequence, this leads to the typical clinical picture of acromegaly, which is mainly characterized by above average growth of extremities and acres: fingers, toes, nose, chin and ears. Clearly recognizable are the typical features of acromegaly in the portrait of American actor Richard Kiel (1939-2014) (Figure 1).

***Figure 1**: Richard Kiel (1939-2014): Was an American actor. He became known for his role as „Jaws“ in the James Bond movies.*

In 1886, the disease was described for the first time as “acromegaly” by the French neurologist Pierre Marie (1853-1940) and for this reason, it is sometimes called Pierre-Marie-Syndrome. Furthermore, he used the greek words akron- (“extremity”) and mega (“large”) to describe the typical clinical findings (Carroll PV et el. 2016). However, clinical descriptions of the disease already existed before (de Herder, 2009).

In 1909, Harvey Cushing observed clinical remission in patients with acromegaly after performing hypophysectomy. Therefore, he suspected the cause of acromegaly must be an overproduction of a hormone which is produced in the pituitary and is responsible for growth (Mammis, Eloy, & Liu, 2010).

1.1.2 Epidemiology

The epidemiology of acromegaly is well described and the disease has an incidence of 0.3 cases in a population of 100,000 in one year (Tjornstrand et al., 2014). Typically, patients are affected in the fourth and fifth life decade (Fernandez, Karavitaki, & Wass, 2010). Patients with acromegaly have an increased mortality rate compared to the normal population. However, this can be reduced to normal values by lowering the GH-values through therapy (Bates, Van't Hoff, Jones, & Clayton, 1993). Especially complications of the cardiovascular and respiratory system are responsible for increased mortality in patients with acromegaly (Colao, Ferone, Marzullo, & Lombardi, 2004). Although the majority of diagnoses are made in middle-aged patients, there is also a large number of younger patients. These younger patients tend to develop more aggressive tumors with higher growth hormone concentrations (Holdaway & Rajasoorya, 1999).

1.1.3 Pathophysiology

Usually, Acromegaly presents itself with a growth-hormone secreting tumor that originates from somatotrophic cells in the pituitary gland (Melmed, 2006). This leads to increased basal growth hormone concentrations due to uncontrolled continuous release of GH (Hartman et al., 1990). The vast majority of somatotrophic tumors (>90%) develop sporadically but some hereditary forms of somatotroph adenomas associated with familial isolated pituitary adenomas (FIPA), X-LAG syndrome,

multiple endocrine neoplasia type 1 (MEN 1) and other types of hereditary endocrine syndromes are also known.

The pathogenesis of somatotroph adeomas is still poorly understood. About 40% of patients with sporadic somatotroph adenomas are bearing mutations of the G protein alpha chain of the stimulating G protein, which leads to continuous activation of the GHRH receptor-dependent signaling cascade and results in increased, GHRH-independent GH synthesis and secretion (Landis et al., 1989; Melmed, 2006).

The increased production of GH is finally responsible for augmented serum levels of Insulin-like growth factor 1 (IGF-1), which mediates the growth-promoting effects of GH to peripheral organs (Laron, 2001). The result are anabolic and growth-promoting metabolic effects which consequently causes the typical clinical appearance of acromegaly (Melmed, 2006).

1.1.4 Growth hormone

GH is a polypeptide that is formed in the pituitary gland and is composed of 191 amino acids. Besides that, it contains two disulfide bridges and has a molecular weight of 22 kilodaltons (Li & Dixon, 1971).
GH acts by binding to somatotropin receptors that can be found in a variety of tissues (Postel-Vinay & Finidori, 1995). Especially in the liver, it stimulates the formation of Insulin-like growth factor (IGF-1).

GH has anabolic effects on bone and mineral metabolism (Tritos & Klibanski, 2016). It can also cause abnormalities in the cardiovascular system (Colao, 2008). In addition, GH increases the blood glucose level by stimulating glycogenolysis and it has a fat-reducing effect by stimulating lipolysis (Ghanaat & Tayek, 2005; Sakharova et al., 2008).

Moreover it has been clearly established that the anabolic and growth-promoting metabolic effects are not determined directly by GH but are mainly mediated indirectly by IGF-1 (Laron, 1999).

Due to regulatory effects of GH on glucose metabolism, hypoglycaemia can lead to compensatory hyperglycaemia and in case of permanently pathologically increased releases of GH, this can lead to insulin resistance and thus to diabetes mellitus (Holly, Amiel, Sandhu, Rees, & Wass, 1988). IGF-1 modulates insulin receptors and can stimulate beta-cells of the pancreas to increase insulin secretion. Therefore, pathologically high levels of IGF-1 can lead to disorders of beta-cell function and thus to insulin resistance. (Moller et al., 1991).

1.1.4.1 Regulation of GH synthesis

The gene for growth hormone is located on the long arm of chromosome 17 (q22-q24) and transcribed exclusively in the somatotropic cells of the pituitary anterior lobe (Miyachi, Yakushiji, & Terazono, 1993).

Growth hormone production is stimulated by GH releasing hormone (GHRH). GHRH consists of 40 amino acids and is formed in the hypothalamus. Via the pituitary portal system GHRH is transported into the pituitary gland (Bloch et al., 1983). In the pituitary gland, it stimulates somatotropic cells to secrete GH in pulsatile rhythm (Cataldi et al., 1994) (Figure 2). Secretion increases during sleep und puberty (Minuto et al., 1982).

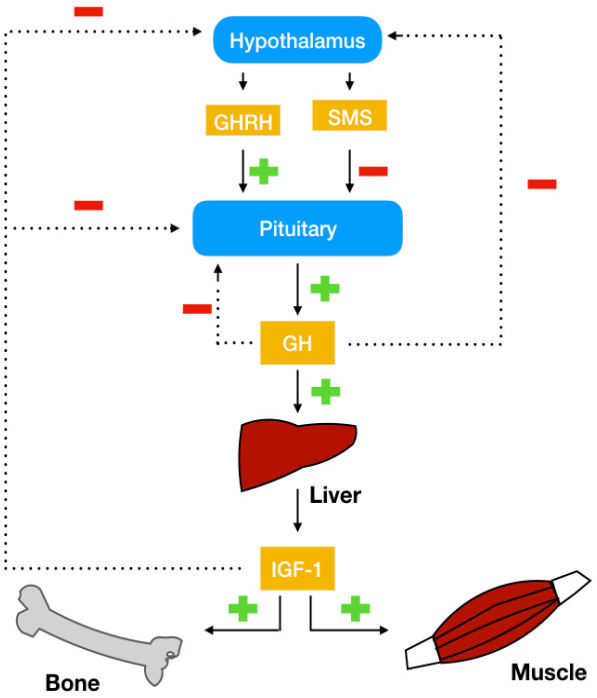

Figure 2: Control loop and effect of GH and IGF-1 on the metabolism. *GH is produced in the pituitary after stimulation by GHRH. It stimulates the formation of IGF-1 in the liver which has anabolic effects on bone and muscle. GH inhibits its secretion through a negative feedback mechanism.*

Somatostatin (SMS) represents the counterpart to GHRH and inhibits secretion of GH (Ge, Tsagarakis, Rees, Besser, & Grossman, 1989). SMS (somatostatin) is a peptide hormone synthesized in the pancreas as well as in the gastrointestinal tract and hypothalamus. In the pituitary, SMS acts as an inhibitor on somatotroph cells and suppresses growth hormone secretion (Brazeau, Rivier, Vale, & Guillemin, 1974). It also inhibits release of glucagon and insulin in the pancreas (Strowski, Parmar, Blake, & Schaeffer, 2000). The distribution of somatostatin is regulated in the cells of the hypothalamus by the second messenger cAMP, which activates the protein kinase A (PKA). PKA phosphorylates the intracellular transcription factor CREB, which binds to the promoter of the somatostatin gene and the expression of the hormone follows (Montminy & Bilezikjian, 1987).

Insulin-like growth factor 1 inhibits the GH release by a negative feedback mechanism on pituitary level. IGF-1 causes enhanced SMS synthesis in the

hypothalamus, which in turn suppresses GH secretion (Berelowitz et al., 1981).
IGF-1 has 70 amino acids and is similar in structure to insulin. It is primarily produced in the liver in response to GH (Daughaday & Rotwein, 1989).

Hypoglycemia, physical exertion and stress have an excitatory effect on GH secretion (Greenwood & Landon, 1966; Jaffe, Huffman, & Demott-Friberg, 1999).
Apart from that, serotonin, testosteron and dopamine stimulate GH secretion and besides IGF-1, hyperglycemia, hyper- and hypothyroidism, fatty acids, nicotine and age have an inhibitory effect on GH secretion (Root & Diamond, 2000).

Ghrelin, a peptide that is formed in the hypothalamus and gastrointestinal tract has a stimulating effect on GH release (Tannenbaum, Epelbaum, & Bowers, 2003)

1.1.5 Diagnostics

On average, diagnosis of acromegaly is made only between six and ten years after onset of the disease (Holdaway & Rajasoorya, 1999). Late diagnoses usually affect patients older than 50 years and less frequently younger patients under 30 years of age (Nabarro, 1987).
Due to typical clinical findings, acromegaly is often a visual diagnosis of an experienced endocrinologist. In order to make the diagnosis, it is also very important to get blood samples to determine GH and IGF-1 values. Here it is of special importance that not only GH values are determined, because GH serum levels alone are not sufficient since they fluctuate during the day (Winer, Shaw, & Baumann, 1990). For this reason, IGF-1 values should always be checked as well because IGF-1 directly correlates with GH activity and is elevated in most initial diagnoses (Barkan, Beitins, & Kelch, 1988).
Another cornerstone of the diagnosis is the oral glucose tolerance test (oGTT). With an oGTT, measurements of the suppression of GH secretion can be performed. After completion of the oGTT, acromegaly can be diagnosed if GH is not suppressed to < 1 µg/L after a 2 hour oGTT (Freda, Post, Powell, & Wardlaw, 1998).
Eventually, to confirm the suspected diagnosis of Acromegaly, neuroradiological imaging with CT or MRI is necessary. MRI is the method of choice in radiological detection and characterization of pituitary adenomas and the goal is to find out its size, location and extent (Rumboldt, 2005) (Figure 3).

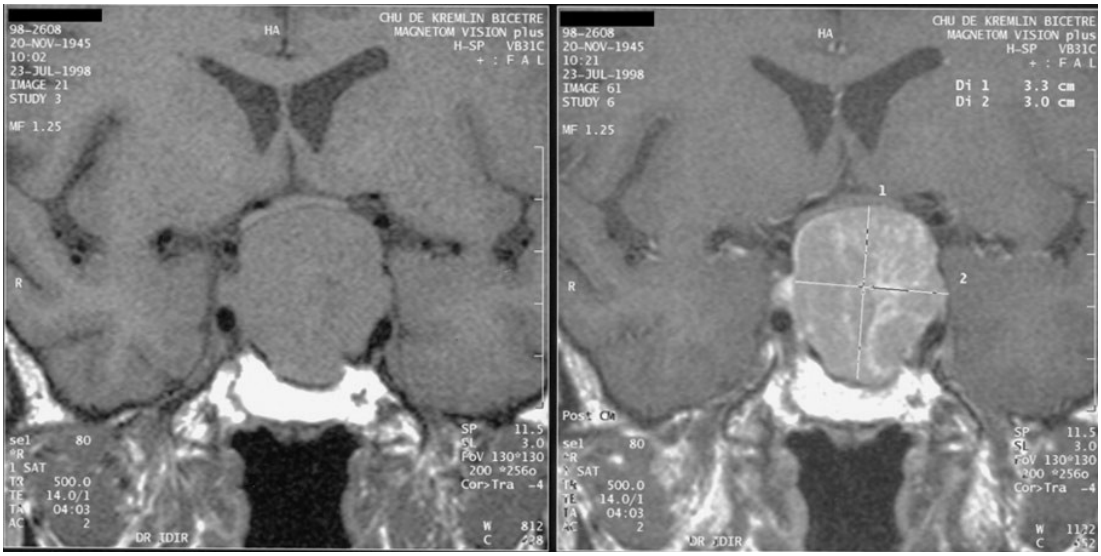

Figure 3: *Pituitary tumor in MRI image: Macroadenoma with compression of optic chiasm.*
(https://en.wikipedia.org/wiki/Acromegaly#/media/File:Acromegaly_pituitary_macroadenoma.JPEG)

Here the classification of Hardy is used to measure the size of the tumor. According to this classification, tumors < 10mm are microadenomas and tumors 10 > mm are macroadenomas. It is important to determine tumor volume because it correlates with visual field defects (Kan, Kan, Atmaca, Atmaca, & Colak, 2013). Visual field defects can be detected with a perimetry test.

1.1.6 Clinical presentation

It is often observed in younger patients that when overproduction of growth hormone occurs before the end of length growth, then gigantism can result (Melmed, 2006). These young patients can reach body sizes of more than two meters (Creo & Lteif, 2016). In adults, epiphyseal plates are already closed and length growth is no longer possible.

Figure 4: *Fedor Machnow (1878-1912): He grew up to a height of 2.39 m. He travelled Europe around 1900, and the USA to exhibit his unusual size at fairs. https://de.wikipedia.org/wiki/Riesenwuchs#/media/File:Fedor_Machnow_01.jpg*

Therefore, in adults, symptoms are particularly evident by changes in face, skeleton as well as organs, and a gradual development of the symptoms takes place with a diagnosis only after about seven to ten years after onset of the disease (Rajasoorya, Holdaway, Wrightson, Scott, & Ibbertson, 1994).

Direct effects of the tumor can be visual loss, headache, cranial nerve paralysis, hypothyroidism, hypogonadism and hypocortisolism.

Overproduction of GH/IGF-1 can lead to several systemic effects in the form of soft tissue and skin changes, enlargement of the extremities, soft tissue swelling, prognathism, carpal tunnel syndrome and hyperhidrosis (Colao et al., 2004).

Hormone excess can also lead to respiratory and cardiovascular complications (Colao et al., 2004). Respiratory complications, such as sleep apnea and lung diseases, are caused by the effects of GH and IGF-1 and can increase mortality in those patients (Fatti, Scacchi, Pincelli, Lavezzi, & Cavagnini, 2001). Cardiovascular symptoms may be arterial hypertension, valvular disease, arrythmias, and cardiomyopathy (Giustina et al., 2003).

Other typical clinical features are complications in metabolism, such as diabetes mellitus and insulin resistance, as well as alterations of bones and joints, in form of increased articular cartilage thickness, arthropathy and carpal tunnel syndrome (Colao et al., 2004). Especially arthropathy is a common complication of acromegaly and can lead to invalidity (Claessen et al., 2017).

Moreover, endocrine disorders such as hypogonadism and hyperprolactinaemia may appear (Colao et al., 2004). In women there may also be amenorrhea or galactorrhea, and in men, libido or potency disorders (Franks, Jacobs, Martin, & Nabarro, 1978; Goldman & Klinges, 1989). In addition, adrenal gland disorders can occur and the number of thyroid pathologies in patients with acromegaly is high (Roginsky, Shaver, & Christy, 1966).

Many patients with pituitary adenomas have lower quality of life index, poorer sleep quality and increased risk of depression (Leistner et al., 2015). For this reason, depression and feelings of inferiority can also occur over the course of the disease (Geraedts et al., 2014).

Epidemiological studies showed an increased risk for the development of cancer in patients with acromegaly (Holdaway & Rajasoorya, 1999), especially for cancer in the gastrointestinal tract (Colao et al., 2004).

1.1.7 Prognosis

Compared to the normal population, acromegaly has a detrimental effect on well-being and mortality risk. The determining factor of outcome are serum GH levels after therapy (Rajasoorya et al., 1994).
The reason for increased mortality risk is not only the tumor itself, but also cardiovascular diseases caused by acromegaly (Wright, Hill, Lowy, & Fraser, 1970).
Another factor that can determine the prognosis is artropathy and if it cannot be prevented in the initial stages, morbidity and functional impairment increases. In general, if there is no proper therapy for acromegaly, morbidity and mortality rates increase (Colao et al., 2004).

1.2 Diabetes mellitus type 2

In patients with acromegaly, disturbances of glucose metabolism are frequently manifested. In approximately 20-30% of patients with acromegaly, type 2 diabetes mellitus may occur, with age, BMI and hypertension being the most significant risk factors (Fieffe et al., 2011; Giustina et al., 2003; Niculescu, Purice, & Coculescu, 2013). Patients with acromegaly who are additionally affected by type II diabetes, have a poorer prognosis. While GH has an inhibitory effect on insulin and downregulates insulin sensitivity, IGF-1 increases insulin sensitivity, however the effect of GH is stronger and often leads to diabetes mellitus (Clemmons, 2002; Colao et al., 2004).

In a diabetic, typical symptoms of acromegaly can indicate the underlying disease. Fatigue, thirst, polydipsia, weight loss, itching, insomnia and restlessness are common signs of diabetes mellitus. In addition, there may be specific symptoms of the kidney, eye, skin (wound healing disorders, etc.), immune system and nervous system (polyneuropathy etc.).

Weight management and lifestyle modification are the primary therapeutic options for diabetes mellitus type 2. Beneficial effects can be achieved through exercise and diet. Risk factors such as smoking, hypertonus, obesity and fat metabolism disorders should be avoided. In type 2 diabetes, weight loss is the most effective treatment

option. However, since weight control is difficult, monotherapy with Metformin is usually initiated. If the therapeutic target is not achieved, one or more oral antidiabetics (for example gliptins, glinides, sulfonylureas) or insulin can be added in addition to metformin.

1.2.1 Metformin

Metformin is an oral antidiabetic drug which reduces insulin resistance, indirectly lowers insulin levels and is mainly used in patients with type 2 diabetes mellitus. The effect of metformin is based on the reduction of hepatic glucose production by inhibiting gluconeogenesis and lipogenesis (Viollet et al., 2012). This is achieved by inhibiting complex I of the mitochondrial respiratory chain and thereby creating an energy deficit within the cell. This results in a direct inhibition of gluconeogenesis and an activation of the intracellular energy sensor AMPK. Consequently there is a reduced transcription of CREB which is responsible for the intranuclear expression of the gluconeogenesis program (Viollet et al., 2012). By activating AMPK, Sirt1 is also activated and leads to inhibition of gluconeogenesis (Caton et al., 2010). The activation of AMPK by metformin and the ensuing inhibition of CREB is likely exclusive to hepatocytes, since there are opposing effects in other body cells, which leads to the assumption that metformin also acts through other intracellular processes (Faggi, Giustina, & Tulipano, 2017; Viollet et al., 2012) (Figure 5).

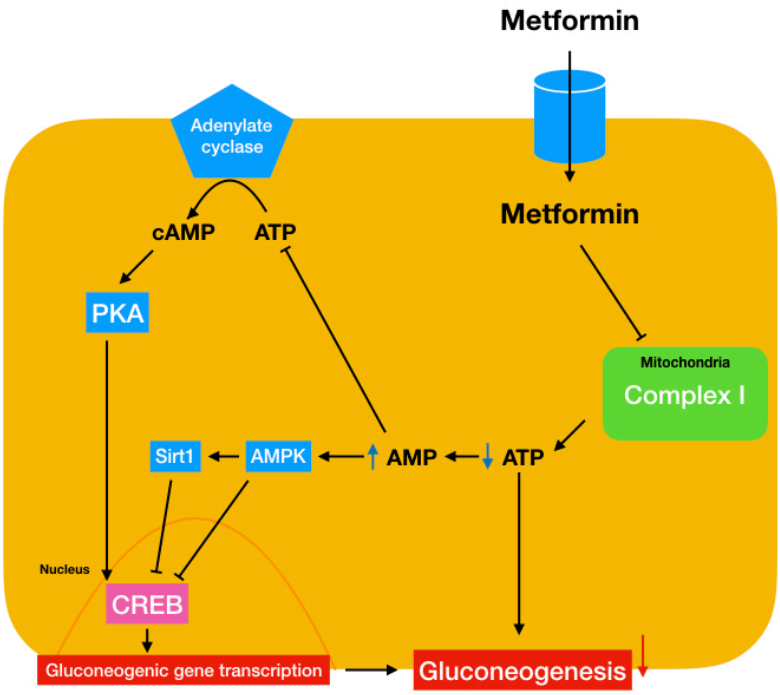

Figure 5: *Effect of Metformin on gluconeogenesis in hepatocytes.*

Furthermore, metformin is believed to increase absorption of glucose in the intestine, as well as in muscle cells (Natali & Ferrannini, 2006). Side effects of therapy with Metformin may be gastrointestinal disorders, such as nausea and diarrhea. However, if doses of metformin are reduced, these side effects usually subside (DeFronzo & Goodman, 1995). Lactate acidosis is a rare but feared side effect of treatment with Metformin. This can take place in hypoperfusion and hypoxia by cardiovascular, pulmonary, hepatic or renal diseases and is characterized by an increased blood lactate concentration (> 45 mg / dL), a reduced blood pH (<7.35) and an increased anion gap (Oliva, 1970; Salpeter, Greyber, Pasternak, & Salpeter, 2010; Stang, Wysowski, & Butler-Jones, 1999).

1.3 Treatment of acromegaly

Primary goal of therapy in acromegaly is reduction of GH and IGF-1 values to normal ranges (Giustina et al., 2000). Biochemical control of acromegaly is defined according to following criteria: 1) Normalization of basal GH below 2.5 ng / mL, 2) suppression of GH <1 ng / mL in the oGTT (or < 0.5 ng / mL), 3) IGF-1 within age- and gender-specific norm ranges (Brabant, 2003; Freda, 2003; Giustina et al., 2000). In patients receiving therapy with somatotropin antagonists, only IGF-1 values are used for control (Lim & Pullan, 2005).

In acromegaly, surgery is the first treatment for most patients. Alternatively, drug therapy with dopamine agonists, somatostatin analogues or GH receptor antagonists is used to block the action or production of GH. Drug therapy can be administered preoperatively or postoperatively, and a combination of two drugs is also possible depending on each individual case. Postoperatively or in patients who do not tolerate a drug therapy, radiotherapy can be performed (Melmed, 2006) (Figure 6).

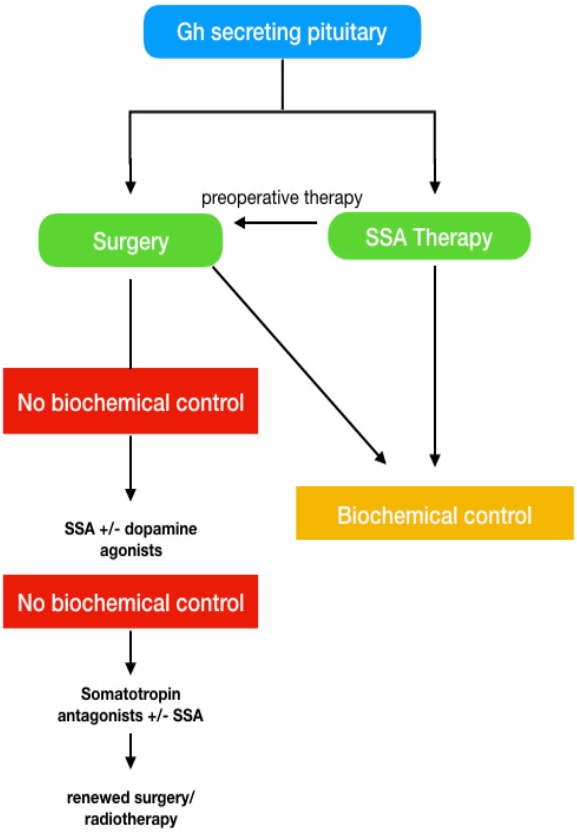

Figure 6: *Therapeutic possibilities.*

The aim of therapy is biochemical and clinical control of the disease. In addition, pituitary function should remain intact and there should not be any injury of the optic chiasm during surgical treatment. If pituitary insufficiency occurs, substitution with the corresponding hormone should be initiated.

1.3.1 Surgery

Transsphenoidal surgery represents the first-line treatment in most patients with acromegaly.

It is indicated for growth-hormone-secreting microadenomas, as well as for decompressions of surrounding tissues, especially the optic tract (Melmed, 2006). Within one hour after surgery, normalization of growth hormone serum levels is possible. For larger macroadenomas and invasive tumors, however, complete resection is rarely possible (Ben-Shlomo & Melmed, 2008).

In transsphenoidal surgery, the surgeon reaches the sphenoid sinus via the nasal cavity, where the hypophysis is adjacent.

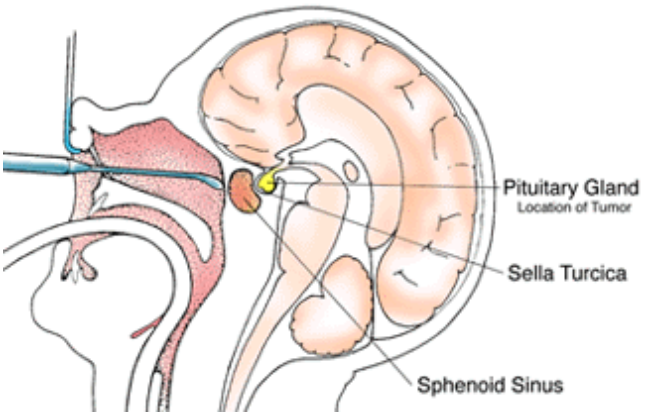

Figure 7: *Transsphenoidal Surgery. (http://www.newhealthadvisor.com/pituitary-adenoma-surgery.html)*

Success in transsphenoidal surgery depends on tumor size, invasiveness of the tumor, and experience of the surgeon (Freda, 2003). Furthermore, as a result of surgery, it was observed in some patients that it had a positive effect on the glucose

metabolism (Helseth et al., 2016). However, in about 10 % of cases, hypopituitarism may occur postoperatively (Melmed, 2006).

1.3.2 Radiotherapy

If not all tumor residues could be removed during surgery, postoperative radiation is considered. In case of intolerance of medical treatment or at the request of the patient, radiotherapy can be initiated. Radiotherapy can be done with Cyberknife, Gammaknife or in conventional stereotactic irradiation. In approximately 60 % of these patients, pituitary insufficiency occurs within 5-10 years, which requires treatment (Melmed et al., 2002).

1.3.3 Hypopituitarism

In some patients pituitary insufficiency may occur. After transsphenoidal surgery approximately 30 % of patients develop hypopituitarism (Colao et al., 2006) and in the space of 10 years after radiotherapy 50 % of patients are affected by it (Melmed, 2006).

For diagnosis of hypopituitarism, measurements of hormone levels are sufficient. However, if there is a lack of growth hormone or corticotropin, a stimulation test is necessary (Schneider, Aimaretti, Kreitschmann-Andermahr, Stalla, & Ghigo, 2007).

Depending on the course of the disease, partial or complete hypopituitarism may occur. In the case of pituitary anterior lobe insufficiency, deficiency of the adenohypophysis with a reduced production of TSH, ACTH, GH etc. can occur. Depending on the affected axis, different symptoms and reduced Quality of Life (QoL) may follow, and therapy should be individualized by hormone replacement, taking into account possible interactions (van Aken & Lamberts, 2005).

1.3.4 Pharmacological therapy

1.3.4.1 Dopamin agonists (DA)

In patients with acromegaly, dopamine agonists have an inhibitory effect on GH secretion. There are many different dopamine subtypes, organized according to their structural homology, e.g. $D_{1,5}$-agonists and $D_{2,3,4}$-agonists. Bromocriptine was the first dopamine agonist used in acromegaly. Although, bromocriptine achieved good effects in controlling acromegaly, it lacked potency and had several side effects (Jaffe & Barkan, 1992).

Today cabergoline a more potent, second-generation D_2 receptor agonist is used as a therapeutic standard, with significantly fewer side effects. Cabergoline is comparatively low priced and can be administered orally (Colao et al., 1997). Cabergoline can be considered in patients with GH-prolactin secreting pituitary adenomas (Muller & Van Der Lely, 2004). In patients, in which therapy with somatostatin analogues is ineffective, cabergoline can normalize IGF-1 values in 40-50 % of patients (Kuhn & Chanson, 2017). Presumably, therapy with cabergoline is beneficial in patients with disturbed glucose tolerance, which is the precursor of diabetes mellitus (Gibson, Karmally, McMahon, Wardlaw, & Korner, 2012).

1.3.4.2 Somatotropin antagonists

Another option to treat acromegaly is pegvisomant, a subcutaneously injected growth hormone receptor antagonist. The monotherapy with pegvisomant is performed in most cases only when surgery, radiotherapy or somatostatin analogues were not successful (Giustina et al., 2011).

Known side effects of the therapy with pegvisomant are reactions at the injection sites, nausea, peripheral edema, chest pain, paresthesia and disorders of glucose metabolism.

Long-term studies showed an efficacy of 85 % after therapy with pegvisomant, but in some cases there was an increase in transaminases as well as an increase in tumor volume (Hodish & Barkan, 2008).

1.3.4.3 Somatostatin analogues

Somatostatin analogues (SSA) are the mainstay in targeted hormonal treatment of acromegaly. Somatostatin (SMS) inhibits the release of many hormones, e.g. GH, insulin, glucagon and TSH (Roelfsema & Frolich, 1991). Moreover, it has an inhibitory effect on the peristalsis of the stomach (Izumi, Honda, Tsuchiya, Ueda, & Hatano, 1980), gastric acid secretion and the exocrine secretion of pancreatic enzymes (Abdu, Hicks, Hennig, Allen, & Grundy, 2002). It also reduces blood pressure via the splanchnic system.

Since the 1980s it is used as a primary, pre- or postoperative therapy of acromegaly. Octreotide (sandostatin) and lanreotide (somatulins) act on the receptor subtypes 2 and 5 (SSTR2, SSTR5), which are responsible for inhibition of growth hormone (Jiang et al., 2011).

Until recently, somatostatin had to be administered subcutaneously daily. Today, only one application every month is possible through depot preparations (somatuline, autogel, Sandostatin LAR). The possibility of oral administration of somatostatin analogues in the therapy of acromegaly is currently under discussion (Biermasz, 2017) as a recent study has shown that patients who have switched from injection therapy to oral therapy have demonstrated adequate efficacy with regard to controlled GH and IGF-1 levels (Melmed et al., 2015).

Whether SSA can be administered depends on therapy resistance and tolerability of the therapy. The most common side effects include digestive disorders, gall stones and reactions at the injection site (Chakravarty, Ajmani, Manchanda, Kulshreshtha, & Chopra, 2012). Moreover, a deterioration of glucose metabolism was observed during preoperative SSA treatment (Helseth et al., 2016).

SSA have a very good response rate in acromegaly and provide an effective treatment option (Vance & Harris, 1991). It has also been shown that SSA can alleviate symptoms such as headache in patients with acromegaly in the shortest time possible (Levy, Bejon, Barakat, Goadsby, & Meeran, 2003).

Optimization of surgical results was achieved by preoperative treatment with SSA (Bevan et al., 2002; Losa et al., 2006). Moreover, a positive effect on tumor shrinkage has been observed in several studies both in macro- as well as in microadenomas (Bevan, 2005; Losa et al., 2006).

The exact mechanisms of how SSA causes a reduction in tumor cells in pituitary adenomas is not yet fully understood. It is known, however, that SSA has an antiproliferative effect on pituitary tumor cells via the tumor suppressor gene Zac1 (Theodoropoulou et al., 2006).

Pasireotide (SOM230) is a new SSA which has a high affinity to the somatostatin receptor type 5 (SSTR5) and is therefore used as a means of second choice in acromegaly. In patients with octreotide resistance, it can suppress GH (van der Hoek et al., 2004). In patients who do not achieve biochemical control in a first-line therapy with somatostatin analogues, Pasireotide could become standard medication because of its efficacy (Gadelha et al., 2014)

1.3.4.4 Somatostatin analogue resistance

Although SSA are the mainstay in medical therapy of acromegaly, many patients are resistant to their anti-secretory and tumor shrinking effects. The efficacy of SSA treatment is dependent on age, gender, initial GH and IGF-1 levels, tumor size and the expression of somatostatin receptors (Colao, Auriemma, Lombardi, & Pivonello, 2011).
A possible marker for the efficacy of the therapy could be the transcription factor Zac1, whose immunoreactivity correlates positively with tumor shrinkage and reduced IGF-1 levels after SSA therapy and was not affected by age at diagnosis, gender or duration of SSA treatment (Theodoropoulou et al., 2009).

1.4 Aim of the study

Treatment of somatotroph pituitary tumors by transspenoidal surgery and/or treatment with somatostatin analogues (SSA) is the standard therapy for acromegaly. During therapy, a considerable proportion of the acromegalic patients develops partial pituitary insufficiency and thus receives corresponding hormone replacement therapy. In up to 30% of the patients with acromegaly the disturbed glucose metabolism may finally lead to type 2 diabetes mellitus which is treated by the anti-diabetic drug metformin. As acromegalic patients respond differently to SSA treatment the question arises whether hormone replacement or metformin treatment has an influence on the success of SSA therapy.

To address these questions the acromegalic patient cohort of the NeoExNET database was used and statistically screened for correlations between the degree of pituitary insufficiency and SSA treatment response. Moreover it was approved, whether anti-diabetic metformin treatment in acromegalic patients had an influence on SSA responsiveness. The effect of metformin on SSA treatment was not only studied in the NeoExNET cohort but also in vitro in primary cell cultures of human somatotroph tumors and in the lactosomatotroph rat pituitary tumor cell line.

In sum the aim of the study was to clarify the influence of hormone replacement therapy and metformin treatment on the efficacy of SSA therapy in acromegaly.

2. Materials & Methods

2.1 Patients

2.1.1 Database NeoExNet^{M}

Retrospective data analysis with patient data from the NeoExNet^{M} was performed. NeoExNet^{M} is a database for neuroendocrine tumors in Munich. The following clinics in Munich are involved: Medical Clinic Center in Zimssensstraße 1, Medical Clinic and Polyclinic II in Marchionistrasse, as well as the Max Planck Institute for Psychiatry in Munich. Only patients with neuroendocrine disorders were added to the NeoExNet^{M}. These included patients with prolactinoma, Cushing's disease, clinically non-functioning adenomas (NFPA) and acromegaly. In this study, only data from acromegaly patients were used (Table 1).

Table 1: *Patient data from NeoExNet^{M}*

Base data	• Name • Gender • Date of Birth • Address • Register assignment (Acromegaly, M.Cushing, NFPA, prolactinoma) • Consent form
Comorbidities	**Cardiovasular** • Arrhythmias • Cardiomyopathy • Cerebrovascular diseases • Arterial hypertension • Coronary heart disease • Myocardial infarction **Metabolic** • Diabetes mellitus • Pathological glucose tolerance

	• Pituitary insufficiency • Diabetes insipidus **Respiratory** • Sleep apnea syndrome
Anamnesis	• Patient history • Family history • Drug history other than acromegaly
Symptoms	• Growth of hands / feet • Soft tissue swelling • Carpal tunnel syndrome • Arthralgia • Fatigue • Headache • Paraesthesia • Hyperhidrosis • Visual impairment • Cycle malfunctions (Gyn.) • Libido and potency loss • Depression
Physical examination	• Height (cm) • Body weight (kg) • BMI • Waist and hip circumference • Blood pressure (mmHg) • Pulse
Laboratory	**General Laboratory** • Leukocytes, erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, thrombocytes, eosinophilia • Sodium, potassium, creatinine, GFR, urea, calcium, phosphate,

	GOT, GPT • Fasting Glucose, HbA1c, fasting Insulin • Total cholesterol, triglycerides, HDL, LDL, vitamin B12, CRP • fT3, fT4, TSH, TPO-AK, 25-OH-Vit. D3, PTH **Urine Stix, urine quantitatively** **OGTT (oral glucose tolerance test)** • fasting glucose / insulin • glucose / insulin after 60 min • glucose / insulin after 120 min **Hormonal diagnostics** • Prolactin • Cortisol • Stimulated cortisol • LH, FSH • Testosterone, estradiol • GH basal, GH nadir in OGTT, Stimulated GH • IGF-1, IGF-BP3
Imaging	**Pituitary** • MRI/CT • Contrast medium • Valuation • Max. tumor size **Imaging bone density** **Echocardiography** **Tyhroid sonography** **Range of vision** **Device diagnostics**
Therapy	**Medicines** • Somatostatin analogues (SSA)

	• Dopaminagonists • Pegvisomant • Other Medicines **Surgery** • Date • Number of surgeries • Type of surgery • Surgery Center • Histology **Radiation** • Date • Type of radiation • Place of radiation
Outcome	• Clinically controlled • Biochemically controlled • Change of tumor size

2.1.2 Data collection

Of 49 patients with acromegaly, 36 patients received therapy with somatostatin analogues at any time. In order to assess the effect of SSA-therapy, various parameters were compared during initial diagnosis and during the course of the disease. These contained the GH and IGF-1 values in the initial diagnosis and after somatostatin analog therapy. We then calculated the difference between the two values and checked whether SSA therapy resulted in a normalization of the values. The difference between the two values was then calculated and checked whether SSA therapy resulted in a normalization of the values. In addition, pituitary adenoma size was reported. We classified the tumor size according to Hardy, where pituitary adenomas are subdivided into 4 grades: grade I for microadenomas (<10 mm in diameter), grade II for macroadenomas (> 10 mm in diameter) that could reach the

suprasellar region but no bony structures, Grade III for locally invasive tumors, and grade IV for large invasive tumors that may affect the hypothalamus or cavernous sinus. We also investigated the presence of pituitary insufficiency. It was examined whether this was an insufficiency of the corticotrophic, thyreotrophic, gonadotrophic or somatotrophic function. Combinations of the malfunctions were also considered. Information on the therapy of pituitary insufficiency was taken from the NeoExnet^{M}.
Differentiation was made here between corticosteroid therapy in the event of failure of the corticotrophic function, testosterone / estradiol therapy in the event of failure of the gonadotropic function, thyroxine therapy in the event of a failure of the thyreotropic function or growth hormone therapy in the case of somatotropic insufficiency.
Furthermore, NeoExnet^{M} provided information on whether metformin therapy was performed in the acromegalic patients with diabetes mellitus.

2.1.3 Data processing and statistical evaluation

Data processing and statistical evaluation of the results were carried out using SPSS (Statistical Package for Social Sciences).
To assess the contribution of both surgery and SSA suppletion on pituitary insufficiency (dichotomous), a binary logistic regression analysis was performed. The model carried an additional correction for age, gender, disease duration and tumor size, as well as the main variables of interest surgery (dichotomous) and SSA (dichotomous). A forced entry method was used.
In addition, a logistic regression was performed to investigate whether there were interactions between therapy with somatostatin analogues and treatment of hypopituitarism.
To assess the contribution of treatment modalities to the outcome change in IGF1, a linear regression analysis was used using three blocks. Block 1 contained: age, gender, disease duration and tumor size. Block 2 contained the treatment modalities / comorbidities (dichotomous): somatostatin analogues, corticosteroid supplementation, testosterone / estrogen supplementation, growth hormone supplementation, thyroid hormone supplementation and pituitary insufficiency. Block 3 contained the main variable of interest (dichotomous): metformin application. A forced entry method was used for all blocks.

In this study, P-values with $p < 0.05$ are considered statistically significant and P-values with $p < 0.01$ as highly significant. For P-values with $p < 0.1$, one speaks of a trend towards statistical significance.
In the case of a variable inflation factor VIF < 1, predictors do not correlate with one another; in the case of 1 < VIF > 5, the predictors correlate moderately with one another and with a VIF > 5 predictors strongly correlate with each other.

2.2 In vitro studies

2.2.1 Reagents

Table 2: *Reagents.*

Product	**Manufacturer**
Dulbecco's modified Eagle medium (DMEM)	Invitrogen Corp. (Paisley, Scotland, UK)
Dimethyl sulfoxide (DMSO)	Sigma (St. Luis, MO, USA)
Fetal Scalf Serum (FCS)	Gibco (Karlsruhe, Germany)
L-Glutamin	Biochrom AG (Berlin, Germany)
Metformin	Sigma (St.Luis. MO, USA)
ONPG	Sigma (St. Luis, MO, USA)
Amphotericin B	Biochrom AG (Berlin, Germany)
Penicillin+Steptomycin-Mix	Biochrom AG (Berlin, Germany)
Phosphate based buffer (PBS)	Life Technologies (Paisley, Scotland, UK)
Resveratrol	Calbiochem (Darmstadt, Germany)
Superfect	Qiagen (Hilden, Germany)
Trypsin	Sigma (St.Luis. MO, USA)
WST-1 assay	Roche (Mannheim, Germany)

2.2.2 Solutions

Table 3: *Solutions.*

Solution	**Composition**
HDB^+ Buffer	Glukose : 10 mM NaCl : 137 mM KCl : 5 mM Na_2HPO_4 : 0.7 mM HEPES : 25 mM pH-adjustment to 7.3 with NaOH Partricin : 500 µg/L Penicillin/Streptomycin : 10^5 U/L
Collagenase mixture	Prepared in HDB^+ Buffer Collagenase 1000 units/ml Trypsin inhibitor 100 µg/ml Hyaluronidase 1 mg/ml BSA 4 mg/ml DNAse II 5 µl/ml
Tumor medium	500 ml DMEM (4,5 g/l Glucose, +Gln) + 50 ml FCS, heat-inactivated + 5 ml Glutamine solution + 5 ml Penicillin/streptomycin solution + 5 ml Amphotericin B solution + 5 ml MEM-vitamins solution

2.2.3 Plasmids

Table 4: *Plasmids.*

Plasmid	**Origin**
ß-Galaktosidase	D. Spengler, Max-Planck-Institut für Psychiatrie, Deutschland
CRE-luc	Clontech Laboratories Inc., Palo Alto, CA, USA
Flag-SIRT1	Addgene. M. Greenberg; Brunet, Cambridge, MA, USA
rGH-luc	Gutierrez-Hartmann, University of Colorado, USA

2.2.4 Cell-lines

In this study, GH3 cell lines from rats (American Type Culture Collection) were used. The cells were cultured in tumor medium at 37 ° C in a humid atmosphere with 5% CO2. When confluence was reached, cells were washed with PBS, trypsinized and centrifuged at 1200 x g for 4 minutes. Finally, they were seeded into multiwell plates for the respective experiment.

2.2.5 Primary cell cultures of human GH-secreting pituitary adenomas

In this part of the study, with approval of the Ethics Committee of the Max Planck Institute, GH-secreting pituitary adenomas from 7 patients were examined. From every patient, or their relatives, informed consent was given. The tumors were diagnosed using clinical, radiological, surgical and biochemical findings. Transsphenoidal surgery was the resection method of choice. According to the classification of Hardy, pituitary adenomas are subdivided in microadenomas (<1 cm) and macroadenomas (> 1 cm).

After surgical excision, the tumors were immediately given in cooled tumor medium and further processed within 24 hours. For this purpose, the tumor was washed with HDB+ buffer, whereupon fibers and deposits were removed and the tumor was divided into two pieces. For morphological analysis, some of the tumor was frozen with dry ice. The other part of the tumor was either frozen for RNA and protein

extraction or processed as follows for cell culture: After mechanical division into small fragments and processing with a collagenase mixture, the cells were counted, mixed in tumor medium to achieve the appropriate cell density and seeded into multiwell plates for the respective experiment. To identify possible resection-related contamination with normal anterior pituitary cells, an RT-PCR was performed using specific primers for SF-1 (steroidogenic factor 1). Expression of SF-1 is found only in the gonadotrophic cell lineage of the anterior pituitary (Zafar et al., 1995). When the expression of SF-1 is detected, it indicates the presence of normal anterior pituitary cells in the tumor material. SF-1 positive tumors were not included in this study.

2.2.6 Transfection

Transfection of the cells was carried out with SuperFect (Qiagen, Hilden, Germany). Cells (3 x 10^5) were transfected for 3 hours with 1 µg Flag-SIRT1 and 0,5 µg GH-luc, and left for 48 hours in 2 % FCS DMEM. The dual luciferase reporter assay (Promega) was used according to the manufacturer's instructions and the luciferase activity was determined by Berthold TriStar luminometer. For effective control of transfection, 0.3 µg plasmids were added with the Rous sarcoma virus promoter. This promoter drives the β-galactosidase gene.
The following procedure was carried out to measure the activity of β-galactosidase:
30 µg of distilled water was added to 20 µg of supernatant and finally spread to a 96 well plate. Thereupon, 50 µg of ONPG buffer (2x) were pipetted into the mixture.
At 37° C the 96-well plate was incubated in a light-protected container until the individual "wells" (shaft) showed a light yellow colour. The β-galactosidase activity was then measured in an ELISA plate reader at 420 nm. The values represent the ratio of the relative luciferase activity of the plasmids to the β-galactosidase activity.

2.2.7 Plasmid preparation

Competent Top10 bacteria (Invitrogen, Paisley, UK) were transformed with the desired plasmid (see table) according to insctructions of the manufacturer and applied to a LB agar of a petri with 50 µg/µL ampicillin. The petri was incubated overnight at 37 ° C. A single colony was removed from the plate and placed in a 250 mL LB medium containing 50 µg / µL ampicillin. Overnight the colony was incubated

at 37 ° C. The plasmid was purified using Qiagen HiSpeed Purification System (Qiagen, Hilden, Germany). The rGH-luc construct has the proximal rat GH promoter upstream of the reporter gene luciferase. The cAMP response element is located above the TATA box of the thymidine kinase promoter of the herpes simplex virus and the reporter gene luciferase in the CRE-luc construct (Mercury pathway profiling system, Clontech Laboratories, Inc., Palo Alto, CA).

2.2.8 GH determination

Rat lactosomatotroph GH3 cells were placed in 96 well plates ($1x10^4$ cells / well) with 10% FCS DMEM medium. After 24 hours, the cells were treated with octreotide and/or metformin for 24 hours in a 0% FCS DMEM medium. After completion of the treatment, the supernatants of the individual wells were collected and stored frozen for rat GH measurement. Human somatotrophinoma cells were seeded into 48 well plates ($1x10^5$ cells/well) in tumor medium. After 48 hours of incubation, the cells were treated with octreotide and/or metformin for 24 h and then the cell culture supernatants were collected and frozen for later human GH analysis

In GH3 cell culture supernatants, rat GH (rGH) was measured by radioimmunoasay (RIA) as described (Monteserin-Garcia, 2013). In this assay, radioactively labelled rGH (^{125}I-rGH; BIOTREND, Cologne, Germany) competes with non-radioactive rGH in the cell culture sample for binding to a primary specific anti-rGH antibody. After addition of a secondary antibody and precipitation of the immunocomplex with 6% PEG, the radioactivity of the precipitate was measured with a gamma-counter and rGH concentrations in the samples could be calculated by comparing them with a standard curve made with different concentrations of a rGH standard.

The human GH (hGH) concentration in the cell culture supernatants was determined with IMMULITE 2000 Growth Hormone, which is a solid-phase, two-site chemiluminescent immunometric assay (Monteserin-Garcia et al., 2013). In this assay, a solid phase (bead) covered with monoclonal anti-hGH antibodies binds the hGH of the sample. A second antibody also against hGH but containing the enzyme alkaline phophatase is added to the reaction mixture and forms an antibody-sandwich complex with the solid phase and the hGH. After washing the antibody excess by centrifugation, a chemiluminescent substrate is added to the reaction. The intensity of the signal is proportional to hGH concentration.

3. Results

3.1 Clinical Study

3.1.1 Patient group

The 49-membered group consisted of 26 men and 23 women. Mean age at diagnosis was 48 years. In the male group, diagnosis was made on average at 44.5 years and in the female group at 53.2 years. At the time of data collection, average age of male patients was 55.7 years and that of women 63.9 years.

Furthermore, there were differences in tumor size, therapy form and therapy response.

In our patient group, 35 patients had a diagnosed macroadenoma and 9 patients had a microadenoma. No information about tumor size was available in a total of 4 patients.

A total of 18 patients developed pituitary insufficiency during the course of their disease. In our patient cohort, 12 of the 49 (24.5%) patients had diabetes mellitus and all of these patients were treated with Metformin

Table 5: *Study cohort.*

Patients	**n =**
Total	49
Females	23
Males	26
Median age at diagnosis	**years =**
Entire group	48
Females	53,2
Tumor Size	**n =**
Makro	35
Mikro	9
n.a.	4
Treatment	**n =**
Surgery	
- Total	45
- Primary therapy	39

Somatostatin analogues	
- Total	36
- Primary therapy	11
Pegvisomant	4
Dopamine agonists	4
Radiotherapy	13

Biochemical control after SSA-Therapy	**normalized hormone values (%)**
Growth hormone	0.611
IGF-1	0.472

Hypopituitarism	**n =**
Total	18
Women	3
Men	15
Insufficiency	
- gonadotropic	15
- thyreotropic	8
- corticotropic	6
Hormone replacement	
- Sex hormones	23
- Glucocorticoids	22
- Thyroid hormones	29

Metformin treated DM2	**n =**
Patient cohort	12
Patients with SSA-Therapy	8

3.1.2 Therapy modalities

In 45 patients (92 %), transsphenoidal surgery was performed and 35 of these patients received this as primary therapy. Somatostatin analogs were applied in 36 patients. In eleven patients, this was used as a primary therapy. In 36 patients, somatostatin analogue therapy was initiated and 11 patients received it as primary therapy.
In addition to transsphenoidal surgery and somatostatin analog therapy, some patients were also used for other types of therapy. Radiotherapy was performed postoperatively in 13 patients. A total of four patients received an additional therapy with Pegvisomant and another four patients were treated with a dopamine agonist.

3.1.3 Hypopituitarism

In the patient group, 18 patients were affected by hypopituitarism, of which n=15 were men and n=3 were women.
In 15 patients, gonadotropic axis was affected, thyreotropic axis in eight, and corticotropic axis in six patients. Nine patients had an isolated disorder of one of the axes. In most patients, however, there was a combined insufficiency of several axes.
In patients with hypopituitarism, glucocorticoids, sex hormones and thyroid hormones, either individually or in combination, were used therapeutically. A total of 23 patients (47 %) received sex hormones, 22 patients (45 %) glucocorticoids and 29 patients (59 %) thyroid hormones.

Binary logistic regression (SPSS software) showed that there is no significant correlation between factors such as sex, age, duration of the disease, tumor size, SSA therapy, transsphenoidal operation, and the incidence of hypopituitarism.

Table 6: Correlation between different *variables and hypopituitarism.*

	p-value
Gender	0.16
Age	0.439
Duration of disease	0.378
Tumor size	0.814
SSA-Therapy	0.710
Thranssphenoidal surgery	0.541

Of 36 patients who received SSA therapy, 15 patients were treated with corticosteroids. In seven patients normalization of the IGF-1 was observed during the course of the study. A linear regression analysis could not establish a correlation between decreasing IGF-1 by SSA therapy and treatment with corticosteroids ($p = 0.173$). From the group of patients treated with SSA, 15 patients received additional therapy with testosterone and estrogen preparations. Four of these patients showed a normalization of IGF-1. The linear regression analysis showed no correlation between decreasing IGF-1 by SSA therapy and treatment with estrogens / progestogens and androgens ($p = 0.085$). From the group of patients who received SSA, 19 patients were additionally treated with thyroxine. In nine of these patients, IGF-1 decreased to normal levels. However, no correlation between treatment with thyroxine and decreasing IGF-1 in SSA therapy could be demonstrated within the framework of linear regression analysis ($p = 0.721$). A further regression analysis also showed no correlation between decreasing IGF-1 after SSA therapy and substitution treatment with corticosteroids, testosterone or L-thyroxine (VIF <3).

3.1.4 SSA-Therapy effect and the influence of Metformin

In a large number of 36 patients treated with SSA, hormone levels were normalized. Overall, the GH value was normalized in 22 patients (61.1 %) and the IGF-1 value in 17 patients (47.2%).

Gender (p = 0.775) and duration of disease (p = 0.827) did not show a statistically significant correlation with decreasing IGF-1 in somatostatin analog therapy after performing a linear regression analysis. However, age (p = 0.075) showed a trend towards statistical significance.

In 8 of the 36 patients (22.2 %) treated with SSA, there was also diabetes mellitus, which was treated in all cases with Metformin.

In linear regression analysis, a correlation between metformin therapy and IGF-1 levels could be shown during concurrent therapy with SSA (p = 0.031; R-square change: 0.135; R-square: 0.321). In patients who received metformin in addition to SSA therapy, there was a stronger suppression of IGF-1 values (see Figure 8).

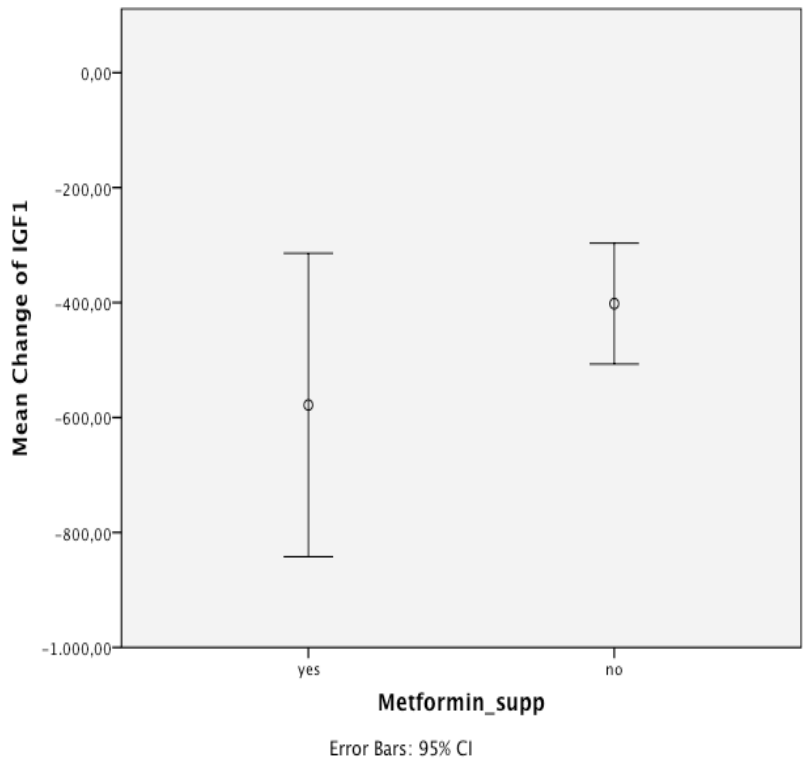

Figure 8: *Stronger reduction of IGF-1 levels under therapy with metformin during concurrent therapy with somatostatin analogues.*

3.2 In vitro studies: Influence of Metformin on GH-synthesis

3.2.1 Metformin inhibits GH secretion in immortalized Rat-GH3-cells

To determine whether metformin inhibits the secretion of GH on pituitary level, rat GH3 cells were treated for 24 h with increasing concentrations of metformin. Metformin dose-dependently inhibited GH secretion and thus showed a direct effect on GH production by lactosomatotroph tumor cells (Figure 9).

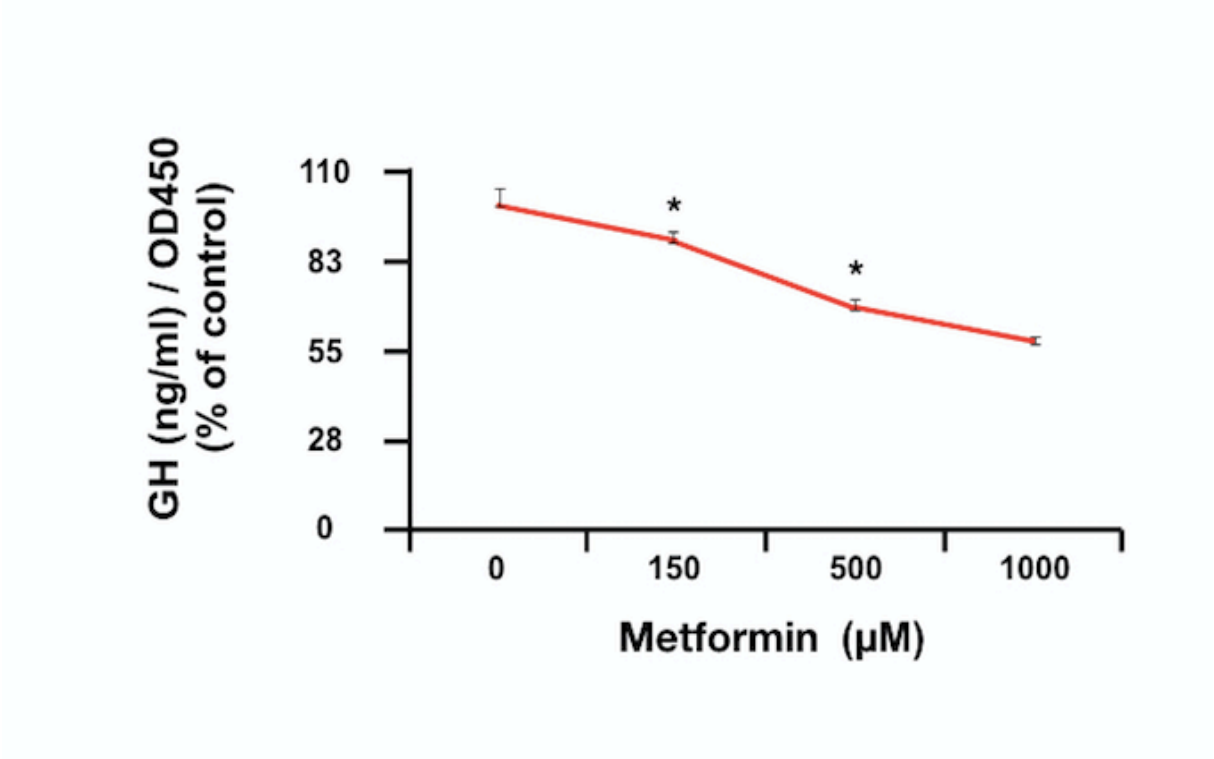

Figure 9: *Increasing concentrations of metformin lead to an inhibition of GH (in % of control).*

3.2.2 Metformin inhibits GH promoter activity

To test the possibility that metformin inhibits GH production by impairing GH promoter activity and thus GH mRNA synthesis, GH3 cells were transiently transfected with a plasmid containing the luciferase gene downstream from the GH promoter (GH-luc). Treatment with metformin suppressed the activity of the GH-promoter suggesting that Metformin lowers GH secretion by directly inhibiting its transcription.

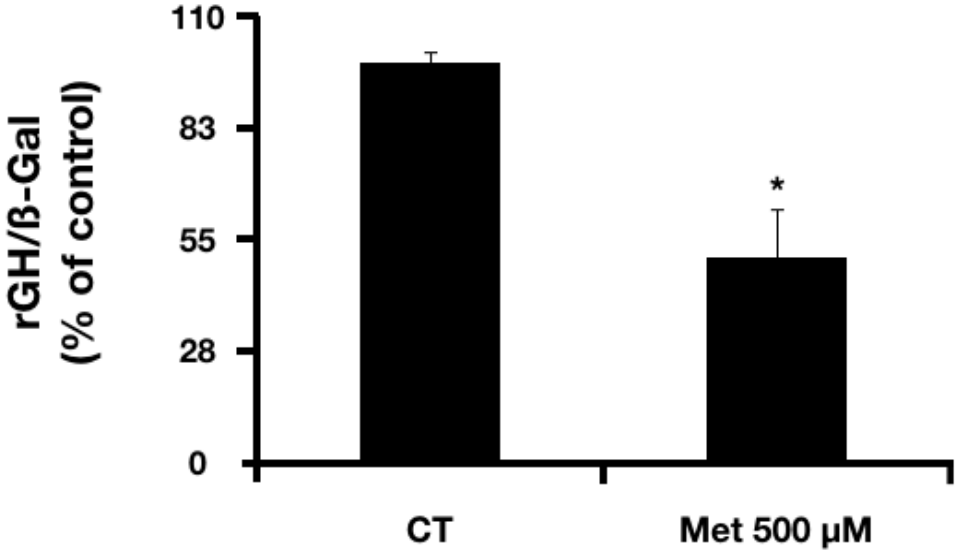

Figure 10: *Metformin has an inhibitory effect on GH promoter activity (in % of control).*

3.2.3 Influence of metformin on GH secretion in human somatotroph tumors

To confirm that metformin also inhibits GH secretion by human somatotroph tumors, metformin (500 µM, 1000 µM) was added to primary cell cultures of 7 human somatotroph adenomas. 24 h treatment of the tumors with metformin in a concentration of 500µM resulted in a slight but not yet significant suppression of GH compared to untreated cells in all adenoma cell cultures studied. In contrast, 1000µM metformin significantly reduced GH production in all somatotroph adenoma cell cultures as shown in a representative example in figure 11.

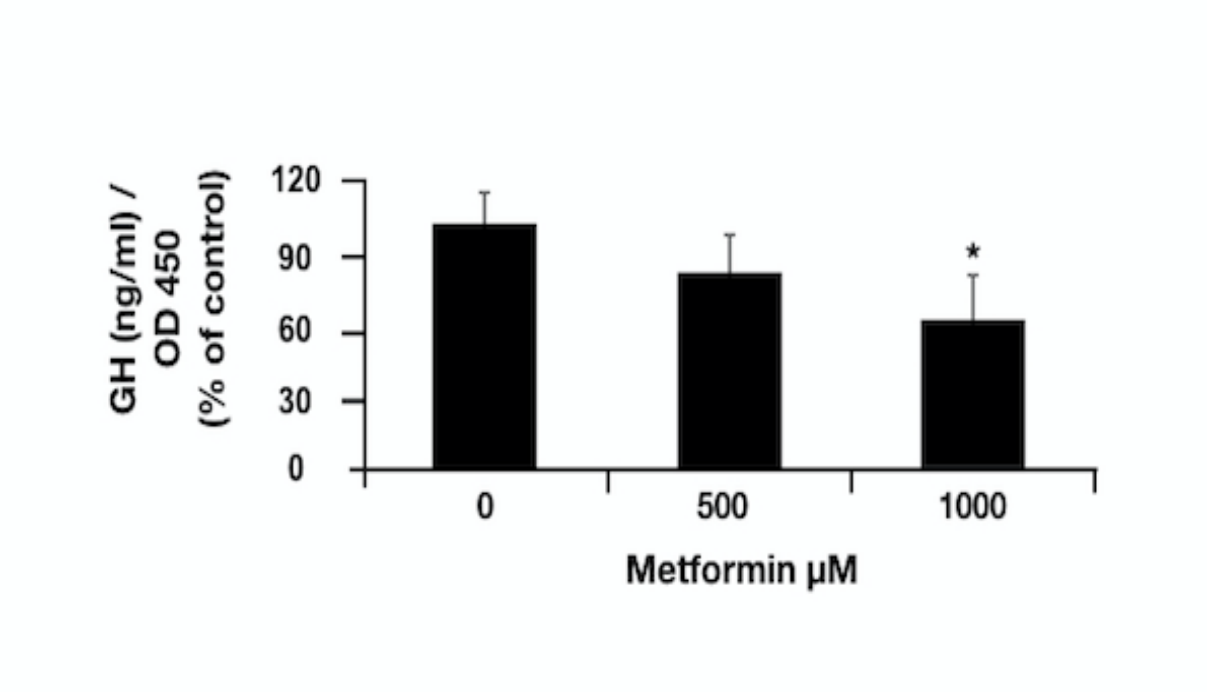

Figure 11: *Metformin significantly suppresses GH secretion in a human somatotroph adenoma cell culture at a concentration of 1mM (64±13% vs. basal=100%).*

3.2.4 Effects of combined metformin and octreotide treatment on GH secretion by human somatotroph adenomas

To study the effect of metformin on octreotide-mediated suppression of GH secretion in human somatotroph adenomas, 7 primary cell cultures of somatotroph tumors were treated for 24 h with octreotide (1 nM) and metformin (500 µM) either alone or in combination. Whereas neither treatment with octreotide nor with metformin alone had a significant effect on GH secretion, combined octreotide/metformin application significantly suppressed GH release in all human somatotroph adenoma cell cultures studied as shown by a representative example (Figure 12). The data suggest that metformin has an additive suppressive effect on octreotide-mediated GH inhibition.

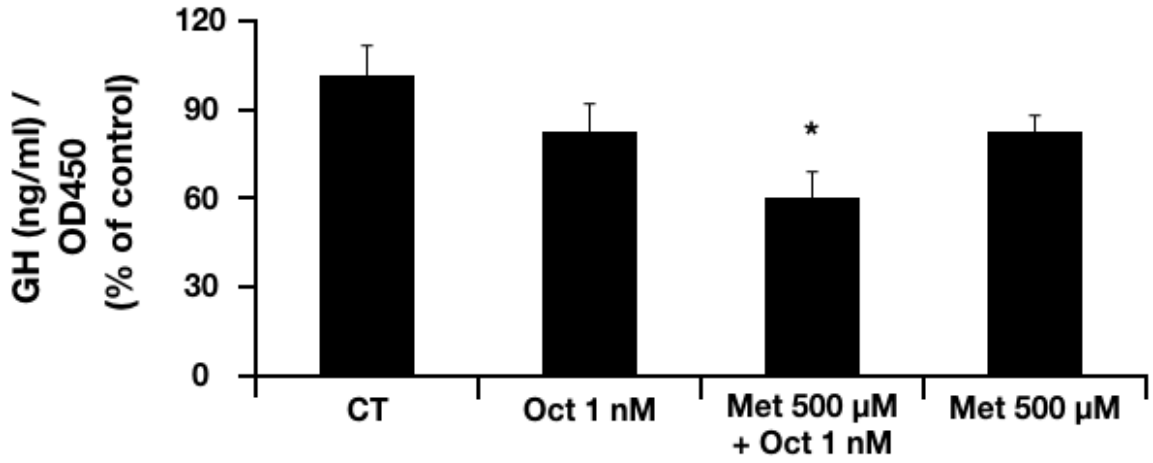

Figure 12: *In vitro studies on 7 human acromegaly tumors. Metformin significantly enhances the GH suppressive effect of octreotide (% of control 63 ± 13 versus 81 ± 12).*

4. Discussion

This study aimed to investigate the effects of different therapeutic modalities on the outcome of the treatment of acromegaly with the somatostatin analogue octreotide. Both the interference with hormone replacement therapy and with anti-diabetic metformin treatment was investigated. For this purpose, a combined clinical and - in case of metformin - an *in vitro* study were carried out.

In the clinical part of the study a retrospective data analysis was performed. In contrast to prospective studies, it is possible to obtain the results of data analysis in a shorter period of time as the data have already been measured and collected.
In this study, all results were based on already collected data from patients with acromegaly that where included in the NeoExNet^{M} database. Due to the typical nature of retrospective studies corresponding known difficulties were encountered, for instance incomplete or scarce patient data. Despite uniform criteria of the NeoExNet^{M}, the data of the patients were variable because patients have come to control examinations with varying degrees of frequency and different forms of the disease. For this reason, visits, blood sampling, functional tests and other entered data varied. It is shown in Table 1, that there was a broad range of data for our patients, which could theoretically have been included in the study. However, data and information were lacking in many patients and therefore not all patients had data on the individual parameters from Table 1. We opposed this with conscientiousness and selective exclusion of non-significant data sets. Therefore we only used data that were available for a sufficient number of patients and were therefore accessible for statistical analysis. Table 5 gives a good overview about the data which were available in most patients and could therefore be examined. Nevertheless, it is important to emphasize that the results of the study must be viewed critically, since as already mentioned a lot of the data showed a considerable degree of variation, in particular the dosages of medication and the treatment periods.

In the following, patient data from our study are compared with data from larger epidemiological studies. In our cohort of 49 patients the male/female distribution was almost identical which has also been described in literature (Lavrentaki, Paluzzi, Wass, & Karavitaki, 2017). It is worth mentioning that in our cohort women received

the diagnosis of acromegaly on average a little later than men. This has also been reported in other epidemiological studies (Fernandez et al., 2010; Lavrentaki et al., 2017).

With regard to the development of pituitary insufficiency, it is worth mentioning that in this study the prevalence of hypopituitarism was slightly higher than described in common literature (Mestron et al., 2004). In a large-scale study investigating the prevalence and incidence of hypopituitarism in the general population, a lack of LH / FSH was most common and in most cases three or more hormones were deficient (Regal, Paramo, Sierra, & Garcia-Mayor, 2001). This is comparable with our patient group where the LH / FSH axis was also most frequently affected.

Transsphenoidal surgery and radiotherapy are the most common risk factors for developing hypopituitarism. In contrast, the pre- or postoperative treatment of the patients with somatostatin analogues, growth hormone receptor antagonists or dopamine agonists do usually cause no pituitary insufficiency (Melmed, 2006; Prabhakar & Shalet, 2006). However, this study did not show any correlation between patient characteristics like gender, age, disease duration, tumor size as well as therapy modality and the development of hypopituitarism. Nonetheless, it is quite possible that one of these factors could have led to pituitary insufficiency, but due to our small cohort statistically no significant correlation could be established.

It is known that resistance to therapy during treatment with SSA is a problem in some patients with acromegaly. For this reason, it was important to see how many patients would achieve biochemical control under SSA therapy. The percentage of patients in this study that achieved normalization of GH and IGF-1 levels was slightly below the remission rates reported in the literature (Melmed, 2006). Due to this relatively frequent resistance to somatostatin analogue therapy in some patients, we also wanted to know whether there are any other drugs that are already used in the treatment of acromegaly and its comorbidities and may have a positive influence on the effectiveness of SSA.

As mentioned, hormone replacement is used for the treatment of hypopituitarism in acromegalic patients with one or more disturbed hormone axes. In this context a disturbed hypothalamic-pituitary adrenal axis may be most critical as the affected acromegalic patients receive cortisone, which is believed to have an inhibitory effect on the somatostatin type 2 receptor (Petersenn, Rasch, Presch, Beil, & Schulte,

1999). For this reason, it was of particular interest to find out whether the treatment with hormone substitutes such as cortisone had an influence on the effectiveness of SSA therapy in our patient cohort. The statistical tests, that were carried out showed that treatment with corticosteroids or any other kind of hormone replacement had no effect on the responsiveness of SSA therapy in terms of IGF-1 values in patients with acromegaly. Thus, although this has to be confirmed in larger studies, there was no evidence for an effect of hormone replacement therapy on the efficacy of SSA treatment in patients with acromegaly.

In our study cohort, about a quarter of patients with acromegaly also suffer from diabetes mellitus type 2 and are usually treated with metformin. Statistical analysis of patient data showed that treatment with metformin lead to significant lower IGF-1 values in patients treated with SSA. It was also shown that patient's age also played a role in patients treated with regard to lower IGF-1 values. In statistical analysis patient's age showed a trend towards statistical significance, what is in line with a publication that assumes that IGF-1 values depend partly on age (Franco et al., 2014). However, other factors not considered in this study could have also led to a reduction in IGF-1 levels. Systemic diseases including a catabolic metabolism, liver or kidney failure and malnutrition can lower IGF-1 levels and lead to false-negative IGF-1 levels (Katznelson et al., 2011).

The observation that metformin may improve the inhibitory action of SSAs in the treatment of acromegalic patients is new, but is in line with studies in which it was shown that in addition to its antidiabetic action, metformin probably has also other potentially useful effects. It has been reported that metformin reduces the risk of developing cancer in patients with diabetes mellitus type 2 (Evans, Donnelly, Emslie-Smith, Alessi, & Morris, 2005; Hawley et al., 2003; Lizcano et al., 2004; Musi et al., 2002). This effect of metformin is linked with its direct effect on tumor cells and its ability to keep insulin levels low by inhibiting gluconeogenesis and promoting glucose uptake in muscles (Dowling, Niraula, Stambolic, & Goodwin, 2012). For instance, potential antitumor activity of metformin is mainly due to activation of AMP-activated protein kinase, which is a promising factor in therapy of tumor diseases as a sensor of energy levels of the cell (Ben Sahra, Le Marchand-Brustel, Tanti, & Bost, 2010; G. Zhou et al., 2001). Another potentially useful effect of metformin could be an improvement in atherosclerosis (Forouzandeh et al., 2014). However, presumed

positive effects on blood pressure have not yet been demonstrated (Snorgaard, Kober, & Carlsen, 1997).

The in vivo findings about the enhanced inhibitory action of SSAs in acromegalic patients treated with metformin prompted me to investigate in the second part of my thesis the action of metformin on GH secretion alone and in combination *in vitro*. To this end the lactorsomatotroph GH3 rat pituitary cell line, which is a classical model to study the regulation of GH synthesis and secretion, was used to investigate the effect of metformin on GH promoter activity and GH secretion. Subsequently, a small series of primary cell cultures obtained from human somatotroph tumor tissues was used to prove the GH inhibitory effect of metformine alone and in combination with octreotide in human somatotroph tumor cells.

Metformin suppressed GH promotor activity in GH3 cells, which means that it inhibits GH mRNA synthesis. Moreover, metformin dose-dependently suppressed GH secretion in GH3 cells and within 24 h the highest metformin dosage applied (1 µM) led to a nearly 50% reduction of GH secretion. As GH3 cells may contain considerable amounts of GH, the rapid inhibitory effect on GH release may not only be explained by its suppressive effect on GH mRNA synthesis but may indicate that metformin acts in parallel also on the GH secretory machinery. The underlying molecular and cellular mechanisms of metformin on GH synthesis and secretion have not further been studied as this would have been far beyond the scope of a medical thesis.

Metformin alone also dose-dependently suppressed the GH secretion in human somatotroph adenoma cell cultures. When applied together with octreotide, an additive inhibitory effect of the two compounds was observed which would support the in vivo findings in acromegalic patients in which a stronger GH suppression was achieved in the subgroup of diabetic patients that received both octreotide and metformin in comparison to non-diabetic acromegalic patients that were treated with octreotide alone.

When starting to write the present thesis, a paper was published by An and colleagues, in which the effect of metformin on GH production by GH3 cells and human somatotroph tumor cells has also been studied (An et al., 2017). Using five times higher dosages of metformin as applied in the present thesis, they also showed

that metformin inhibited GH secretion by somatotroph tumor cells, but did not study its effect on the GH promoter and in combination with octreotide (An et al., 2017). They focused their interest on the growth inhibitory role of metformin and could show that metformin inhibited the proliferation and stimulated the apoptosis in somatotroph tumor cells both in vitro in cell cultures but also in vivo in experimental GH3 tumors in nude mice suggesting that metformin has a more general anti-tumorigenic role in somatotroph tumors (An et al., 2017). Their findings were in part confirmed in a very recent study, in which it was demonstrated that the growth both of GH3 cells and GH1 cells, another somatotroph rat pituitary tumor cell line, could be inhibited by metformin (Faggi et al., 2018). The anti-tumorigenic action of metformin was also demonstrated very recently in another pituitary tumor cell type, in corticotroph AtT20 mouse pituitary tumor cells. In this cell type metformin inhibited proliferation, induced apoptosis and suppressed ACTH production (Jin et al., 2018). However, due to its known effects on metabolic mechanisms it is unclear whether metformin will be used as a drug for the treatment of pituitary adenomas or other types of tumors responding to metformin in the future. But it is also evident that patients with diabetes type 2 will not only benefit from the anti-diabetic action of metformin but in addition from its anti-tumorigenic effects.

With respect to the putative mechanisms of action of metformin on GH production, it is necessary to keep in mind how metformin operates within the cell and how these mechanisms interfere with the signaling pathways regulating GH synthesis and secretion. One of the biomolecular functions of metformin is inhibition of Complex I of the mitochondrial respiratory chain in liver cells (Wheaton et al., 2014). This results in reduction of ATP-synthesis and reduction of chemical energy within the cell which leads to activation of AMP-activated protein kinase A (Stephenne et al., 2011). AMPK is known as a sensor of energy balance within the cell and plays an important role in inhibition of gluconeogenesis (Hardie, 2011; Jeon, 2016). Suppression of gluconeogenesis through metformin results on the one hand by an inhibition of the adenylate cyclase by reduction of ATP and on the other hand by induction of Sirt1 after increased expression of the AMP-activated protein kinase (Caton et al., 2010; Viollet et al., 2012).

It has been shown that energy sensors within the cell could have an effect on endocrine and metabolic processes and the energy sensors AMPK and Sirt1 are of

particular interest here. In a current study, AMPK has been investigated as a potential target in the therapy of GH secreting pituitary tumors (Faggi et al., 2017). It has also been shown that AMPK influences the function in somatotrophic cells by inhibiting the growth of pituitary adenoma cells and their secretion of growth hormone (Tulipano et al., 2011). Another study showed that Sirt1 inhibits the GH/IGF-1 axis by inhibiting the GH secretion at pituitary level (Monteserin-Garcia et al., 2013).

To understand the context, it is important to illustrate the intracellular processes of GH synthesis and the transcription factor CREB. GHRH binds to somatotroph cells via the GHRH-receptor, a stimulatory G protein-coupled receptor that is predominantly expressed in somatotroph cells of the pituitary gland (Mayo et al., 2000). After binding to the G protein-coupled receptor, an increase in cAMP results and activation of the protein kinase A occurs. Possibly, PKA enters the nucleus and activates the transcription factor CREB which is responsible for GH transcription (Cohen, Hashimoto, Zanger, Wondisford, & Radovick, 1999). Subunits of PKA activate the co-activator of transcription, the CREB binding protein (CBP). Activated CBP is mobilized by the DNA binding proteins Pit1 and CREB to the GH-promoter (GH-luc) where expression of GH takes place (Monteserin-Garcia et al., 2013). In contrast, GH secretion was shown to decline by the activation of the regulatory enzyme Sirt1 and the subsequent inhibition of CREB and Pit1 (Monteserin-Garcia et al., 2013). It was demonstrated, that activation of Sirt1 led to an inhibition of GH-luc (GH promoter) and an inactivation of Sirt1 led to an increased activity of the promoter (Monteserin-Garcia et al., 2013).
In contrast to GHRH, that activates cAMP/PKA, somatostatin suppresses through 5 different somatostatin receptors (SSTR1 to SSTR5) the production of cAMP and down-regulates PKA expression and activity (Theodoropoulou & Stalla, 2013). Somatostatin binds to its receptors that are linked to inhibitory G-proteins. The latter suppress the adenyl cyclase activity which reduces intracellular cAMP levels and thus suppress the subsequent cAMP signaling cascade including CREB phosphorylation and activation. In this context octreotide is mainly acting through the SSTR2 and SSTR5 (Theodoropoulou & Stalla, 2013). Thus octreotide and metformin affect GH Production in somatotroph tumor cells through different pathways, octreotide through inhibiting the cAMP/PKA pathway and metformin through activating AMPK/Sirt1 signaling. The independent GH inhibitory effects of octreotide

and metformin may explain their additive GH-suppressive effect when applied together.

However, very recently an alternate GH-suppressive action of metformin has been suggested in which this compound is inhibiting GH production through suppressing STAT3 phosphorylation (An et al., 2017). Only a couple of years ago, it was shown that in somatotroph pituitary adenoma cells, STAT3 upregulation induces GH hypersecretion through mechanisms that are not yet completely understood. Consequently, STAT3 inhibitors were able to reduce GH secretion *in vitro* in human somatotroph adenoma cell cultures and in GH3 cells as well as *in vivo* in experimental GH3 tumors in nude mice (C. Zhou et al., 2015). When studying the mechanism of action of metformin in GH3 cells, An and colleagues could show, that the effects of metformin on proliferation/apoptosis and on GH secretion are mediated through two different mechanisms (An et al., 2017). Whereas the effects of metformin on GH3 cell proliferation and apoptosis involved AMPK activation, the suppressive action of metformin on GH secretion was mediated through down-regulation of STAT3 phosphorylation (An et al., 2017).

For this reason, it is actually not yet completely clear, whether the inhibitory effect of metformin on the secretion of GH and consequently IGF-1 is triggered by the activation of energy sensors such as AMPK and Sirt1 or whether the effect is mediated through STAT3 inhibition. Thus more work is needed to clarify the precise mechanism of action of metformin. However, as our clinical results suggest that the combined application of metformin and octreotid lowers IGF-1 levels significantly better than octreotide alone in acromegalic patients, future studies on the role of metformin on GH suppression are recommended to improve the treatment of patients with acromegaly.

5. Summary

Somatostatin analogues (SSA) constitute the mainstay of pituitary-targeted pharmacological treatment in acromegaly, but half of the patients are resistant to their anti-secretory and tumor shrinking effects. The successful management of acromegaly in addition to targeting biochemical control involves the treatment of the metabolic comorbidities and hypopituitarism that is managed with hormone replacement. The aim of this study was to analyze the impact of the concomitant antidiabetic treatment with metformin and hormone replacement on the response to SSA. Data were collected from 49 acromegalic patients: 45 had transsphenoidal surgery (35 as primary therapy), 36 SSA (octreotide or lanreotide; 11 as primary treatment). All acromegalic patients with diabetes mellitus (25%) received metformin. Hypopituitarism affected 18 patients. Binary logistic regression analysis (SPSS software) showed no correlation between SSA (p=0.71) and transsphenoidal surgery (p=0.541) on the incidence of pituitary insufficiency. Regression analysis showed no correlation between IGF-1 lowering response to SSA and substitution treatment with hydrocorticosterone, testosterone, or L-thyroxin (variance inflation factor <3). Linear regression analysis showed no correlation between age, gender, disease duration, other treatment modalities (hydrocorticosterone, testosterone, or L-thyroxin) and change of IGF-1 levels after SSA treatment. However, the same analysis showed correlation between metformin therapy and IGF-1 levels after SSA treatment (p=0,031; R-square change: 0,135; R-square: 0,321). In a series of in vitro experiments, metformin alone significantly suppressed GH secretion at the 1mM concentration (64±13) and similar observations were obtained on the rat GH promoter activity and GH secretion from rat immortalized GH-secreting GH3 cells. *In vitro* investigation on 7 human acromegalic tumours showed that metformin (500nM) enhances the GH-suppressive effect of octreotide (1nM) (63±13% versus 81±12). These preliminary observations indicate that hormone replacement does not affect SSA response, but metformin treatment improves SSA response in terms of IGF-1 reduction most likely through directly suppressing GH production in somatotroph tumor cells.

6. Zusammenfassung

Somatostatin-Analoga (SSA) sind bei weitem die wichtigsten Medikamente bei der pharmakologischen Behandlung der Akromegalie, jedoch sind die Hälfte der Patienten resistent gegen ihre antisekretorischen und tumorschrumpfenden Effekte. Die erfolgreiche Behandlung der Akromegalie beinhaltet neben der klinischen Kontrolle zusätzlich die Behandlung von Komorbiditäten, u.a. Diabetes Typ 2 und Hypophyseninsuffizienz, welche mit Metformin bzw. Hormonersatzmedikamenten therapiert werden. Ziel dieser Studie war es, die Auswirkungen der begleitenden Therapie mit dem Antidiabetikum Metformin und Hormonersatzmedikamenten in Hinblick auf die Wirkung von GH-inhibierenden SSA zu untersuchen. Es wurden von 49 Patienten mit Akromegalie Daten erhoben. 35 dieser Patienten erhielten eine transsphenoidale Operation als primäre Therapieform. 36 Patienten erhielten SSA in Form von Octreotide oder Lanreotide und 11 aus dieser Gruppe erhielten SSA als primäre Therapie. Alle akromegalen Patienten mit Diabetes mellitus erhielten Metformin (25%). Bei 18 Patienten wurde eine Hypophyseninsuffizienz festgestellt. Mittels SPSS Software wurde eine binäre logistische Regressionsanalyse durchgeführt, welche keine Korrelation zwischen der SSA-Therapie ($p=0.71$) und transphenoidaler Operation ($p=0.541$) im Hinblick auf die Inzidenz einer Hypophyseninsuffizienz offenbarte. Die Regressionsanalyse wies keinen Zusammenhang zwischen sinkenden IGF-1-Werten bei SSA-Therapie und der Behandlung mit Hormonersatzmedikamenten wie Hydrocortison, Testosteron oder L-Thyroxin bei Hypophyseninsuffizienz auf (Variance inflation factor < 3). Bei der linearen Regressionsanalyse konnte keine Korrelation zwischen Alter, Geschlecht, Erkrankungsdauer und anderen Therapieformen wie Hydrocortison, Testosteron oder L-Thyroxin im Hinblick auf sinkende IGF-1-Werte bei SSA Therapie festgestellt werden. Jedoch zeigte die gleiche Analyse eine Korrelation zwischen Metformin-Therapie und IGF-1-Werten nach SSA-Therapie ($p=0,031$; R-square change: 0,135; R-square: 0,321). *In vitro* Untersuchungen in somatotropen GH3 Tumorzellen zeigten, dass Metformin (1 mM) alleine die GH-Sekretion signifikant supprimierte (64±13%) und die Aktivität des GH-Promotors inhibierte. *In vitro* Untersuchungen an 7 akromegalen Tumoren zeigten, dass Metformin (500 nM) die GH-suppressive Wirkung von Octreotide (1nM) verstärkt (63±13% vs. 81±12%). Diese Beobachtungen zeigen, dass der Hormonersatz die SSA-Wirkung nicht beeinflusst, während Metformin die SSA-Wirkung im Hinblick auf IGF-1-Reduktion verbessert.

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8. Bibliography

Abdu, F., Hicks, G. A., Hennig, G., Allen, J. P., & Grundy, D. (2002). Somatostatin sst₂ receptors inhibit peristalsis in the rat and mouse jejunum. *American Journal of Physiology - Gastrointestinal and Liver Physiology, 282*(4), G624-G633. doi:10.1152/ajpgi.00354.2001

An, J., Pei, X., Zang, Z., Zhou, Z., Hu, J., Zheng, X., . . . Yang, H. (2017). Metformin inhibits proliferation and growth hormone secretion of GH3 pituitary adenoma cells. *Oncotarget, 8*(23), 37538-37549. doi:10.18632/oncotarget.16556

Ayuk, J., & Sheppard, M. C. (2006). Growth hormone and its disorders. *Postgrad Med J, 82*(963), 24-30. doi:10.1136/pgmj.2005.036087

Barkan, A. L., Beitins, I. Z., & Kelch, R. P. (1988). Plasma insulin-like growth factor-I/somatomedin-C in acromegaly: correlation with the degree of growth hormone hypersecretion. *J Clin Endocrinol Metab, 67*(1), 69-73. doi:10.1210/jcem-67-1-69

Bates, A. S., Van't Hoff, W., Jones, J. M., & Clayton, R. N. (1993). An audit of outcome of treatment in acromegaly. *Q J Med, 86*(5), 293-299.

Ben Sahra, I., Le Marchand-Brustel, Y., Tanti, J. F., & Bost, F. (2010). Metformin in cancer therapy: a new perspective for an old antidiabetic drug? *Mol Cancer Ther, 9*(5), 1092-1099. doi:10.1158/1535-7163.mct-09-1186

Ben-Shlomo, A., & Melmed, S. (2008). Acromegaly. *Endocrinol Metab Clin North Am, 37*(1), 101-122, viii. doi:10.1016/j.ecl.2007.10.002

Berelowitz, M., Szabo, M., Frohman, L. A., Firestone, S., Chu, L., & Hintz, R. L. (1981). Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. *Science, 212*(4500), 1279-1281.

Bevan, J. S. (2005). Clinical review: The antitumoral effects of somatostatin analog therapy in acromegaly. *J Clin Endocrinol Metab, 90*(3), 1856-1863. doi:10.1210/jc.2004-1093

Bevan, J. S., Atkin, S. L., Atkinson, A. B., Bouloux, P. M., Hanna, F., Harris, P. E., . . . Wardlaw, J. M. (2002). Primary medical therapy for acromegaly: an open, prospective, multicenter study of the effects of subcutaneous and intramuscular slow-release octreotide on growth hormone, insulin-like growth factor-I, and tumor size. *J Clin Endocrinol Metab, 87*(10), 4554-4563. doi:10.1210/jc.2001-012012

Biermasz, N. R. (2017). New medical therapies on the horizon: oral octreotide. *Pituitary, 20*(1), 149-153. doi:10.1007/s11102-016-0785-3

Bloch, B., Brazeau, P., Ling, N., Bohlen, P., Esch, F., Wehrenberg, W. B., . . . Guillemin, R. (1983). Immunohistochemical detection of growth hormone-releasing factor in brain. *Nature, 301*(5901), 607-608.

Brabant, G. (2003). Insulin-like growth factor-I: marker for diagnosis of acromegaly and monitoring the efficacy of treatment. *Eur J Endocrinol, 148 Suppl 2*, S15-20.

Brazeau, P., Rivier, J., Vale, W., & Guillemin, R. (1974). Inhibition of growth hormone secretion in the rat by synthetic somatostatin. *Endocrinology, 94*(1), 184-187. doi:10.1210/endo-94-1-184

Cataldi, M., Magnan, E., Guillaume, V., Dutour, A., Conte-Devolx, B., Lombardi, G., & Oliver, C. (1994). Relationship between hypophyseal portal GHRH and somatostatin and peripheral GH levels in the conscious sheep. *J Endocrinol Invest, 17*(9), 717-722. doi:10.1007/bf03347767

Caton, P. W., Nayuni, N. K., Kieswich, J., Khan, N. Q., Yaqoob, M. M., & Corder, R. (2010). Metformin suppresses hepatic gluconeogenesis through induction of SIRT1 and GCN5. *J Endocrinol, 205*(1), 97-106. doi:10.1677/joe-09-0345

Chakravarty, A. A., Ajmani, A., Manchanda, S., Kulshreshtha, B., & Chopra, S. (2012). Incidence of gall stone formation in acromegalic patients on octreotide therapy. *Indian J Endocrinol Metab, 16*(3), 406-408. doi:10.4103/2230-8210.95683

Claessen, K., Canete, A. N., de Bruin, P. W., Pereira, A. M., Kloppenburg, M., Kroon, H. M., & Biermasz, N. R. (2017). Acromegalic arthropathy in various stages of the disease: an MRI study. *Eur J Endocrinol, 176*(6), 779-790. doi:10.1530/eje-16-1073

Clemmons, D. R. (2002). Roles of insulin-like growth factor-I and growth hormone in mediating insulin resistance in acromegaly. *Pituitary, 5*(3), 181-183.

Cohen, L. E., Hashimoto, Y., Zanger, K., Wondisford, F., & Radovick, S. (1999). CREB-independent regulation by CBP is a novel mechanism of human growth hormone gene expression. *J Clin Invest, 104*(8), 1123-1130. doi:10.1172/jci7308

Colao, A. (2008). The GH-IGF-I axis and the cardiovascular system: clinical implications. *Clin Endocrinol (Oxf), 69*(3), 347-358. doi:10.1111/j.1365-2265.2008.03292.x

Colao, A., Auriemma, R. S., Lombardi, G., & Pivonello, R. (2011). Resistance to somatostatin analogs in acromegaly. *Endocr Rev, 32*(2), 247-271. doi:10.1210/er.2010-0002

Colao, A., Ferone, D., Marzullo, P., Di Sarno, A., Cerbone, G., Sarnacchiaro, F., . . . Lombardi, G. (1997). Effect of different dopaminergic agents in the treatment of acromegaly. *J Clin Endocrinol Metab, 82*(2), 518-523. doi:10.1210/jcem.82.2.3648

Colao, A., Ferone, D., Marzullo, P., & Lombardi, G. (2004). Systemic complications of acromegaly: epidemiology, pathogenesis, and management. *Endocr Rev, 25*(1), 102-152. doi:10.1210/er.2002-0022

Colao, A., Pivonello, R., Auriemma, R. S., Briganti, F., Galdiero, M., Tortora, F., . . . Lombardi, G. (2006). Predictors of tumor shrinkage after primary therapy with somatostatin analogs in acromegaly: a prospective study in 99 patients. *J Clin Endocrinol Metab, 91*(6), 2112-2118. doi:10.1210/jc.2005-2110

Creo, A. L., & Lteif, A. N. (2016). Pituitary gigantism: a retrospective case series. *J Pediatr Endocrinol Metab, 29*(5), 597-602. doi:10.1515/jpem-2015-0269

Daughaday, W. H., & Rotwein, P. (1989). Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr Rev, 10*(1), 68-91. doi:10.1210/edrv-10-1-68

de Herder, W. W. (2009). Acromegaly and gigantism in the medical literature. Case descriptions in the era before and the early years after the initial publication of Pierre Marie (1886). *Pituitary, 12*(3), 236-244. doi:10.1007/s11102-008-0138-y

DeFronzo, R. A., & Goodman, A. M. (1995). Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. *N Engl J Med, 333*(9), 541-549. doi:10.1056/nejm199508313330902

Dowling, R. J., Niraula, S., Stambolic, V., & Goodwin, P. J. (2012). Metformin in cancer: translational challenges. *J Mol Endocrinol, 48*(3), R31-43. doi:10.1530/jme-12-0007

Evans, J. M., Donnelly, L. A., Emslie-Smith, A. M., Alessi, D. R., & Morris, A. D. (2005). Metformin and reduced risk of cancer in diabetic patients. *Bmj, 330*(7503), 1304-1305. doi:10.1136/bmj.38415.708634.F7

Faggi, L., Giustina, A., & Tulipano, G. (2017). Effects of metformin on cell growth and AMPK activity in pituitary adenoma cell cultures, focusing on the interaction with adenylyl cyclase activating signals. *Mol Cell Endocrinol*. doi:10.1016/j.mce.2017.09.030

Fatti, L. M., Scacchi, M., Pincelli, A. I., Lavezzi, E., & Cavagnini, F. (2001). Prevalence and pathogenesis of sleep apnea and lung disease in acromegaly. *Pituitary, 4*(4), 259-262.

Fernandez, A., Karavitaki, N., & Wass, J. A. (2010). Prevalence of pituitary adenomas: a community-based, cross-sectional study in Banbury (Oxfordshire, UK). *Clin Endocrinol (Oxf), 72*(3), 377-382. doi:10.1111/j.1365-2265.2009.03667.x

Fieffe, S., Morange, I., Petrossians, P., Chanson, P., Rohmer, V., Cortet, C., . . . Delemer, B. (2011). Diabetes in acromegaly, prevalence, risk factors, and evolution: data from the French Acromegaly Registry. *Eur J Endocrinol, 164*(6), 877-884. doi:10.1530/eje-10-1050

Forouzandeh, F., Salazar, G., Patrushev, N., Xiong, S., Hilenski, L., Fei, B., & Alexander, R. W. (2014). Metformin beyond diabetes: pleiotropic benefits of metformin in attenuation of atherosclerosis. *J Am Heart Assoc, 3*(6), e001202. doi:10.1161/jaha.114.001202

Franco, L., Williams, F. M., Trofimov, S., Malkin, I., Surdulescu, G., Spector, T., & Livshits, G. (2014). Assessment of age-related changes in heritability and IGF-1 gene effect on circulating IGF-1 levels. *Age (Dordr), 36*(3), 9622. doi:10.1007/s11357-014-9622-7

Franks, S., Jacobs, H. S., Martin, N., & Nabarro, J. D. (1978). Hyperprolactinaemia and impotence. *Clin Endocrinol (Oxf), 8*(4), 277-287.

Freda, P. U. (2003). How effective are current therapies for acromegaly? *Growth Horm IGF Res, 13 Suppl A*, S144-151.

Freda, P. U., Post, K. D., Powell, J. S., & Wardlaw, S. L. (1998). Evaluation of disease status with sensitive measures of growth hormone secretion in 60 postoperative patients with acromegaly. *J Clin Endocrinol Metab, 83*(11), 3808-3816. doi:10.1210/jcem.83.11.5266

Gadelha, M. R., Bronstein, M. D., Brue, T., Coculescu, M., Fleseriu, M., Guitelman, M., . . . Colao, A. (2014). Pasireotide versus continued treatment with octreotide or lanreotide in patients with inadequately controlled acromegaly (PAOLA): a randomised, phase 3 trial. *Lancet Diabetes Endocrinol, 2*(11), 875-884. doi:10.1016/s2213-8587(14)70169-x

Ge, F., Tsagarakis, S., Rees, L. H., Besser, G. M., & Grossman, A. (1989). Relationship between growth hormone-releasing hormone and somatostatin in the rat: effects of age and sex on content and in-vitro release from hypothalamic explants. *J Endocrinol, 123*(1), 53-58.

Geraedts, V. J., Dimopoulou, C., Auer, M., Schopohl, J., Stalla, G. K., & Sievers, C. (2014). Health Outcomes in Acromegaly: Depression and Anxiety are Promising Targets for Improving Reduced Quality of Life. *Front Endocrinol (Lausanne), 5*. doi:10.3389/fendo.2014.00229

Ghanaat, F., & Tayek, J. A. (2005). Growth hormone administration increases glucose production by preventing the expected decrease in glycogenolysis seen with fasting in healthy volunteers. *Metabolism, 54*(5), 604-609. doi:10.1016/j.metabol.2004.12.003

Gibson, C. D., Karmally, W., McMahon, D. J., Wardlaw, S. L., & Korner, J. (2012). Randomized pilot study of cabergoline, a dopamine receptor agonist: effects on body weight and glucose tolerance in obese adults. *Diabetes Obes Metab, 14*(4), 335-340. doi:10.1111/j.1463-1326.2011.01534.x

Giustina, A., Barkan, A., Casanueva, F. F., Cavagnini, F., Frohman, L., Ho, K., . . . Melmed, S. (2000). Criteria for cure of acromegaly: a consensus statement. *J Clin Endocrinol Metab, 85*(2), 526-529. doi:10.1210/jcem.85.2.6363

Giustina, A., Bronstein, M. D., Casanueva, F. F., Chanson, P., Ghigo, E., Ho, K. K., . . . Melmed, S. (2011). Current management practices for acromegaly: an international survey. *Pituitary, 14*(2), 125-133. doi:10.1007/s11102-010-0269-9

Giustina, A., Casanueva, F. F., Cavagnini, F., Chanson, P., Clemmons, D., Frohman, L. A., . . . Melmed, S. (2003). Diagnosis and treatment of acromegaly complications. *J Endocrinol Invest, 26*(12), 1242-1247.

Goldman, M. H., & Klinges, K. (1989). Acromegaly as the amenorrhea-galactorrhea syndrome. *N J Med, 86*(11), 887-888.

Greenwood, F. C., & Landon, J. (1966). Growth hormone secretion in response to stress in man. *Nature, 210*(5035), 540-541.

Hardie, D. G. (2011). AMP-activated protein kinase—an energy sensor that regulates all aspects of cell function. *Genes Dev, 25*(18), 1895-1908. doi:10.1101/gad.17420111

Hartman, M. L., Veldhuis, J. D., Vance, M. L., Faria, A. C., Furlanetto, R. W., & Thorner, M. O. (1990). Somatotropin pulse frequency and basal concentrations are increased in acromegaly and are reduced by successful therapy. *J Clin Endocrinol Metab, 70*(5), 1375-1384. doi:10.1210/jcem-70-5-1375

Hawley, S. A., Boudeau, J., Reid, J. L., Mustard, K. J., Udd, L., Makela, T. P., . . . Hardie, D. G. (2003). Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade. *J Biol, 2*(4), 28. doi:10.1186/1475-4924-2-28

Helseth, R., Carlsen, S. M., Bollerslev, J., Svartberg, J., Oksnes, M., Skeie, S., & Fougner, S. L. (2016). Preoperative octreotide therapy and surgery in acromegaly: associations between glucose homeostasis and treatment response. *Endocrine, 51*(2), 298-307. doi:10.1007/s12020-015-0679-6

Hodish, I., & Barkan, A. (2008). Long-term effects of pegvisomant in patients with acromegaly. *Nat Clin Pract Endocrinol Metab, 4*(6), 324-332. doi:10.1038/ncpendmet0831

Holdaway, I. M., & Rajasoorya, C. (1999). Epidemiology of acromegaly. *Pituitary, 2*(1), 29-41.

Holly, J. M., Amiel, S. A., Sandhu, R. R., Rees, L. H., & Wass, J. A. (1988). The role of growth hormone in diabetes mellitus. *J Endocrinol, 118*(3), 353-364.

Izumi, Y., Honda, M., Tsuchiya, M., Ueda, Y., & Hatano, M. (1980). Effect of somatostatin on plasma renin activity and blood pressure in patients with essential hypertension. *Endocrinol Jpn, 27*(4), 505-511.

Jaffe, C. A., & Barkan, A. L. (1992). Treatment of acromegaly with dopamine agonists. *Endocrinol Metab Clin North Am, 21*(3), 713-735.

Jaffe, C. A., Huffman, B. W., & Demott-Friberg, R. (1999). Insulin hypoglycemia and growth hormone secretion in sheep: a paradox revisited. *Am J Physiol, 277*(2 Pt 1), E253-258.

Jeon, S. M. (2016). Regulation and function of AMPK in physiology and diseases. *Exp Mol Med, 48*(7), e245-. doi:10.1038/emm.2016.81

Jiang, H., Deng, X. F., Duan, C. M., Chen, C., Xiang, J. L., Lu, Y. L., & Ma, Q. F. (2011). Somatostatin receptors SSTR2 and SSTR5 are expressed in the human thoracic duct. *Lymphology, 44*(1), 21-28.

Jin, K., Ruan, L., Pu, J., Zhong, A., Wang, F., Tan, S., . . . Yang, G. (2018). Metformin suppresses growth and adrenocorticotrophic hormone secretion in mouse pituitary corticotroph tumor AtT20cells. *Mol Cell Endocrinol*. doi:10.1016/j.mce.2018.07.007

Kan, E., Kan, E. K., Atmaca, A., Atmaca, H., & Colak, R. (2013). Visual field defects in 23 acromegalic patients. *Int Ophthalmol, 33*(5), 521-525. doi:10.1007/s10792-013-9733-7

Katznelson, L., Atkinson, J. L., Cook, D. M., Ezzat, S. Z., Hamrahian, A. H., & Miller, K. K. (2011). American Association of Clinical Endocrinologists medical guidelines for clinical practice for the diagnosis and treatment of acromegaly--2011 update. *Endocr Pract, 17 Suppl 4*, 1-44.

Kuhn, E., & Chanson, P. (2017). Cabergoline in acromegaly. *Pituitary, 20*(1), 121-128. doi:10.1007/s11102-016-0782-6

Landis, C. A., Masters, S. B., Spada, A., Pace, A. M., Bourne, H. R., & Vallar, L. (1989). GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. *Nature, 340*(6236), 692-696. doi:10.1038/340692a0

Laron, Z. (1999). Somatomedin-1 (recombinant insulin-like growth factor-1): clinical pharmacology and potential treatment of endocrine and metabolic disorders. *BioDrugs, 11*(1), 55-70.

Laron, Z. (2001). Insulin-like growth factor 1 (IGF-1): a growth hormone. *Mol Pathol, 54*(5), 311-316.

Lavrentaki, A., Paluzzi, A., Wass, J. A., & Karavitaki, N. (2017). Epidemiology of acromegaly: review of population studies. *Pituitary, 20*(1), 4-9. doi:10.1007/s11102-016-0754-x

Leistner, S. M., Klotsche, J., Dimopoulou, C., Athanasoulia, A. P., Roemmler-Zehrer, J., Pieper, L., . . . Sievers, C. (2015). Reduced sleep quality and depression associate with decreased quality of life in patients with pituitary adenomas. *Eur J Endocrinol, 172*(6), 733-743. doi:10.1530/eje-14-0941

Levy, M. J., Bejon, P., Barakat, M., Goadsby, P. J., & Meeran, K. (2003). Acromegaly: a unique human headache model. *Headache, 43*(7), 794-797.

Li, C. H., & Dixon, J. S. (1971). Human pituitary growth hormone. 32. The primary structure of the hormone: revision. *Arch Biochem Biophys, 146*(1), 233-236.

Lim, E. M., & Pullan, P. (2005). Biochemical Assessment and Long-Term Monitoring in Patients with Acromegaly: Statement from a Joint Consensus Conference of The Growth Hormone Research Society and The Pituitary Society.1. *Clinical Biochemist Reviews, 26*(2), 41-43.

Lizcano, J. M., Goransson, O., Toth, R., Deak, M., Morrice, N. A., Boudeau, J., . . . Alessi, D. R. (2004). LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *Embo j, 23*(4), 833-843. doi:10.1038/sj.emboj.7600110

Losa, M., Mortini, P., Urbaz, L., Ribotto, P., Castrignano, T., & Giovanelli, M. (2006). Presurgical treatment with somatostatin analogs in patients with acromegaly: effects on the remission and complication rates. *J Neurosurg, 104*(6), 899-906. doi:10.3171/jns.2006.104.6.899

Mammis, A., Eloy, J. A., & Liu, J. K. (2010). Early descriptions of acromegaly and gigantism and their historical evolution as clinical entities. *Neurosurg Focus, 29*(4), E1. doi:10.3171/2010.7.focus10160

Mayo, K. E., Miller, T., DeAlmeida, V., Godfrey, P., Zheng, J., & Cunha, S. R. (2000). Regulation of the pituitary somatotroph cell by GHRH and its receptor. *Recent Prog Horm Res, 55*, 237-266; discussion 266-237.

Melmed, S. (2006). Medical progress: Acromegaly. *N Engl J Med, 355*(24), 2558-2573. doi:10.1056/NEJMra062453

Melmed, S., Popovic, V., Bidlingmaier, M., Mercado, M., van der Lely, A. J., Biermasz, N., . . . Strasburger, C. (2015). Safety and efficacy of oral octreotide in acromegaly: results of a multicenter phase III trial. *J Clin Endocrinol Metab, 100*(4), 1699-1708. doi:10.1210/jc.2014-4113

Melmed, S., Vance, M. L., Barkan, A. L., Bengtsson, B. A., Kleinberg, D., Klibanski, A., & Trainer, P. J. (2002). Current status and future opportunities for controlling acromegaly. *Pituitary, 5*(3), 185-196.

Mestron, A., Webb, S. M., Astorga, R., Benito, P., Catala, M., Gaztambide, S., . . . Gilabert, M. (2004). Epidemiology, clinical characteristics, outcome, morbidity and mortality in acromegaly based on the Spanish Acromegaly Registry (Registro Espanol de Acromegalia, REA). *Eur J Endocrinol, 151*(4), 439-446.

Minuto, F., Barreca, A., Ferrini, S., Mazzocchi, G., Del Monte, P., & Giordano, G. (1982). Growth hormone secretion in pubertal and adult subjects. *Acta Endocrinol (Copenh), 99*(2), 161-165.

Miyachi, Y., Yakushiji, F., & Terazono, T. (1993). [Synthesis and regulation of growth hormone secretion]. *Nihon Rinsho, 51*(10), 2585-2591.

Monteserin-Garcia, J., Al-Massadi, O., Seoane, L. M., Alvarez, C. V., Shan, B., Stalla, J., . . . Theodoropoulou, M. (2013). Sirt1 inhibits the transcription factor CREB to regulate pituitary growth hormone synthesis. *Faseb j, 27*(4), 1561-1571. doi:10.1096/fj.12-220129

Montminy, M. R., & Bilezikjian, L. M. (1987). Binding of a nuclear protein to the cyclic-AMP response element of the somatostatin gene. *Nature, 328*(6126), 175-178. doi:10.1038/328175a0

Muller, A. F., & Van Der Lely, A. J. (2004). Pharmacological therapy for acromegaly: a critical review. *Drugs, 64*(16), 1817-1838.

Musi, N., Hirshman, M. F., Nygren, J., Svanfeldt, M., Bavenholm, P., Rooyackers, O., . . . Goodyear, L. J. (2002). Metformin increases AMP-activated protein kinase activity in skeletal muscle of subjects with type 2 diabetes. *Diabetes, 51*(7), 2074-2081.

Nabarro, J. D. (1987). Acromegaly. *Clin Endocrinol (Oxf), 26*(4), 481-512.

Natali, A., & Ferrannini, E. (2006). Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: a systematic review. *Diabetologia, 49*(3), 434-441. doi:10.1007/s00125-006-0141-7

Niculescu, D., Purice, M., & Coculescu, M. (2013). Insulin-like growth factor-I correlates more closely than growth hormone with insulin resistance and glucose intolerance in patients with acromegaly. *Pituitary, 16*(2), 168-174. doi:10.1007/s11102-012-0396-6

Oliva, P. B. (1970). Lactic acidosis. *Am J Med, 48*(2), 209-225.

Petersenn, S., Rasch, A. C., Presch, S., Beil, F. U., & Schulte, H. M. (1999). Genomic structure and transcriptional regulation of the human somatostatin receptor type 2. *Mol Cell Endocrinol, 157*(1-2), 75-85.

Postel-Vinay, M. C., & Finidori, J. (1995). Growth hormone receptor: structure and signal transduction. *Eur J Endocrinol, 133*(6), 654-659.

Prabhakar, V. K. B., & Shalet, S. M. (2006). Aetiology, diagnosis, and management of hypopituitarism in adult life. *Postgrad Med J, 82*(966), 259-266. doi:10.1136/pgmj.2005.039768

Rajasoorya, C., Holdaway, I. M., Wrightson, P., Scott, D. J., & Ibbertson, H. K. (1994). Determinants of clinical outcome and survival in acromegaly. *Clin Endocrinol (Oxf), 41*(1), 95-102.

Regal, M., Paramo, C., Sierra, S. M., & Garcia-Mayor, R. V. (2001). Prevalence and incidence of hypopituitarism in an adult Caucasian population in northwestern Spain. *Clin Endocrinol (Oxf), 55*(6), 735-740.

Roelfsema, F., & Frolich, M. (1991). Pulsatile thyrotropin release and thyroid function in acromegalics before and during subcutaneous octreotide infusion. *J Clin Endocrinol Metab, 72*(1), 77-82. doi:10.1210/jcem-72-1-77

Roginsky, M. S., Shaver, J. C., & Christy, N. P. (1966). A study of adrenal cortical function in acromegaly. *J Clin Endocrinol Metab, 26*(10), 1101-1108. doi:10.1210/jcem-26-10-1101

Root, A. W., & Diamond, F. B., Jr. (2000). Regulation and clinical assessment of growth hormone secretion. *Endocrine, 12*(2), 137-145. doi:10.1385/endo:12:2:137

Rumboldt, Z. (2005). Pituitary adenomas. *Top Magn Reson Imaging, 16*(4), 277-288. doi:10.1097/01.rmr.0000224684.76006.cf

Sakharova, A. A., Horowitz, J. F., Surya, S., Goldenberg, N., Harber, M. P., Symons, K., & Barkan, A. (2008). Role of Growth Hormone in Regulating Lipolysis, Proteolysis, and Hepatic Glucose Production during Fasting. *J Clin Endocrinol Metab, 93*(7), 2755-2759. doi:10.1210/jc.2008-0079

Salpeter, S. R., Greyber, E., Pasternak, G. A., & Salpeter, E. E. (2010). Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. *Cochrane Database Syst Rev*(4), Cd002967. doi:10.1002/14651858.CD002967.pub4

Schneider, H. J., Aimaretti, G., Kreitschmann-Andermahr, I., Stalla, G. K., & Ghigo, E. (2007). Hypopituitarism. *Lancet, 369*(9571), 1461-1470. doi:10.1016/s0140-6736(07)60673-4

Snorgaard, O., Kober, L., & Carlsen, J. (1997). The effect of metformin on blood pressure and metabolism in nondiabetic hypertensive patients. *J Intern Med, 242*(5), 407-412.

Stang, M., Wysowski, D. K., & Butler-Jones, D. (1999). Incidence of lactic acidosis in metformin users. *Diabetes Care, 22*(6), 925-927.

Stephenne, X., Foretz, M., Taleux, N., van der Zon, G. C., Sokal, E., Hue, L., . . . Guigas, B. (2011). Metformin activates AMP-activated protein kinase in primary human hepatocytes by decreasing cellular energy status. *Diabetologia, 54*(12), 3101-3110. doi:10.1007/s00125-011-2311-5

Strowski, M. Z., Parmar, R. M., Blake, A. D., & Schaeffer, J. M. (2000). Somatostatin inhibits insulin and glucagon secretion via two receptors subtypes: an in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice. *Endocrinology, 141*(1), 111-117. doi:10.1210/endo.141.1.7263

Tannenbaum, G. S., Epelbaum, J., & Bowers, C. Y. (2003). Interrelationship between the novel peptide ghrelin and somatostatin/growth hormone-releasing hormone in regulation of pulsatile growth hormone secretion. *Endocrinology, 144*(3), 967-974. doi:10.1210/en.2002-220852

Theodoropoulou, M., & Stalla, G. K. (2013). Somatostatin receptors: from signaling to clinical practice. *Front Neuroendocrinol, 34*(3), 228-252. doi:10.1016/j.yfrne.2013.07.005

Theodoropoulou, M., Tichomirowa, M. A., Sievers, C., Yassouridis, A., Arzberger, T., Hougrand, O., . . . Stalla, G. K. (2009). Tumor ZAC1 expression is associated with the response to somatostatin analog therapy in patients with acromegaly. *Int J Cancer, 125*(9), 2122-2126. doi:10.1002/ijc.24602

Theodoropoulou, M., Zhang, J., Laupheimer, S., Paez-Pereda, M., Erneux, C., Florio, T., . . . Stalla, G. K. (2006). Octreotide, a somatostatin analogue, mediates its antiproliferative action in pituitary tumor cells by altering phosphatidylinositol 3-kinase signaling and inducing Zac1 expression. *Cancer Res, 66*(3), 1576-1582. doi:10.1158/0008-5472.can-05-1189

Tjornstrand, A., Gunnarsson, K., Evert, M., Holmberg, E., Ragnarsson, O., Rosen, T., & Filipsson Nystrom, H. (2014). The incidence rate of pituitary adenomas in western

Sweden for the period 2001-2011. *Eur J Endocrinol, 171*(4), 519-526. doi:10.1530/eje-14-0144

Tritos, N. A., & Klibanski, A. (2016). Effects of Growth Hormone on Bone. *Prog Mol Biol Transl Sci, 138*, 193-211. doi:10.1016/bs.pmbts.2015.10.008

Tulipano, G., Giovannini, M., Spinello, M., Sibilia, V., Giustina, A., & Cocchi, D. (2011). AMP-activated protein kinase regulates normal rat somatotroph cell function and growth of rat pituitary adenomatous cells. *Pituitary, 14*(3), 242-252. doi:10.1007/s11102-010-0288-6

van Aken, M. O., & Lamberts, S. W. (2005). Diagnosis and treatment of hypopituitarism: an update. *Pituitary, 8*(3-4), 183-191. doi:10.1007/s11102-006-6039-z

van der Hoek, J., de Herder, W. W., Feelders, R. A., van der Lely, A. J., Uitterlinden, P., Boerlin, V., . . . Lamberts, S. W. (2004). A single-dose comparison of the acute effects between the new somatostatin analog SOM230 and octreotide in acromegalic patients. *J Clin Endocrinol Metab, 89*(2), 638-645. doi:10.1210/jc.2003-031052

Vance, M. L., & Harris, A. G. (1991). Long-term treatment of 189 acromegalic patients with the somatostatin analog octreotide. Results of the International Multicenter Acromegaly Study Group. *Arch Intern Med, 151*(8), 1573-1578.

Viollet, B., Guigas, B., Sanz Garcia, N., Leclerc, J., Foretz, M., & Andreelli, F. (2012). Cellular and molecular mechanisms of metformin: an overview. *Clin Sci (Lond), 122*(6), 253-270. doi:10.1042/cs20110386

Wheaton, W. W., Weinberg, S. E., Hamanaka, R. B., Soberanes, S., Sullivan, L. B., Anso, E., . . . Chandel, N. S. (2014). Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. *Elife, 3*, e02242. doi:10.7554/eLife.02242

Winer, L. M., Shaw, M. A., & Baumann, G. (1990). Basal plasma growth hormone levels in man: new evidence for rhythmicity of growth hormone secretion. *J Clin Endocrinol Metab, 70*(6), 1678-1686. doi:10.1210/jcem-70-6-1678

Wright, A. D., Hill, D. M., Lowy, C., & Fraser, T. R. (1970). Mortality in acromegaly. *Q J Med, 39*(153), 1-16.

Zafar, M., Ezzat, S., Ramyar, L., Pan, N., Smyth, H. S., & Asa, S. L. (1995). Cell-specific expression of estrogen receptor in the human pituitary and its adenomas. *J Clin Endocrinol Metab, 80*(12), 3621-3627. doi:10.1210/jcem.80.12.8530610

Zhou, C., Jiao, Y., Wang, R., Ren, S. G., Wawrowsky, K., & Melmed, S. (2015). STAT3 upregulation in pituitary somatotroph adenomas induces growth hormone hypersecretion. *J Clin Invest, 125*(4), 1692-1702. doi:10.1172/jci78173

Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., . . . Moller, D. E. (2001). Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest, 108*(8), 1167-1174. doi:10.1172/jci13505

Eidesstattliche Versicherung

Ich, Moritz Thorben Ole Winkelmann, erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Thema:

Evidence for better response to somatostatin analogue treatment in acromegalic patients treated with metformin

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

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Tübingen, 05.06.2019	Moritz Winkelmann
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