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Novel approaches in the management of comorbidities and biochemical control in acromegaly

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Introduction

Acromegaly: definition, epidemiology and clinical presentation

Acromegaly is a rare and severe systemic endocrine disease due to the prolonged exposure to high levels of growth hormone (GH) and its peripheral mediator, insulin-like growth factor-1 (IGF-1). It is caused in more than 95% of cases by the presence of a GH-secreting pituitary adenoma, which generally develops as a result of monoclonal expansion of a mutated somatotropic cell. In a very small percentage of cases acromegaly may be caused by neuroendocrine tumors secreting ectopic GH-RH (growth hormone releasing hormone) or, even more rarely, GH. Moreover, it may be one of the manifestations of certain genetically determined syndromes, including multiple endocrine neoplasia syndrome (MEN 1), McCune-Albright syndrome, and the Carney-Complex.

According to the latest epidemiological data, acromegaly has an estimated prevalence of 2.8-13.7 cases/100000 people and an annual incidence of 0.2-1.1 cases/100000 people [1, 2].

The clinical presentation of the disease is linked both to the signs and symptoms related to the presence of the pituitary adenoma and its mass effect, and to those related to the systemic effects of chronic hypersecretion of GH and thus IGF-1. Symptoms caused by the compressive effect of the adenoma on the surrounding structures are mainly headache, visual symptoms (field deficits with varying degrees of severity, diplopia, reduced visual acuity) and hypopituitarism due to the effect of compression on the remaining healthy glandular parenchyma. In the latter case, the symptomatology will vary depending on the deficit in production and secretion of the specific hormones concerned. The deficit most frequently observed is central hypogonadism, which occurs in up to 70% of cases in women and up to 50% in men, and can contribute to worsening body composition, bone health and the patient's state of well-being.

The typical somatic and metabolic signs and symptoms referable to hormonal hypersecretion (in particular IGF-1 increase) include acral growth, physiognomic changes (prognathism, dental diastasis, nasal pyramid enlargement and accentuation of the frontal bosses), soft tissue hypertrophy and hyperplasia, asthenia, hyperhidrosis, arthralgias, decreased libido and/or sexual potency, mood swings, etc [3].

Table 1. Clinical Features of Acromegaly.

Local tumor effects	**Visceromegaly**
Pituitary enlargement	Tongue
Visual-field defects	Thyroid gland
Cranial-nerve palsy	Salivary glands
Headache	Liver
Somatic systems	Spleen
Acral enlargement, including thickness of soft tissue of hands and feet	Kidney
	Prostate
Musculoskeletal system	**Endocrine and metabolic systems**
Gigantism	Reproduction
Prognathism	Menstrual abnormalities
Jaw malocclusion	Galactorrhea
Arthralgias and arthritis	Decreased libido, impotence, low levels of sex hormone–binding globulin
Carpal tunnel syndrome	
Acroparesthesia	Multiple endocrine neoplasia type 1
Proximal myopathy	Hyperparathyroidism
Hypertrophy of frontal bones	Pancreatic islet-cell tumors
Skin and gastrointestinal system	Carbohydrate
Hyperhidrosis	Impaired glucose tolerance
Oily texture	Insulin resistance and hyperinsulinemia
Skin tags	Diabetes mellitus
Colon polyps	Lipid
Cardiovascular system	Hypertriglyceridemia
Left ventricular hypertrophy	Mineral
Asymmetric septal hypertrophy	Hypercalciuria, increased levels of 25-hydroxyvitamin D_3
Cardiomyopathy	Urinary hydroxyproline
Hypertension	Electrolyte
Congestive heart failure	Low renin levels
Pulmonary system	Increased aldosterone levels
Sleep disturbances	Thyroid
Sleep apnea (central and obstructive)	Low thyroxine-binding–globulin levels
Narcolepsy	Goiter

Figure 1: Clinical features af acromegaly (Melmed S., *Acromegaly*, 2006)

Diagnosis

Diagnosis of acromegaly thus arises from clinical suspicion. The presence of typical phenotypic manifestations, or the presence of concomitantly associated clinical conditions such as hypertension, diabetes mellitus type 2, sleep apnea syndrome, debilitating arthritis, carpal tunnel syndrome and hyperhidrosis, even in the absence of clear clinical manifestations, should suggest a requirement for an IGF-1 assay. IGF-1 levels vary physiologically over the years, so it is important to use age-adjusted normal ranges. Conditions that cause a falsely increased or reduced value should also be excluded. In particular, advanced liver disease, nutritional deficiencies or prolonged fasting, renal failure, alcohol consumption and oral oestrogen intake lead to reduced IGF-1 levels, whereas

puberty, pregnancy and the action of testosterone and dehydroepiandrosterone sulphate (DHEA-s) lead to an increase. If the IGF-1 value is high for age, diagnostic confirmation is carried out by the oral glucose tolerance test (OGTT). Failure to suppress GH (GH nadir >1 µg/L or, according to the latest Consensus Statements, >0.4 µg/L) confirms the diagnosis of acromegaly. Although the sensitivity of current GH assay methods has improved, sufficient accuracy for values below 1 µg/L has not yet been achieved in many laboratories, so the cut-off of 1 µg/L is still considered sufficient to exclude the diagnosis. Random GH dosing is not recommended as a high or borderline value could depend on a plasma GH peak, which has a pulsatile production. Furthermore, GH is also subject to physiological or pathological conditions that can increase or decrease its plasma levels, such as puberty, pregnancy, severe hepatopathy or renal failure, malnutrition and prolonged fasting, psycho-physical stress, poorly compensated diabetes mellitus, glucocorticoids, testosterone or oestrogen therapy. Once biochemical diagnosis of acromegaly has been confirmed, magnetic resonance imaging (MRI) of the sella turcica is the gold standard for detecting pituitary adenoma, as well as providing information on its size and densitometric characteristics, which is necessary to define patient's subsequent management [4].

Comorbidities

Because GH and IGF-1 receptors are ubiquitously expressed, deleterious effects of both IGF-1 and growth hormone excess occur in multiple organ systems.

Patient with acromegaly are burdened by a wide spectrum of cardiovascular, respiratory, metabolic, musculoskeletal, neurological and neoplastic comorbidities, often already present at diagnosis due to a frequent diagnostic delay. If untreated, they show an increased mortality risk compared to the general population, tightly linked to long-term exposure to GH and IGF-1 excess [5]. Comorbidities are not always reversible once disease control is achieved; however, reaching biochemical control may result in a significant reduction of patient mortality rate, as well as in the restoration of normal life expectancy.

Cardiovascular disease is present in more than 50% of patients with acromegaly: hypertension affects 18–55% of patients, probably mediated by direct kidney growth hormone action, while hypertrophic cardiomyopathy, including earlier concentric cardiac remodelling, is due to both hypertrophy of myocardial cells and volume overload and is observed in 13–79% of patients. Hypertrophy (usually biventricular concentric hypertrophy) might be partly reversed in some

patients by achieving biochemical control; congestive heart failure is instead rare (<3%) and usually not reversible even with disease control [6, 7].

Obstructive sleep apnoea occurrs in about 27–88% of acromegalic patients; contributing factors include upper airway narrowing caused by macroglossia, swelling of the uvula and pharyngeal wall, and mandibular overgrowth. Although treatment can induce regression of tissue hypertrophy and tongue volume, it might not sufficient to alleviate obstructive sleep apnoea after biochemical control [8, 9].

GH and IGF-1 have opposite actions on glucose and lipid metabolism. Overall, growth hormone excess increases insulin resistance and might lead to hyperglycaemia and overt diabetes in approximately 30% of patients, even increasing expression of proinflammatory cytokine in the adipose tissue. Hyperlipidaemia occurs in 13–80% of patients, tipically represented by hypertriglyceridaemia, low amounts of high-density lipoprotein, and elevated concentrations of apolipoprotein B. Biochemical control usually improves insulin resistance, but there may be a residual increase in intrahepatic lipid content and abdominal adiposity and a decrease in muscle mass. Surgical adenoma resection might reverse the lipodystrophy pattern associated with acromegaly [9].

Joint pain is common, affecting more than 75% of patients, and it is caused by a degenerative joint disease characterised by chondrocyte hypertrophy and osteophytosis. Although any joint might be affected, hip and spine are almost universally involved. Biochemical control might help reduce cartilage thickness, especially in early arthropathy, but degenerative disease persists and worsens over time despite normalisation of GH and IGF-1.

Bone disease associated with acromegaly is characterised by altered trabecular architecture and increased cortical bone, predisposing patients to vertebral fractures, with a risk 3-8 times higher than in normal population, affecting approximately 40% of acromegalic patients. Concomitant hypogonadism, diabetes, and differential expression of IGF-binding proteins contribute to the severity of bone disease. Vertebral fractures could be an early consequence of acromegaly, directly related to modestly elevated GH concentrations, since they are often present at diagnosis [10]. The Fracture Risk Assessment Tool, which relies on bone mineral density (BMD) assessments, is not reliable in assessing risk of vertebral fractures in patients with acromegaly. In fact, BMD in these patients is often within normal ranges. A more useful measure might be the trabecular bone score (TBS) as a low score value reflects disrupted and fracture-prone bone microarchitecture. The aim of our recent study was precisely to investigate the superiority of TBS over BMD in identifying patients

with impaired trabecular microarchitecture of the lumbar spine [11]. Biochemical control reduces but does not eliminate fracture risk, and 20% of patients show further decrease in vertebral height despite IGF-1 normalisation. Previous vertebral fractures, disease duration, and active disease are among the most relevant factors contributing to skeletal fragility.

Effects of GH excess on skeletal muscle tissue and muscle function are emerging, as skeletal muscle is one of the main targets of GH and IGF-1. Given the known anabolic functions of GH and the inhibition of IGF-1-mediated proteolysis, an increase in skeletal muscle mass should be assumed in patients with active disease. In uncontrolled acromegaly actually both body cell mass and proximal muscle strength might increase, but with reduced grip strength. With regard to muscle quality and performance, it has largely been shown that, despite an increase in muscle mass, patients with acromegaly experience myopathy with weakness and pain, along with a reduction in muscle endurance. Biochemical remission may normalise body composition and improves hand grip strength, but increases proximal muscle fatigue. Increased thigh muscle adiposity might also be linked to muscle dysfunction, specifically slower gait speed. A gold standard for assessing muscle characteristics in patients with acromegaly has not yet been identified. In fact, the limited number of published studies and the presence of multiple confounding factors (e.g. the heterogeneity of the radiological techniques used in the different studies, but also the different medical therapies, the presence of comorbidities that may impact on the muscle, or the different degree of physical activity of the patients) contribute to providing conflicting results, especially in relation to skeletal muscle mass. In this regard, we conducted a systematic review of the literature including 15 studies [*Milioto A, Corica G, et al. J Clin Endocrinol Metab, Under Review*].

The aim of another study was to investigate whether the measure of temporal and masseter muscle thickness (easily visible at the brain/sella turcica MRIs routinely performed by acromegalic patients), was consistent and whether it correlated with demographic characteristics associated with skeletal muscle mass. We also investigated the potential correlations between disease activity, time from diagnosis, and muscle thickness *[Gatto G, Milioto A, Corica G et al., Manuscript in preparation]*.

Nerve compression as a result of soft tissue hypertrophy and bone overgrowth might cause peripheral neuropathy, including carpal tunnel syndrome, which is observed in 19–64% of patients. Headaches, reported in two-thirds of patients, might be due to direct effect of GH or due to mass effect of the adenoma from cavernous sinus invasion and irritation of the trigeminal nerve upon stretch of the dura. Biochemical control improves headache in most patients.

GH and IGF-1 promote cellular proliferation, potentially leading to carcinogenesis and tumour progression. The exact mechanisms underlying neoplastic processes in acromegaly are not yet fully elucidated; however, it is known that high circulating levels of GH and IGF-1 exert mitogenic and anti-apoptotic effects in many tissues through the regulation of different intracellular transduction mechanisms. The incidence of both benign tumor and malignancies, particularly of the colon, rectum and thyroid, appears to be increased in patients with acromegaly, approximately twice as high than in people without acromegaly, although the intensity of screening compared to the general population may influence incidence rates. Biochemical control of acromegaly in patients diagnosed with malignancy is crucial to manage its outcome [12].

	Cardiovascular	Respiratory	Musculoskeletal	Metabolic and endocrine	Neurological	Neoplastic	Mass effects	Quality of life
Screen	Hypertension, hypertrophic cardiomyopathy, valvulopathy, systolic and diastolic dysfunction, and arrythmias	Obstructive and central sleep apnoea and respiratory insufficiency	Arthropathy, vertebral fractures, and jaw malocclusion	Diabetes or prediabetes, dyslipidaemia (elevated triglyceride and reduced high-density lipoprotein concentrations), hepatic steatosis, polycystic ovary syndrome or hyperandrogenism, and hyperprolactinaemia	Nerve entrapment and headache	Colorectal polyps, thyroid nodules, cancer (colon, breast, thyroid, and renal), and skin tags	Headache, hypopituitarism, and cranial nerve palsy	Negative body image or low self-esteem, cognitive dysfunction, psychological symptoms, pain or headache, treatment burden, and growth hormone deficiency
Test	Blood pressure, echocardiography, and electrocardiogram	Epworth Sleepiness scale and STOP-Bang score (both used for obstructive sleep apnoea), polysomnography, and pulmonary function test	Spine x-rays; dual-energy x-ray absorptiometry, and trabecular bone score; measurement of 25-hydroxy vitamin D concentrations, parathyroid hormone, calcium concentrations in urine, and bone markers if needed; and gonadal hormones	Fasting glucose and oral glucose tolerance test, HbA_{1c} measurement, lipid profile, measurement of alanine transaminase and aspartate transaminase, and prolactin measurement	Electromyography or nerve conduction studies	Colonoscopy, physical examination, and thyroid ultrasound (if palpable nodule)	MRI, visual field testing, and hormonal testing for pituitary deficiency	Quality of life and disease impact
Refer	Cardiologist	Pulmonologist	Bone endocrinologist, rheumatologist, orthopaedic surgeon, and maxillofacial surgeon	Diabetologist and hepatologist for non-alcoholic fatty liver disease and non-alcoholic steatohepatitis	Neurologist	Oncologist, gastroenterologist, and dermatologist	Ophthalmologist, neurosurgeon, and radiation oncologist	Psychotherapist or counsellor, and psychiatrist
Treat	Repeat echocardiography according to general population guidelines and treat with antihypertensive drugs (diuretics, β-blockers)	Continuous positive airway pressure, mouth guard, and uvulopalatoplasty	Intake of calcium and vitamin D, sex hormone replacement therapy, anti-osteoporotic therapy, pain management, joint injections, and joint replacement; avoid over-replacement of glucocorticoids	Metformin, incretin-based therapy, insulin secretagogues, insulin, statins, and monitoring for weight gain after treatment for growth hormone excess	Headache management and octreotide subcutaneous injection if needed	Specific to the disease; follow-up colonoscopy according to general guidelines, unless IGF-1 concentrations are elevated; and fine-needle aspiration for thyroid nodules according to general guidelines	Debulking surgery, radiation therapy, and hormonal replacement	Support groups, reliable educational resources, psychological support, cognitive–behavioural therapies, pain management, and growth hormone replacement for select patients with growth hormone deficiency

IGF=insulin-like growth factor.

Table 2: Complications of acromegaly

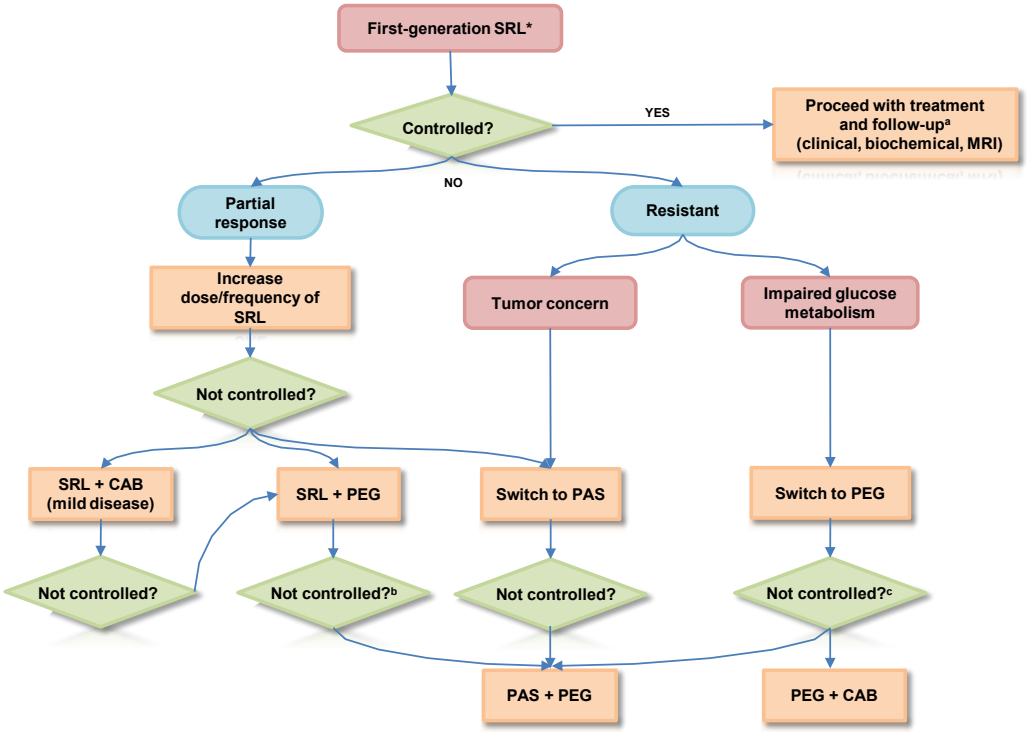
Figure 2. Complications of acromegaly (Fleseriu M., *Acromegaly: pathogenesis, diagnosis, and management,* 2022)

Therapeutic goals and treatment strategies

Achieving biochemical control (namely, glucose-suppressed GH levels <0.4 µg/L and normal sex and age-adjusted IGF-1 values), is the primary therapeutic goal; other treatment goals include volumetric reduction of the pituitary tumour, preservation of the remaining pituitary function, symptoms relief, management of complications, reduction of morbidity and improvement of quality of life (QoL).

Pituitary surgery remains the first-line treatment for acromegaly, but nowadays, given the array of novel medical treatment options, much attention has been paid to identify treatment algorithms that suggest the best strategies for that patients requiring medical therapy, considering individual patients' unique characteristics and preferences [13, 14].

The use of first-generation somatostatin receptor ligands (fg-SRLs) is recommended for selected patients who have persistent disease after surgery, who are not candidates for surgical treatment, who refuse surgery, or who are at risk for low surgical success rates. According to the most recent data, approximately 40% of patients treated with the commercially available fg-SRLs octreotide (OCT) and lanreotide (LAN) achieve biochemical control.



Abbreviations and legend: SRL, somatostatin receptor ligand; MRI, magnetic resonance imaging; CAB, cabergoline; PEG, pegvisomant; PAS, pasireotide; *As adjuvant therapy and/or neoadjuvant treatment; [a] If significant tumor shrinkage after neoadjuvant SRL treatment, consider surgery; [b] Non-diabetic patients; [c] PAS + PEG if risk related to tumor concern and uncontrolled disease is greater than the worsening of glucose unbalance

Figure 3. Proposed algorithm for second-line medical treatment in acromegaly. (Corica G., *Octreotide-resistant acromegaly: challenges and solutions,* 2019)

The latest treatment algorithms proposed in Consensus Statements and guidelines suggest various treatment strategies based on patient-specific characteristics, even for patients who partially respond or are resistant to fg-SRL therapy. In addition to disease control, personalized treatment may improve compliance to therapy and reduce direct costs associated with the disease. As a first step, in case of partial response, it is suggested to increase the dose of OCT or LAN as much as possible (e.g.,40 mg/4 weeks for OCT) or to shorten the dosing interval (e.g.,120 mg/3 weeks for LAN). On the other hand, in patients with mild disease activity, cabergoline (CAB), a dopamine agonist (DA), can be successfully added to fg-SRL therapy. In patients with impaired glucose metabolism, it is strongly recommended to combine fg-SRL with pegvisomant (PEG), a GH receptor antagonist (GHRA), or switch to PEG monotherapy.

Not only is pasireotide (PAS) more effective than fg-SRL, but because of the significant incidence of drug-induced hyperglycemia and diabetes associated with PAS therapy in early studies, switching to pasireotide (PAS) is now recommended primarily in patients with normal glucose metabolism. However, data from a recent clinical trial focusing on adverse events with PAS revealed that when hyperglycemia does occur, most patients are successfully managed with first-line antidiabetic agents (such as metformin) and reach target glycated hemoglobin levels (HbA1c <7%) without the need for additional therapy. Although actual data confirm that the degree of hyperglycemia associated with PAS depends on glycemic control at baseline, it is unlikely that impaired glucose metabolism is an absolute contraindication to treatment, since the effects of PAS are immediately reversible and few patients have been reported to discontinue treatment due to this problem. Besides evaluation of glycemic status, PAS therapy is suggested as a second-line medical therapy in cases where a (residual) pituitary tumor mass may be of concern to the patient, or in cases of headache unresponsive to fg-SRL [15]. Pharmacologically, PAS is a second-generation somatostatin receptor ligand with a higher binding affinity for somatostatin receptor type 5 (SST5) and slightly lower binding affinity for SST2 compared to OCT. Furthermore, when bound to SST2, PAS may exhibit different functional properties compared to fg-SRL and naive somatostatin [16]. Over the past 20 years, PAS has been shown in preclinical studies to be superior to OCT and LAN in suppressing hormone secretion and cell proliferation, and to achieve biochemical control and tumor volume reduction in acromegaly patients naive to medical therapy or who are not adequately controlled with fg-SRL. Since 2018, few studies have been published describing the efficacy of PAS treatment and its impact on glucose metabolism in real-world clinical practice. By definition, these studies were conducted in different patient populations, with varying treatment courses leading up

to the initiation of PAS therapy. Building on the experience of the PAPE clinical trial, Lasolle and colleagues switched a group of patients previously treated with combination therapy (fg-SRLs + PEG or fg-SRL + CAB) to PAS monotherapy and found that PAS monotherapy was successful in maintaining biochemical control, with slight improvement compared to previous therapy, despite a higher incidence of hyperglycemia [17]. In our study, we retrospectively evaluated biochemical outcomes along with glucose metabolism profiles in a cohort of acromegalic patients previously treated with medical combination therapy or unconventional doses of fg-SRL and subsequently switched to PAS therapy. Notably, the switch to PAS therapy was made both in patients who had had an inadequate response to prior therapy and in patients who were biochemically controlled, with the aim of simplifying the on going treatment schedule [18].

The need for a personalised approach in the management of patients with acromegaly, such as those delivered by multidisciplinary teams in Pituitary Tumor Centres of Excellence, is certainly a hot topic in pituitary disease research. In this scenario, "real-life" studies can play a key role in better understanding the correct positioning of the different therapeutic strategies currently available in the management of acromegaly, including medical therapy with PAS.

Recent advances in acromegaly disease control and comorbidity management have led to a reduction in disease-related mortality, which now approaches (in controlled patients) that of the general population. Centres of Excellence for pituitary pathology ensure multidisciplinary management of the biochemical dysfunction and mass effects of the pituitary lesion, as well as the availability of advanced procedures for the diagnosis, monitoring and treatment of disease-related comorbidities. Despite newer and more precise GH and IGF-1 assays and imaging advances, substantial gaps remain indeed in elucidation of diagnosis and morbidity management. Comorbidities are important to recognise and require rigorous screening, diagnosis, and targeted management.

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New approaches to acromegaly comorbidities

Trabecular Bone Score as a Reliable Measure of Lumbar Spine Bone Microarchitecture in Acromegalic Patients

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Abstract: Although GH and IGF-1 excess has a controversial impact on bone mineral density (BMD), acromegalic patients display variable degrees of bone structure impairment. In this study, we aim to investigate the usefulness of trabecular bone score (TBS), compared to BMD, in identifying acromegalic patients with impaired lumbar spine trabecular microarchitecture. Forty-four acromegalic patients were investigated for disease control, metabolic and gonadal status, bone metabolism parameters, and the presence of vertebral fractures (VFs). Patients and matched healthy controls underwent BMD and TBS examination. Mean TBS values were lower in patients than in controls ($p < 0.001$), without significant differences in mean lumbar and femoral BMD. TBS values were significantly higher in controlled patients compared to the uncontrolled ones ($p = 0.012$). No significant differences were found in bone markers with respect to disease control. Mean TBS or lumbar BMD did not significantly differ in patients with or without VFs (prevalence 11.4%). TBS and BMD levels were lower in hypogonadal patients compared to the eugonadal ones ($p = 0.030$ and $p < 0.001$, respectively). In conclusion, TBS values are significantly lower in patients than in controls, confirming the presence of impaired lumbar spine trabecular bone in acromegaly. Both uncontrolled disease and hypogonadism contribute to TBS deterioration in acromegaly.

Introduction

Acromegaly has long been considered a cause of secondary osteoporosis, although bone mineral density (BMD) is not always reduced in acromegalic patients. Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) have a predominantly anabolic effect on the bone. However, their long-term excess seems to activate bone reabsorption mechanisms, and to increase bone turnover with a controversial final effect on BMD [1–4]. Currently available data from the literature report both normal, increased, and also reduced bone density in acromegalic patients, compared to the general population. Furthermore, in patients with acromegaly, other factors can affect bone metabolism, particularly the presence of hypogonadism (with or without hyperprolactinemia), a condition often associated with the disease [5–10]. The effect of GH excess on bone can vary in relation to the analyzed sites, with normal or reduced bone density at the lumbar spine (70% trabecular bone), and increased in the appendicular bone (90% cortical bone) [11,12]. The final result is bone tissue characterized by an altered trabecular structure, which is more prone to the risk of fractures. Despite a number of studies reporting an increased incidence of vertebral fractures (VFs) in acromegalic patients, a significant correlation between BMD values and the risk of fractures has never been found [6,13–20]. In order to better evaluate the trabecular bone structure in acromegalic patients, different techniques have been used, including peripheral quantitative computed tomography (pQTC) on iliac crest biopsy, quantitative vertebral computed tomography (QCT), high-resolution peripheral quantitative computed tomography (HR-pQCT), cone-beam computed tomography, histomorphometry of bone biopsy, and trabecular bone score (TBS) [6,20–25]. Among the recently developed methods to study the trabecular bone microarchitecture, TBS measurement is particularly accurate in identifying post-menopausal women at high risk of bone frailty and fractures, as well as in monitoring the efficacy of medical therapies [26–29]. TBS is an indirect quantitative index that classifies the state of trabecular bone microarchitecture, and it is calculated jointly with the densitometric results. The advantages of TBS compared to the other proposed techniques mainly resides in the use of a non-invasive investigation, performed in the same place as the standard densitometric examination, without exposing the patient to additional radiations, with a (very) low burden on health care costs. The evaluation of TBS has been proposed, in association with dual-energy x-ray absorptiometry (DXA), for the study of bone quality in various endocrine disorders such as primary hyperaldosteronism, Cushing's syndrome and subclinical hypercortisolism, primary hyperparathyroidism, GH deficiency, as well as diabetes mellitus, proving in some cases more reliable than BMD alone in identifying patients with a higher risk of fractures

[30–39]. Currently, few studies have analyzed TBS in patients with acromegaly [23,24,40–47]. In most cases, the authors reported lower TBS values in acromegalic patients compared to healthy subjects. However, the impact of disease control on TBS values is still controversial, and studies mainly focused on the effects of medical treatment on bone microarchitecture in patients with long-term follow-up are still lacking.

Therefore, the main aims of the present study are: (i) to evaluate BMD (lumbar and femoral) and lumbar TBS values in a cohort of acromegalic patients compared to a control group of healthy subjects; (ii) to investigate the impact of disease control on both BMD and TBS values; (iii) to assess the prevalence of VFs in acromegalic patients, correlating the presence of VFs with BMD and TBS values. Furthermore, we investigated the role of RANK/RANK-L/OPG and DKK-1/sclerostin systems in our cohort, trying to elucidate the correlation between bone metabolism serum markers and TBS values, disease activity, as well as the presence of VFs.

Patients and Methods

Study Design and Patients

An observational cohort study involving acromegalic patients with active follow-up at a single tertiary center for pituitary diseases (Endocrinology Unit, IRCCS Ospedale Policlinico San Martino, Genoa, Italy). Diagnosis of acromegaly was made based on clinical features, biochemical evidence of GH hypersecretion (lack of suppression of GH to <1 µg/L after a 2-h oral glucose tolerance test), IGF-1 levels above the age-adjusted upper limit of normality range (>1 xULN), and the presence of a pituitary adenoma at magnetic resonance imaging (MRI).

Forty-four acromegalic patients (28 females, age range 31–75 years) were included in the study, and 44 healthy volunteers comparable for age, sex, and BMI (35 females, age range 27–77 years) were enrolled as a control group. Pregnancy status was excluded for women of childbearing age. Patients with diseases or clinical conditions possibly leading to osteoporosis (hyperthyroidism/ thyrotoxicosis, hypercortisolism, primary or secondary hyperparathyroidism, chronic renal failure, malabsorption, bedridden), as well as current or previous therapies that can impact bone structure (glucocorticoids as antiinflammatory/immunosuppressive therapy, GnRH analogues, immunosuppressive drugs, antiretrovirals, anticoagulants, anticonvulsants, pioglitazone) were excluded from the study. The presence of uncontrolled diabetes mellitus (HbA1c ≥8%), active malignant neoplasms, and previous traumas represented additional exclusion criteria.

Detailed clinical information was collected for all patients. Particularly, the presence of familial history of osteoporosis, lifestyle, smoking habits, and alcohol intake, as well as the time from diagnosis of acromegaly was investigated. As for the control group, the presence of primary or secondary osteoporosis, as well as previous or ongoing osteoporosis treatments were carefully investigated in the clinical history, and they were considered as exclusion criteria.

At the time of data collection (data censoring: time of DXA and TBS assessment), IGF- 1 values were used to evaluate patient biochemical control. In detail, according to recent clinical studies and consensus statements, we considered as having an acceptable biochemical control (controlled patients) those study subjects with sex- and age-adjusted IGF-1 values < 1.2 the upper limit of normality (ULN), while patients with IGF-1 levels ≥1.2 xULN were defined as uncontrolled [48–50]. Time from diagnosis was defined as the timeframe from the diagnosis and the time of data collection, irrespective of disease control.

As concerns the gonadal status, patients were considered hypogonadal in case of low total testosterone levels and associated symptoms (men), or low levels of estradiol accompanied by the absence of menstrual cycles (women). Women with menopause were included in the hypogonadal group, as well.

The study was conducted in accordance with the recommendations of the Declaration of Helsinki and all patients gave written informed consent to use clinical data for research purposes.

Laboratory Methods

The following hormonal and metabolic parameters were investigated in all acromegalic patients at the time of DXA and TBS assessment: GH, IGF-1, fasting plasma glucose, glycated hemoglobin (HbA1c), thyroid stimulating hormone (TSH), free thyroxine (fT4), prolactin (PRL), testosterone (men), estradiol (women), sex-hormone binding globulin (SHBG), albumin, morning plasma cortisol, urinary free cortisol (UFC), parathyroid hormone (PTH), 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [1,25(OH)2D], electrolytes, creatinine, bone ALP and osteocalcin. All the above-mentioned parameters were determined by the routine automatic methods in use at the Medicine Laboratory of our Institution (IRCCS Ospedale Policlinico San Martino, Genova, Italy). Additional biomarkers of bone function (osteoprotegerin (OPG), sclerostin, Dickkopf-related protein 1 (DKK-1), RANK-L) were tested in acromegalic patients, being assessed at the Research Laboratory of Clinical Rheumatology of our hospital (by use of Enzyme Immunoassays; Biomedica Medizinprodukte GmbH, Wien, Austria).

BMD, TBS, and Vertebral Fracture Assessment

All study participants (both patients and controls) underwent DXA to evaluate the bone quantity and bone quality using BMD and TBS, respectively. Densitometry values were detected at the lumbar spine (L1-L4), and at all femoral sites (neck, ward's triangle, trochanter, total hip), and they were computed using the Lunar Prodigy Advance densitometer (GE Lunar, enCORE software GE Healthcare version 16, Madison, WI, USA). BMD values were expressed as grams per square centimeter (g/cm2 ±SD), and as T-score, a measure of the bone quantity of the study subject compared with a young adult of the same gender with peak bone mass. A T-score below -2.5 identifies osteoporosis. We also evaluated the Z-score, which establishes the amount of bone compared with people of the same age and gender group [51]. During the same DXA procedure, TBS was calculated at the lumbar spine, using the region of interest of the BMD measurement, by use of the iNsight software (Medimaps®). All acromegalic patients also underwent dorsal-lumbar spine x-ray morphometric analysis to detect vertebral fractures.

The results were correlated with biochemical control, bone metabolism parameters, and metabolic and gonadal status at the time of data censoring (namely, the time of DXA and TBS assessment).

Statistical Analysis

SPSS 28.0 forWindows (SPSS, Chicago, IL, USA) was used for statistical analyses, while GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA, USA) was used to draw figures. The Kolmogorov-Smirnov test was used to check the normality of the distribution of the continuous variables. Quantitative data are presented as mean ±standard deviation (SD), while categorical variables are presented as frequencies and percentages. Betweengroup comparisons were analyzed by the Student's t-tests (or the Mann–Whitney test) and the one-way ANOVA test (or the Kruskal–Wallis test), where appropriate. Correlations were performed using Pearson's r correlation coefficient or Spearman's rho correlation coefficient for ranks, based on data distribution. The two-sided Fisher's test or the Chisquare test was used to evaluate differences in cross-tables. Differences were taken to be statistically significant at $p < 0.05$.

Results

Patients Characteristics

Forty-four patients with acromegaly (28 females, 16 males) and 44 healthy controls (35 females, 9 males) were included in the study (F/M ratio between patients and controls: p = 0.155). Mean age was 54.2 (±11.5) years for patients, and 51.3 (±11.2) years for controls (p = 0.430). No significant differences were found in BMI values between patients and controls (26.96 ±5.37 Kg/m2 vs. 25.17 ±4.55 Kg/m2, p = 0.128). General and clinical patient characteristics, including time from diagnosis, family history for osteoporosis, alcohol intake, and smoking habits, as well as treatment modalities and biochemical outcomes, are depicted in Table 1.

	Patients (*n* = 44)	Healthy Controls (*n* = 44)	*p*-Value
Sex (F; *n*, %)	28 (63.6)	35 (79.5)	0.155
Age (mean ± SD; years)	54.2 ± 11.5	51.3 ± 11.2	0.430
BMI (mean ± SD; Kg/m^2)	26.96 ± 5.37	25.17 ± 4.55	0.128
Hypogonadism/menopause (*n*, %)	21 (48)	16 (36)	0.388
Familial history of osteoporosis (*n*, %)	5 (11.4)		
Smoking habits (*n*, %)	10 (22.7)		
Alcohol intake[a] (*n*, %)			
-no consumption	13 (29.5)		
-sporadic	24 (54.5)		
-moderate	6 (13.6)		
-high	1 (2.4)		
Lifestyle (*n*, %)			
-sedentary	13 (29.5)		
-mild activity	20 (45.5)		
-moderate activity	9 (20.5)		
-intense activity	2 (4.5)		
Time from diagnosis (mean ± SD; years)	13.98 ± 6.20		
Treatment modalities			
Neurosurgery	29 (65.9)		
Radiotherapy	2 (4.5)		
Medical therapy			
Fg-SRL	26 (59.1)		
Fg-SRL + CAB	4 (9.1)		
Fg-SRL+ PEG	5 (11.4)		
Fg-SRL + CAB + PEG	2 (4.5)		
Biochemical values (last follow-up)			
GH (mean ± SD; µg/L)	2.25 ± 1.92		
IGF-1 absolute (mean ± SD; µg/L)	269.7 ± 201.18		
IGF-1 xULN (mean ± SD)	1.14 ± 0.82		
Biochemical control (IGF-1 < 1.2 xULN; *n*,%)	34 (77.3)		

Table 1. General, clinical characteristics and hormone values of the acromegalic patients included in the study. Available data about healthy controls are reported, as well.
Legend: F, females; SD, standar deviation; BMI, body mass index; fg-SRL, first-generation so-matostatin receptor ligand; PEG, pegvisomant; CAB, cabergoline; GH, growth hormone; IGF-1, insulin-like growth factor 1; ULN, upper limit of normality range. a alcohol intake: sporadic, <2 alcoholic units/day for men and <1 alcoholic unit/day for women; mild consumption, 2 alcoholic units/day for men and 1 alcoholic unit/day for women; high consumption: >2 alcoholic units/day for men and >1 alcoholic unit/day for women.

In detail, osteoporosis was reported in the family history for five patients, while 10 study subjects reported smoking habits. Regarding alcohol consumption, 24 patients reported occasional intake, 6 moderate consumption (two alcoholic units/day for men and one alcoholic unit/day for women), and only 1 patient reported high alcohol consumption.

At the time of DXA and TBS assessment, mean GH and mean absolute IGF-1 values were 2.25 ±1.92 µg/L and 269.70 ±201.18 µg/L, respectively. Mean sex- and age-adjusted IGF-1 levels were 1.14 ±0.83 xULN. Biochemical control, defined as IGF- 1 xULN < 1.2 xULN, was detected in 34 patients (77%, mean IGF-1 0.79 0.20 xULN), while 10 patients were considered as uncontrolled (23%, mean IGF-1 2.34 ±1.03 xULN). Twenty-one out of 44 patients (48%, 5 males and 16 females) had hypogonadism due to menopause or pituitary function impairment.

The mean hormone and biochemical parameters of the patient cohort are reported in Table 2. Overall, free thyroid hormones, morning plasma cortisol, and UFC were within the normal range in all patients, while only one study subject presented with mild hyperprolactinemia (44 µg/L).

Measures	Values (Mean ± SD)	Normal Ranges
Fasting plasma glucose (mg/dL)	93.43 ± 16.81	65–110
HbA1c (%)	5.92 ± 0.48	4.3–5.8
PRL (µg/L)	8.50 ± 7.95	M: 2.64–13.13 F: 3.34–26.72 [1]; 2.74–19.64 [2]
TSH (mIU/L)	1.41 ± 0.83	0.27–4.20
fT4 (ng/L)	12.17 ± 1.99	9.3–17.0
Morning plasma cortisol (µg/dL)	11.28 ± 2.85	3.7–19.4
UFC (µg/24h)	37.50 ± 20.76	4.3–176.0
25(OH)D (ng/mL)	21.97± 7.72	6.0–46.0
1,25(OH)2D (pmoI/L)	148.01 ± 69.66	43–168
PTH (ng/L)	25.58 ± 8.25	6.5–36.8
Ca (mg/dL)	9.61 ± 0.36	8.5–11.0
P (mg/dL)	3.25 ± 0.58	2.5–4.5
Mg (mg/dL)	2.04 ± 0.21	1.9–2.5
Osteocalcin (µg/L)	16.90 ± 6.68	M: 12.0–52.1 F: 6.5–42.3 [1]; 5.4–59.1 [2]
Bone ALP (µg/L)	8.15 ± 3.51	M: 8–16.6 F: 5.8–11.6 [1]; 8.5–17.9 [2]

Table 2. Main hormonal and biochemical parameters investigated in acromegalic patients.
Legend: HbA1c, glycated hemoglobin; PRL, prolactin; M, males; F, females; TSH, thyroid stim-ulating hormone; fT4, free thyroxine; UFC, urinary free cortisol; PTH, parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)2D, 1,25-dihydroxyvitamin D; Ca, calcium, P, phos-phorus; Mg, magnesium; ALP, alkaline phosphatase. 1 normal ranges for pre-menopausal fe-males; 2 normal ranges for post-menopausal females.

As for 25(OH)D, 16/44 patients (36%) had values <20 ng/mL (mean 21.97 ±7.72 ng/mL). However, calcium levels and PTH values were normal in all patients. As expected, 25(OH)D values directly

correlated with both calcium (r = 0.307, p = 0.042) and 1,25(OH)2D levels (rho = 0.439, p = 0.003), while showing an inverse correlation with PTH (r = -0.367, p = 0.014). Furthermore, PTH levels were directly correlated with age (r = 0.398, p = 0.007) and osteocalcin levels (r = 0.352, p = 0.022).

TBS and BMD Values in Acromegalic Patients and Healthy Subjects

Patients and healthy controls were not significantly different in BMD values, T-score, and Z-score at both the lumbar spine (L1-L4) and all femoral sites (Figure 1, values detailed in Table 3).

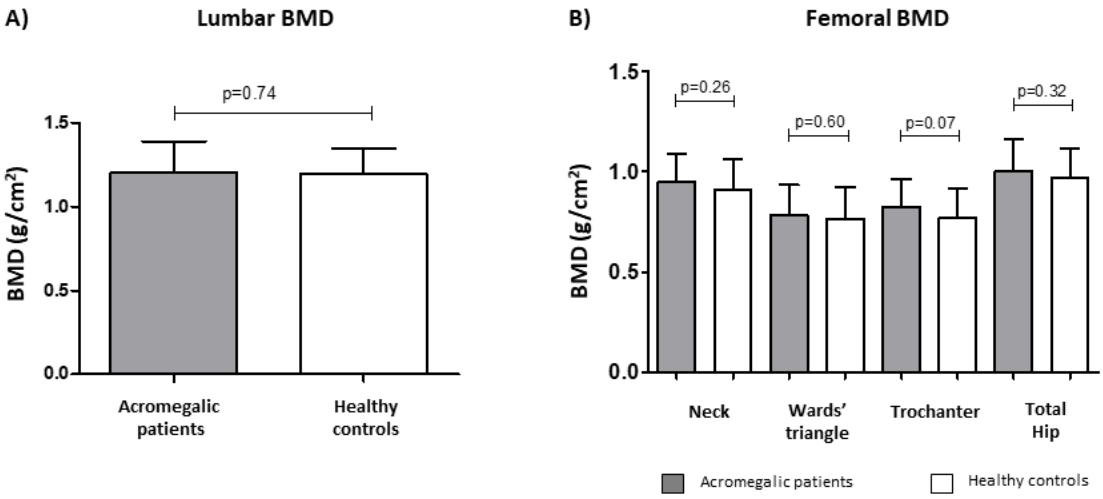

Figure 1. Mean lumbar (A) and femoral (B) bone mass density (BMD) values did not significantly differ in acromegalic patients (n = 44) compared to age- and sex-matched healthy controls (n = 44).

Of note, mean TBS values were significantly lower in acromegalic patients compared to healthy controls (1.18 ±0.15 vs. 1.29 ±0.14, p < 0.001) (Figure 2A, Table 3).

We observed a significant inverse correlation between TBS values and patients' age (r = -0.44, p = 0.002), whereas sex, BMI, lifestyle, smoking habits, alcohol intake, familial history of osteoporosis, and time from diagnosis, did not significantly affect TBS. Interestingly, TBS in the control group was not significantly correlated with age (r = -0.22, p = 0.151), and, similarly to the observation in the patient group, sex and BMI did not affect TBS values, as well.

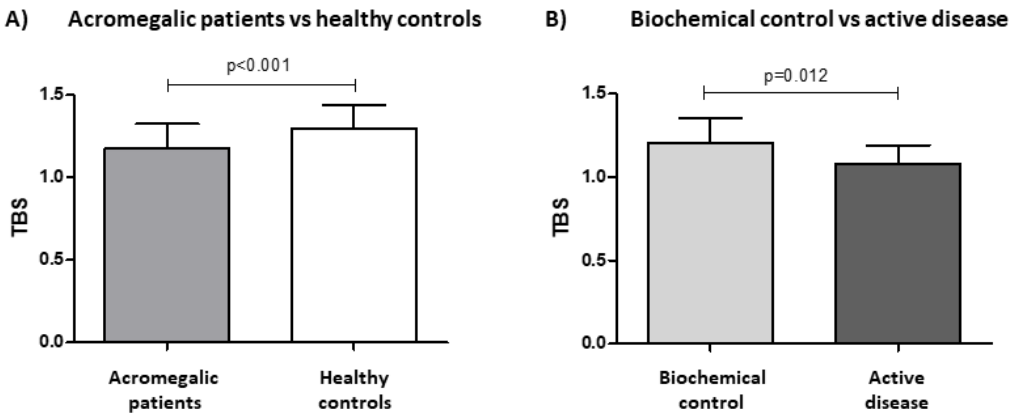

Figure 2. Mean trabecular bone score (TBS) values were significantly lower in acromegalic patients compared to age- and sex-matched healthy controls (A). Patients achieving biochemical control (IGF-1 <1.2 xULN) had higher TBS values compared to the uncontrolled ones (B).

Data	Patients (n = 44)	Healthy Controls (n = 44)	
TBS	1.18 ± 0.15	1.29 ± 0.14	$p < 0.001$
Lumbar BMD (L1–L4)			
BMD (g/cm2)	1.20 ± 0.19	1.19 ± 0.16	$p = 0.74$
T-score	0.09 ± 1.52	−0.07 ± 1.10	$p = 0.61$
Z-score	0.58 ± 1.25	0.56 ± 1.11	$p = 0.93$
Femoral BMD			
Neck BMD (g/cm2)	0.95 ± 0.14	0.91 ± 0.15	$p = 0.13$
Neck T-score	−0.52 ± 1.08	−0.73 ± 1.12	$p = 0.37$
Neck Z-score	0.09 ± 0.82	−0.11 ±1.07	$p = 0.32$
Ward's triangle BMD (g/cm2)	0.78 ± 0.15	0.77 ± 0.16	$p = 0.60$
Ward's triangle T-score	−1.12 ± 1.09	−1.11 ± 1.21	$p = 0.98$
Ward's triangle Z-score	−0.08 ± 0.80	−0.17 ± 1.05	$p = 0.65$
Trochanter BMD (g/cm2)	0.82 ± 0.14	0.77 ± 0.15	$p = 0.07$
Trochanter T-score	−0.12 ±1.13	−0.45 ± 1.12	$p = 0.17$
Trochanter Z-score	0.06 ± 0.99	−0.18 ± 1.03	$p = 0.26$
Total hip BMD (g/cm2)	1.00 ± 0.16	0.97 ± 0.15	$p = 0.17$
Total hip T-score	−0.22 ± 1.17	−0.39 ± 1.04	$p = 0.48$
Total hip Z-score	0.16 ± 0.90	0.03 ± 0.99	$p = 0.54$

Table 3. Mean TBS (lumbar), BMD, T-score, and Z-score (all sites) in acromegalic patients and matched healthy controls. Legend: TBS, trabecular bone score; BMD, bone mass density. Bold text indicates a statistically significant difference with a p-value less than 0.05.

Stratifying acromegalic patients based on IGF-1 levels, we found that controlled subjects (IGF-1 < 1.2 xULN) had higher TBS values compared to the uncontrolled ones (1.21 ±0.15 vs. 1.08 ±0.12, p = 0.013). In this context, we observed a trend for an inverse correlation (although not statistically significant) between TBS values and sexand age-adjusted IGF-1 levels (rho= -0.277, p = 0.068). On the contrary, GH and absolute IGF-1 values did not correlate with TBS (rho = 0.03, p = 0.854 and rho= -0.191, p = 0.213, respectively).

When considering the other hormonal and biochemical parameters evaluated in acromegalic patients (see Laboratory Methods), we found a significant inverse correlation between fasting plasma glucose levels and TBS values (r = -0.408, p = 0.006). On the other hand, HbA1c, thyroid function, cortisol levels, PRL, 25(OH)D, 1,2(OH)2D, Ca, P, PTH, bone ALP, and osteocalcin did not correlate with TBS.

Mean TBS values were significantly lower in hypogonadal patients than in the eugonadal ones (1.13 ±0.12 vs. 1.22 ±0.16, p = 0.016). Similarly, BMD values at all sites were significantly lower in hypogonadal compared to eugonadal patients (lumbar: 1.10 ±0.17 vs. 1.30 ±0.14, p < 0.001; femoral neck: 0.88 ±0.12 vs. 1.01 ±0.14, p < 0.001; ward's triangle: 0.70 ±0.11 vs. 0.86 ±0.15, p < 0.001; trochanter: 0.74 ±0.11 vs. 0.90 ±0.12, p < 0.001; total hip: 0.91 ±0.12 vs. 1.09 ±0.13, p < 0.001). A significant direct correlation was found between estradiol levels and TBS in females (r = 0.668, p = 0.005), whereas it was not detected between total testosterone levels and TBS values in men (r = 0.352, p = 0.181).

Of note, the prevalence of hypogonadism was higher among patients with active disease (8/10, 80%), compared to the controlled ones (13/34, 38%; Fisher's test p = 0.031). Within controlled acromegalic patients, we did not observe any statistically significant difference in TBS values between hypogonadal and eugonadal acromegalic patients (1.16 ±0.11 vs. 1.24 ±0.16, p = 0.131).

At univariate linear regression analysis, both uncontrolled disease (adjusted R^2: 0.117, B: -0.130, β: -0.371, p = 0.013) and hypogonadism (adjusted R^2: 0.086, B: -0.096, β: -0.327, p = 0.030) were significant negative predictors of TBS values. When the concomitant presence of uncontrolled disease and hypogonadism was evaluated using a multivariate regression model, we found an adjusted R^2 value of 0.142. Of note, age was another significant predictor of TBS value, both in univariate and multivariate analysis. Interestingly, when considering uncontrolled disease, hypogonadism, and age in the multivariate regression model, the adjusted R2 value raised to 0.256, with uncontrolled disease and age being still independent significant predictors of TBS (Table 4).

	Univariate Analysis				Multivariate Analysis			
Variable	Adjusted R^2	B	**β**	*p*-value	Adjusted R^2	B	**β**	*p* value
Uncontrolled disease	0.117	−0.130	−0.371	0.013	-	−0.124	−0.353	0.017
Hypogonadism	0.086	−0.096	−0.327	0.030	-	0.029	0.098	0.600
Age	0.179	−0.006	−0.445	0.002	-	−0.006	−0.469	0.010
All variables					0.256			

Table 4. Univariate and multivariate analysis evaluating the main predictors of TBS values in acromegalic patients.
Legend: B, unstandardized B; β, standardized coefficient β. Bold text indicates statis-tical significance (p-value less than 0.05).

RANK-L/Osteoprotegerin and DKK-1/Sclerostin System in Acromegalic Patients

We found that biochemically controlled patients had slightly higher RANK-L levels (357.36 ±297.68 vs. 262.33 ±394.14 pg/mL, p = 0.092) and RANK-L/OPG ratio (18.03 ±14.66 vs. 10.44 ±14.49, p = 0.060), compared to the uncontrolled ones, although these differences were not statistically significant. No differences were found in OPG (16.43 ±9.64 vs. 20.04 ±9.77 pg/mL, p = 0.374), DKK-1 (1841.92 ±786.7 vs. 1329.67 ±1056.98 pg/mL, p = 0.102) and sclerostin (59.60 ±33.73 vs. 51.39 ±37.55 pg/mL, p = 0.529) levels between controlled and uncontrolled patients. Of note, all the bone markers investigated showed a large variability among patients, irrespective of disease control, as demonstrated by the high standard deviation values reported above.

No statistically significant correlations were found between GH, IGF-1 levels, and bone markers, except for an inverse correlation between sclerostin and absolute IGF-1 values (rho= -0.343, p = 0.024).

TBS values in acromegalic patients did not correlate with RANK-L, OPG, DKK1, and sclerostin levels. Overall, bone markers did not correlate with patient BMD, except for a significant direct correlation observed between RANKL/OPG ratio and trochanteric BMD values (rho = 0.340, p = 0.037).

TBS, BMD, Bone Markers, and Vertebral Fractures

In our cohort, the prevalence of silent VFs (assessed by vertebral morphometry) was relatively low (5 out of 44 patients, 11.4%). No significant differences were found in mean lumbar BMD (p = 0.565) and TBS values (p = 0.858) in patients with VFs compared with those without fractures.

General patient characteristics, time from diagnosis, as well as GH, IGF-1 values, and bone markers were not significantly associated with the presence of VFs.

Discussion

In the present study, we found that TBS values were lower in acromegalic patients compared to age, BMI, and sex-matched healthy controls, while no significant differences were observed in BMD values between the two groups, both at lumbar and femoral sites. These data are in line with the results reported by other authors, although some studies did not find significant differences in TBS values between patients and matched controls [23,41–43,45–47]. Furthermore, we observed that both BMD and TBS values were significantly lower in hypogonadal patients compared to the eugonadal ones, thus confirming the pivotal role of gonadal status on both bone density and quality in acromegaly [6,40].

In line with previous findings, we observed a significant inverse correlation between TBS values and age in acromegalic patients, although this correlation was not found in the control group [23,37,43,46].

We observed a slight (not statistically significant) trend for an inverse correlation between TBS and sex- and age-adjusted IGF-1 values, while both GH and absolute IGF-1 levels were not major determinants of TBS values. However, stratifying our patients based on biochemical control, we found that controlled subjects had higher TBS values compared to those with active disease. Of note, the vast majority of our patients achieved biochemical control following medical therapy, and at the time of TBS evaluation had a long disease history (median follow-up 13.98 ±6.20 years).

This finding is of particular interest, since the effect of disease control on bone metabolism in acromegaly remains controversial. Indeed, Calatayud and colleagues reported higher TBS values in patients who underwent post-surgical remission [43], while a previous report from Godang K et al. described a reduction in TBS values one year after surgery, although associated with an increase in BMD levels [24]. Recently, Sala and colleagues failed to demonstrate significant changes in both TBS and BMD values in a prospective study evaluating 18 acromegalic patients at diagnosis and 12 months after achieving cured/controlled disease (66.7% of the patients were treated with somatostatin receptor ligands) [44].

As concerns the role of RANK/RANK-L/OPG and DKK-1/sclerostin systems in acromegaly, many aspects remain to be clarified. We did not find any significant difference in bone markers between controlled and uncontrolled patients. While no significant correlations were found between GH and IGF-1 levels and OPG, DKK-1, and RANK-L, we observed an inverse correlation between IGF-1 and sclerostin values. Interestingly, another study showed an inverse correlation between sclerostin and GH levels, suggesting that the observed decrease in Wnt antagonists' levels (such as sclerostin) could

represent a compensatory mechanism to counteract the increased bone frailty in active acromegaly [52]. However, another study showed a positive correlation between sclerostin, GH, and IGF-1 values [53], while Uygur and colleagues recently reported a lack of correlation between sclerostin with both GH and IGF-1 values [54]. Therefore, the significance of sclerostin levels in acromegaly is still debated, and the impact of disease control on sclerostin is still unknown.

The role of other bone markers in both active and controlled acromegaly is largely debated. In contrast with our results, Ozer and coworkers reported an inverse correlation between OPG and IGF-1 levels [55], while Constantin and colleagues did not find any correlation between GH and IGF-1 with both OPG and RANK-L [56]. High DKK-1 levels have been already reported in patients with acromegaly, and some studies describe an increase of this marker in patients with GH deficiency following GH replacement therapy [40,57]. In this light, Belaya and colleagues recently reported that GH excess results in an increased expression of DKK-1 [58].

The pathogenesis of increased bone resorption in acromegaly remains unclear. Osteoclasts express IGF-1 and IGF-1 receptors, therefore the GH/IGF-1 system can stimulate the production of cytokines involved in osteoclast regulation. Moreover, the complex interaction between osteoclasts and adipocytes observed in acromegaly may also play a role [40]. Studies on animal models and GH-deficient patients suggested that the RANK-L/OPG system might mediate the effects of IGF-1 on osteoclasts [59,60]. In this light, we would expect a decrease in the RANK-L/OPG ratio after acromegaly treatment. However, we found that controlled patients had slightly higher RANK-L and RANK-L/OPG ratios compared to patients with active disease, while no difference was found in OPG levels. Therefore, our results, together with previous data reported by other authors, did not confirm this hypothesis [56]. A possible explanation could be that plasma RANK-L and OPG levels may not reflect local cytokine production at the tissue level.

Our data confirm the presence of silent VFs in acromegalic patients. In our cohort, the prevalence of VFs, assessed bymorphometric examination, was 11.4%, similar to that reported byMadeira and colleagues, but lower compared to other studies [13–20,23,41,61]. Different methods have been used to identify VFs in different studies, performing a radiographic examination of the spine rather than a morphometric examination in most cases. These differences can, at least partially, explain the heterogeneity observed in the reported prevalence of VFs. We did not find significant differences in TBS values and bone markers between patients with or without VFs. However, these data need to be carefully handled, due to the low prevalence of VFs found in our cohort.

Overall, the main strength of our study is the comprehensive evaluation of BMD, TBS, VFs, disease control, biochemical and hormonal parameters, as well as specific bone markers in a well-characterized cohort of acromegalic patients followed-up at a tertiary center for pituitary diseases. The main limitations are represented by the relatively limited number of patients (although carefully selected and characterized), and the absolute low number of subjects with VFs (only five subjects). Due to the complex mechanisms regulating the cross-talk between bone density, bone structure, bone markers, and the GH/IGF-1 system, our results need to be investigated possibly in larger cohorts [62].

In conclusion, we have confirmed that acromegalic patients have significantly lower TBS values than matched healthy subjects, without significant differences in BMD values and scores. These results highlight the impairment of trabecular bone in acromegalic patients, underling how TBS and BMD provide different information about the bone status. The evaluation of TBS is useful in identifying acromegalic patients with deterioration of trabecular structure at the lumbar spine and, therefore, possibly exposed to a higher risk of VFs. TBS evaluation can be carried out in the same session as standard densitometry, saving time, and money, and preventing patients to further exposure to ionizing radiation compared to other methods used to study trabecular bone structure. In this context, particular attention should be given to elderly patients and those with concomitant impairment of gonadal function.

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Informed Consent Statement: The study was conducted in accordance with the recommendations of the Declaration of Helsinki and all patients gave written informed consent to use clinical data for research purposes.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: All authors declare that they do not have any conflict of interest.

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Skeletal muscle evaluation in patients with acromegaly: a systematic review

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Abstract

Context. Patients with acromegaly are characterized by the chronic exposure to high GH and IGF-1 levels, known for their anabolic effect on skeletal muscle. Therefore, an increased skeletal muscle mass could be hypothesized in these subjects. Herein, we have performed a systematic revision of published evidences regarding skeletal muscle mass, quality, and performance in subjects with acromegaly.

Evidence Acquisition. A systematic review of the literature on PUBMED database up to September 1st 2023 has been conducted with the following query: *acromegaly AND ("muscle mass" OR "skeletal muscle")*. We excluded studies that did not compare different disease states or used non-radiological methods for the skeletal muscle analyses, except for bioelectrical impedance analysis.

Evidence Synthesis. N=15 studies meet inclusion criteria. A total of 360 patients were evaluated for skeletal muscle mass, 122 for muscle fatty atrophy, and 192 for muscle performance. No clear evidence of increased skeletal muscle mass in patients with active disease compared to controlled or healthy subjects emerged. As for skeletal muscle quality, we observed a trend towards higher fatty infiltration among patients with acromegaly compared to healthy subjects. Likewise, patients with active disease showed consistent worse physical performances compared to controlled or healthy subjects.

Conclusions. Skeletal muscle in acromegaly has lower quality and performance compared to healthy subjects. The small number of published studies and multiple confounding factors (e.g. use of different radiological techniques) contributed to mixed results, especially regarding skeletal muscle mass. Well-designed prospective studies are needed to investigate skeletal muscle mass in patients with acromegaly.

Introduction

Acromegaly is a chronic and systemic disease characterized by elevated levels of GH and IGF-1, due in the vast majority of cases (>95%) by a GH-secreting pituitary tumor [1]. Long-term exposure to supraphysiological levels of GH and IGF-1 may lead to several comorbidities, such as cardiovascular, respiratory, osteoarticular, and metabolic diseases, among others [2].

Skeletal muscle is one of the main target tissues of both GH and IGF-1 [2]. Under physiological conditions, the activation of the GH/IGF-1 axis inhibits proteolysis and exerts an anabolic action on muscles [2]. In patients with acromegaly, a shift of amino-acid metabolism toward protein synthesis and free-fatty acid toward lipolysis has been described, likely due to the direct activation of GH receptor, and the impairment of insulin signaling [3]. Therefore, differently from the general population in which insulin resistance is often associated with increased body fat [4], active acromegaly constitutes a unique and paradoxical metabolic scenario in which patients harbor insulin resistance despite a relative decrease in adipose tissue [5]. Overall, the skeletal muscle of active acromegaly patients is characterized by decreased protein breakdown and increased protein synthesis. An increase in skeletal muscle mass, and thus a better physical performance would be expected in these patients. However, some of the most prevalent and debilitating comorbidities of acromegaly include musculoskeletal pain and weakness, as well as osteoarticular diseases [6,7]. Nevertheless, there is limited evidence regarding the effect of the disease and its biochemical control on skeletal muscle (including both tissue mass, quality, and function).

Nowadays, skeletal muscle is considered more than a tissue only deputed to mechanical functions (such as mobility). A number of studies have already demonstrated its role as an endocrine organ exerting a variety of functions, including glucose and lipid metabolism regulation [8–16]. Therefore, it is relevant to investigate and properly describe the skeletal muscle status in a complex endocrinological disease like acromegaly, which is tightly linked to metabolic comorbidities.

Patients and methods

Search strategy and selection criteria

We performed a systematic review of the literature on the PUBMED database with the following query: *acromegaly AND ("muscle mass" OR "skeletal muscle")* (search up to September 1st 2023). A total of 67 studies have been identified. We only included English-written studies. We excluded studies that assessed or estimated the entire lean mass without reporting skeletal muscle data. Then, we only included studies comparing different disease statuses (i.e. active acromegaly vs

controlled acromegaly) or studies that compared a specific disease status versus a control group (i.e. active acromegaly vs control group or controlled acromegaly vs control group). Except for bioelectrical impedance analysis (BIA), studies that analyzed or estimated skeletal muscle condition by use of non-radiological methods were excluded. In detail, studies using magnetic resonance imaging (MRI) and ultrasound sonography (US) were included since they provide a direct measure of skeletal muscle mass; likewise, studies employing dual x-ray absorptiometry (DXA) and bioelectric impedance analysis (BIA) have also been included since they allow a - reliable – estimation of the same parameter. As concern the skeletal muscle quality, studies using MRI and proton magnetic resonance spectroscopy (HMRS) were included, since they provide a measurement of inter-muscular adipose tissue (IMAT) and intramyocellular lipids (IMCL), respectively. We then considered studies using physical tests such as gait speed, 30-second chair stand, timed up and go, as well as hand grip, thigh flex and extension to evaluate the skeletal muscle performance. A single study using pennation angle (PA) evaluated through US has been included as a reliable estimation of muscle performance.

To enhance the quality of the systematic review, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was applied. Figure 1 shows a flow diagram of the study identification, screening, and inclusion process. After applying the above criteria, 15 manuscripts were identified and then analyzed.

Patients stratification and control populations

The criteria to define controlled disease (CD) were not consistent among the identified manuscripts. Some authors defined patients as reaching biochemical control in presence of circulating IGF-1 levels below the upper limit of normality (IGF-1 ≤ 1 xULN), adjusted for age in most cases [17–19]. However, other authors preferred to consider GH levels - during fasting condition, after an oral glucose tolerance test, or evaluated as a daily curve - to define the biochemical control of the disease [20–23].

In most studies, the control group (CTR) was selected among “healthy” or “non-acromegalic” subjects matched for several criteria such as age, gender, height, weight, and body mass index. In one manuscript, the control group was composed of subjects affected by clinically non-functioning pituitary tumour [24].

Results

Skeletal muscle mass

Skeletal muscle mass has been assessed in nine studies [17–19,22–27], most of them with a cross-sectional design only [17,18,22,23,25], except four manuscripts that adopted both a cross-sectional and a prospective design [19,24,26,27] (Table 1). MRI was the most employed method (n=5/9) [18,19,25–27], followed by DXA (n=4/9) [17,18,23,25], then BIA (n=2/9) [23,24], and US, this latter only performed once (n=1/9) [22]. As mentioned above, both MRI and US can provide a direct measurement of skeletal muscle tissue based on the area or the volume of a specific region (or the evaluation of the entire body). On the other hand, DXA and BIA can provide an estimate of skeletal muscle mass derived from the measurement of appendicular lean tissue or tissue impedance, respectively (see "Discussion" section).

Computed tomography (CT) was not employed in any of the studies included in our systematic review.

Of note, in three studies more than one technique was used (namely, twelve skeletal muscle mass analyses were performed in nine studies) [18,23,25]. A total body assessment was performed in all but four studies: Eroğlu et al. [18] employed DXA to estimate the appendicular skeletal mass and used MRI to evaluate the abdominal muscle area; Lopes et al. [23] used DXA to estimate the appendicular skeletal mass; Ozturk Gokce et al. [22] performed ultrasound sonography on the thigh and calf; Bredella et al. [19] employed MRI to evaluate the thigh district. A total of 360 patients with acromegaly underwent 478 skeletal muscle mass analyses: DXA was employed in 224 cases, followed by MRI (135 cases) and BIA (80 cases). Lastly, 39 cases have been assessed through US (Fig. 2A).

The results of skeletal muscle mass analyses in patients with active disease (AD), CD, and CTR groups are shown in Figure 3A. The AD group was compared to the CD group in six studies (including eight analyses globally). In two analyses, the AD group showed higher skeletal muscle mass than the CD group [17,19], while the opposite has been reported in one analysis [24]. No differences between the two groups (AD vs CD) have been identified in the remaining five evaluations [18,22,23].

When comparing the AD group with the CTR group, only one analysis found a greater muscle mass in AD vs CTR [24], while two analyses found the opposite (i.e. subjects in the CTR group had higher muscle mass than the AD group) [22,26]. Of note, most analyses (n=6) did not find any significant difference in skeletal muscle mass between AD and CTR [18,19,25,27].

Finally, the skeletal muscle mass of CD and CTR groups were compared in four analyses, showing no significant differences [18,19,22].

Skeletal muscle quality

To evaluate the quality of skeletal muscle, fatty atrophy was assessed (i.e. the higher the fatty atrophy the lower the quality of the muscle, and vice versa). A cross-sectional design was used in all six publications that evaluated skeletal muscle quality [5,19–21,26,27], and three of them sided a prospective study design [19,26,27] (Table 2). The techniques employed were MRI and HMRS, in four [5,21,26,27] and two studies [19,20], respectively. A total of 91 analyses involving 91 patients with acromegaly has been performed by MRI (Fig. 2B). Likewise, HMRS has been used for 43 analyses conducted on 27 patients with acromegaly (Fig. 2B). Of note, HMRS has been performed twice on 16 patients with acromegaly, before and after biochemical control was achieved. In three out of four studies that used MRI, IMAT was measured across all body districts to assess muscle quality [5,26,27], whereas in one study the fat fraction of thighs (i.e. percentage of fatty infiltration in skeletal muscle) was assessed [21]. In the two studies that employed HMRS, IMCL was assessed in the soleus muscle in one case [19] and both in the soleus and tibialis anterior muscles in the other one [20].

Figure 3B shows the results of fatty atrophy analyses among AD, CD, and CTR groups. In one analysis, skeletal muscle fatty atrophy in the AD group has been compared with the CD group, revealing no significant difference [19].

Among the four studies that compared the AD group versus the CTR group, three analyses showed a higher degree of skeletal muscle fatty atrophy in AD patients [5,26,27], while in one analysis no significant difference was found [19].

Furthermore, no difference in skeletal muscle fatty atrophy has been demonstrated between CD and CTR groups in two analyses [19,20]. However, one analysis demonstrated more fatty atrophy in the CD group compared to the CTR [21].

Skeletal muscle performance

A total of five cross-sectional studies used functional exercises [18,21], dynamometers [6,18,21,28], and US [22] to evaluate or estimate the skeletal muscle performance in patients with acromegaly (Table 3). Among these, one study carried a prospective design also [6]. Overall, 477 performance tests were conducted in 209 acromegalic patients; the results are shown in Table 3 and Figure 3C.

In three analyses in which patients with AD were compared to the CD group, active disease patients performed worse than those with controlled disease [6,28]. On the other hand, four performance analyses found no difference between AD and CD [18,22].

When the AD group was compared to CTR, patients with active disease performed worse than controls in two analyses [6,22], while in one evaluation the opposite result was found (AD performed better than CTR) [6]. No difference between AD and CTR groups has been reported in the remaining three analyses [18].

Finally, ten analyses compared CD and CTR groups: in three evaluations the CD group had worse results [21], while in seven analyses no difference was found (i.e. CD and CTR groups performed similarly) [6,18,21,22].

Discussion

Skeletal muscle is one of the main target tissues of GH and IGF-1 activity, and it is typically affected in acromegaly. Despite the first evaluation of skeletal muscle status in patients with acromegaly dates back to 1965 [29], the number of studies primarily focused on this issue is still relatively low.

Overall, performing a critical analysis of our systematic review on available literature data, the assumption that patients with acromegaly and active disease could have more muscle mass than patients with controlled disease (or healthy subjects) does not emerge clearly. Indeed, most analyses performed to compare patients with active disease with both controlled or healthy subjects did not find a significantly higher skeletal muscle mass in AD patients (14 out of 17 evaluations). In other three analyses, the authors reported higher skeletal muscle mass in AD compared to CD or CTR (Figure 3A). Of note, one of these three analyses, carried out by Reid and colleagues [17], included the highest number of patients evaluated in a single study (n=138; Table 1).

On the other hand, a clear trend towards a lower muscle quality in patients with acromegaly comes out. Indeed, in all reported analyses, patients with acromegaly (either AD or CD) had equal or higher muscle fatty atrophy compared to healthy controls (Figure 3B).

A fair degree of consistency was found in the muscle performance assessment, as in all but one analysis the AD group performed worse than both the CD and the CTR groups (Figure 3C). In line with this finding, CD patients performed equally (six analyses) or worse (three analyses) than healthy controls.

Therefore, while data on muscle mass are still conflicting, the evaluated studies are consistent in reporting higher fatty atrophy and lower muscle performance in patients with acromegaly compared to controls, with a further detrimental impact of active disease. Patients with acromegaly presenting high fatty atrophy and reduced muscle performance may show impaired mobility, walking ability, and higher fall risk [21]. Together with the well-known bone impairment and skeletal frailty reported in patients with acromegaly [30,31], these factors may significantly impact patients' quality of life and contribute to the increased fracture risk observed in this peculiar population [2]. Overall, musculoskeletal complications represent one of the most debilitating conditions associated to acromegaly [30].

However, it is crucial to examine the potential confounding factors of the included studies to correctly interpret the reported findings. Firstly, the impairment of skeletal muscle function and the osteoarticular pain often observed in patients with acromegaly may prompt a more sedentary lifestyle compared to age- and sex-matched populations. Nevertheless, physical activity has been included among the matching factors for healthy controls only in one study [21]. Additionally, comorbidities such as diabetes mellitus or impairment of other pituitary axes, such as the gonadal axis or, to a lesser extent, the corticotroph or thyrotroph axis, could affect skeletal muscle metabolism [32–35]. Unfortunately, detailed information on the whole hormonal status of evaluated patients is missing in most of the studies we have analyzed in the present manuscript. The potential impact of distinct imaging modalities and heterogenous treatments (i.e. medical therapy and surgical approach) on the evaluated clinical outcomes has been described in the following sections.

Radiological factors

Recent discoveries on the clinical implications of sarcopenia [8] led several groups to focus on skeletal muscle evaluation. In 2019, the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) indicated MRI and CT as the gold standard techniques to evaluate skeletal muscle mass and quality, due to their precision and reliability. However, the high cost of MRI and the use of ionizing radiation for CT make the use of both techniques not always suitable on a large scale in clinical practice. At the same time, the Group defined various pitfalls of DXA (lack of consistency among different DXA instrument brands, the influence of subject hydratation status) and BIA (lack of consistency among different age and ethnic groups, the influence of subject hydratation status) [36]. Differently from MRI, CT, and US, neither DXA nor BIA can directly assess skeletal muscle mass.

DXA can estimate the body lean mass, which includes organs and soft tissues in addition to muscle tissue [25]. Kim and colleagues [37] proposed an equation that estimates skeletal muscle mass in healthy subjects from DXA-estimated appendicular lean tissue, which strongly correlates with MRI-assessed skeletal muscle mass. Because of the well-established fluid-retentive impact of high IGF-1 and GH, patients with acromegaly represent a special population, with a condition similar to an over-hydratation status. Consistent with this consideration, patients with acromegaly show an increase in lean mass [25], which may lead to an overestimation of skeletal muscle mass by DXA. However, the equation proposed by Kim and colleagues [37] has been later validated also in a group of acromegalic patients with active disease [25].

As mentioned before, even BIA devices cannot directly measure the skeletal muscle mass but rather estimate it through an equation that defines a positive correlation between the measured resistance expressed in Ohms and the estimated skeletal muscle mass. While this equation has been shown to strongly correlate with skeletal muscle mass measured by MRI in healthy adults [38], there is currently no data validating its use in patients with acromegaly. Considering the high water conductivity, the fluid retention present in acromegalic patients, and the direct proportionality between the BIA-estimated muscle mass and the measured resistance, it can be inferred that resistance is under-measured, leading to an underestimation in skeletal muscle mass in these patients. However, Lopes et al. [23] reported a strong correlation in muscle mass estimation among acromegalic patients by comparing DXA with BIA in the AD versus CD groups.

As previously mentioned, among the studies we have identified in our search, a total of four different techniques (MRI, BIA, DXA, US) have been employed to assess or estimate the skeletal muscle mass in patients with acromegaly; while MRI and HMRS have been used for the evaluation of skeletal muscle quality. This heterogeneity in the applied techniques reflects the lack of a standardized method proposed to study muscle mass in subjects with acromegaly in a clinical setting.

Finally, there is also no agreement on how to evaluate muscular performance among patients with acromegaly. Indeed, five different physical tests and one sonographic technique have been performed in the studies we have reported. Again, there is a lack of consensus on how to estimate muscle performance in different populations with various health statuses.

Therapeutical approaches

Nowadays, several medical therapies for acromegaly are available in clinical practice. First-generation somatostatin receptor ligands (fg-SRLs, octreotide and lanreotide) and the second-generation SRL, pasireotide, mainly act at the pituitary level reducing GH secretion and result in the lowering of circulating IGF-1 level [1]. On the other hand, pegvisomant is a recombinant GH analog that acts on blocking GH signaling in the periphery - mainly at the liver -, resulting in a reduced IGF-1 production [1].

As concerns fg-SRLs, an early study showed that s.c. octreotide treatment leads to a reduction in lean body bass estimated by DXA; however, this finding has been attributed to the reduction in soft tissue fluid, more than a direct effect on skeletal muscle mass [39].

To our knowledge, there are no available clinical data on the effects of pasireotide on skeletal muscle; however, one *in vitro* study hypothesized a direct stimulation of this compound on protein synthesis in rat myoblast cells, although this data have not been confirmed so far by other studies [40].

As concern pegvisomant, Kuker and colleagues [26] evaluated the effect of this drug on body composition in 21 patients with acromegaly, reporting no significant changes in skeletal muscle mass and quality after long term treatment.

Furthermore, two studies investigated the impact of the surgical approach on the skeletal muscle in patients with acromegaly. Guo and colleagues [24] reported a decrease in skeletal muscle mass in acromegaly patients one year after surgery compared to the pre-surgical evaluation. Similarly, Reyes-Vidal and colleagues [27] found a decrease in skeletal muscle mass one year after surgery only in male patients and a decrease in fatty atrophy only in female patients at the same time point.

To our knowledge, currently there are no studies directly comparing the various medical therapies each other or versus the surgical approach. Therefore, further research is needed to investigate whether different therapeutic approaches may impact the skeletal muscle characteristics in a different way.

Conclusions

To the best of our knowledge, this is the first systematic review focusing on the skeletal muscle evaluation in patients with acromegaly. We identified a few studies, mostly with a cross-sectional design, that investigated this topic. The rarity of acromegaly, the high prevalence of confounding

factors, and the heterogeneity of the methods used to assess the skeletal muscle characteristics led to mixed results.

However, performing an analysis of the evaluated studies, we found a fair degree of consistency in reporting higher fatty atrophy and lower muscle performance in patients with acromegaly compared to healthy controls, with an additional detrimental effect observed in patients with active disease. On the other hand, data on skeletal muscle mass are still conflicting, particularly as concerns the impact of disease activity.

It is therefore necessary to identify which method could be the most reliable, reproducible, and available on a large scale to properly investigate skeletal muscle mass, quality, and performance in a clinical setting. Then, larger studies with a longitudinal design should be conducted to better define the impact that GH, IGF-1, and the above mentioned confounding factors may have on skeletal muscle in this special population.

Data availability Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Patents, Royalties, Other Intellectual Property: Springer, Elsevier. F.G. has received personal honoraria for lectures, manuscript writing, educational events and consultancy from Ipsen, Novartis, Pfizer and Recordati. F.G. serves on the Executive Committee of the European Neuroendocrine Association (ENEA). The other authors have nothing to disclose.

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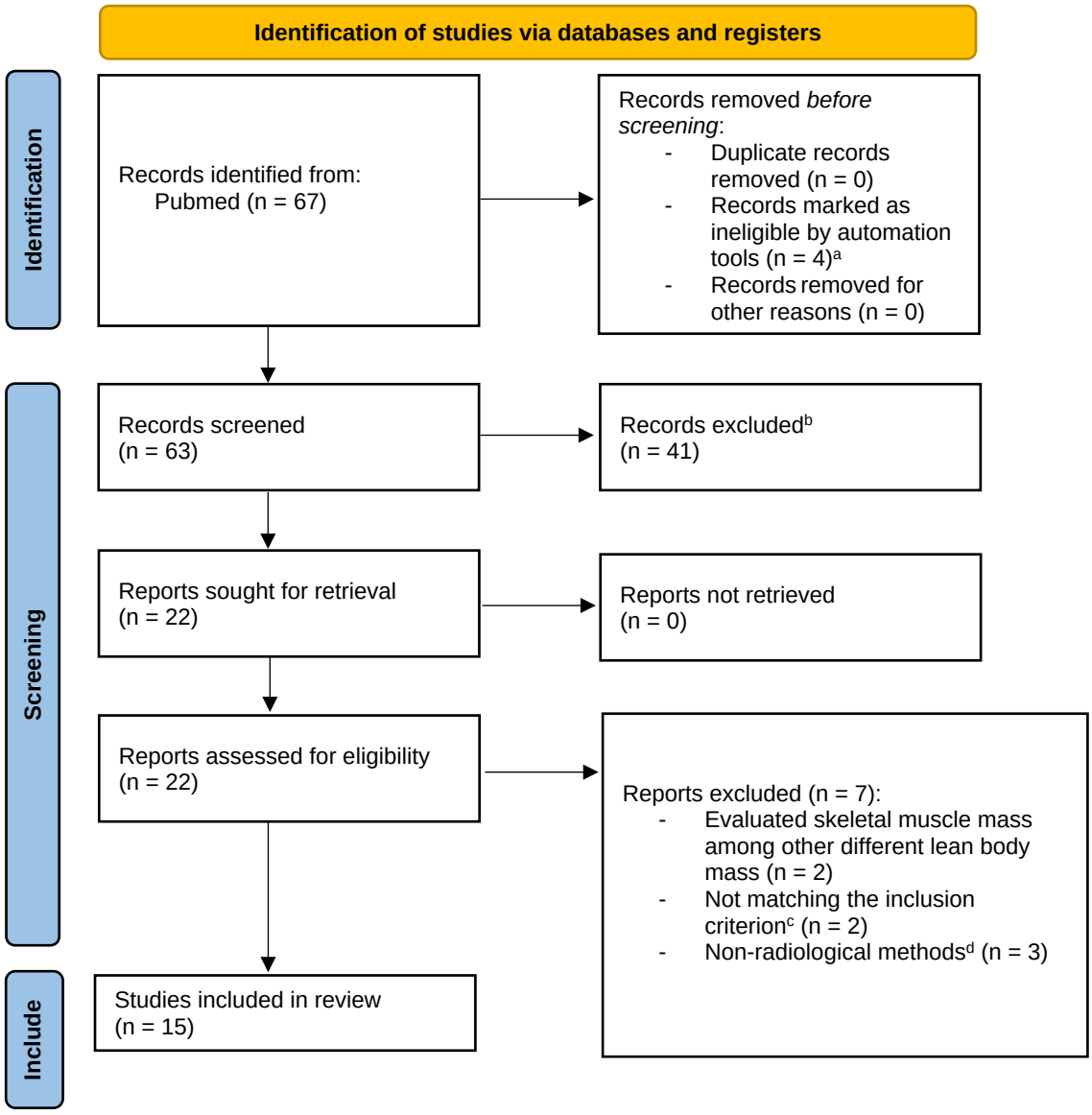

Figure 1 | PRISMA flow-chart.

[a] Not English written.

[b] Excluded by title.

[c] Only studies comparing different disease statuses (i.e. active acromegaly vs controlled acromegaly) or a specific disease status vs control group (i.e. active acromegaly vs control group or controlled acromegaly vs control group) have been included.

[d] Bioelectrical impedance analysis has been included.

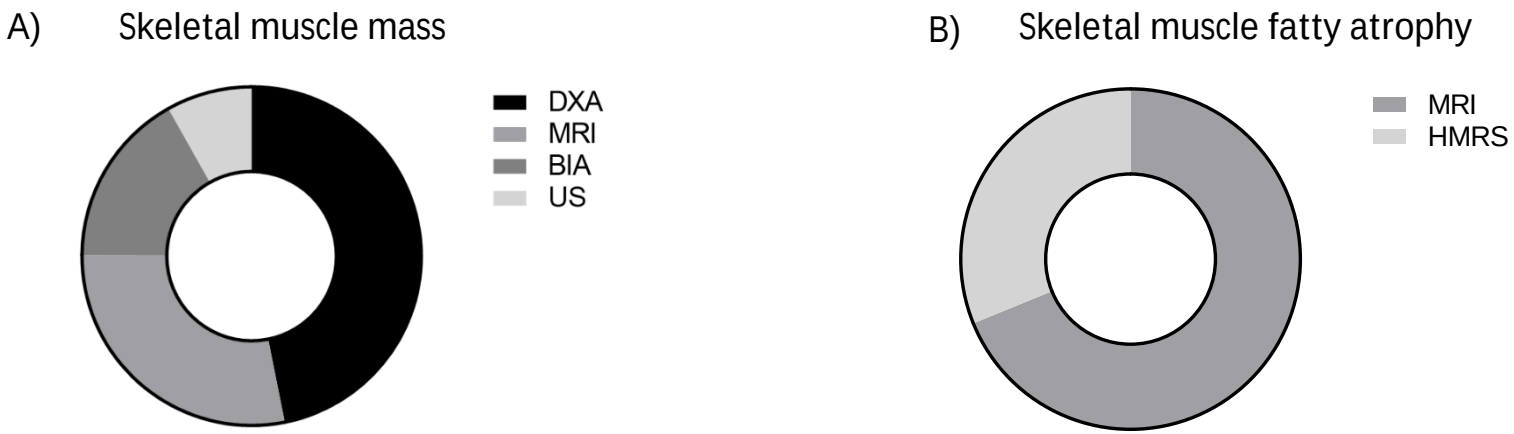

Figure 2 | Techniques employed to assess skeletal muscle mass (A), and fatty atrophy (B).

A technique was counted as one each time it was performed on a patient with acromegaly. If the same technique has been used on the same patient with acromegaly at a different time and with a different disease status, it has been counted as two.

Legend. DXA: dual-energy X-ray absorptiometry; MRI: magnetic resonance imaging; BIA: bioelectrical impedance analysis; US: ultrasound. HMRS: proton magnetic resonance spectroscopy.

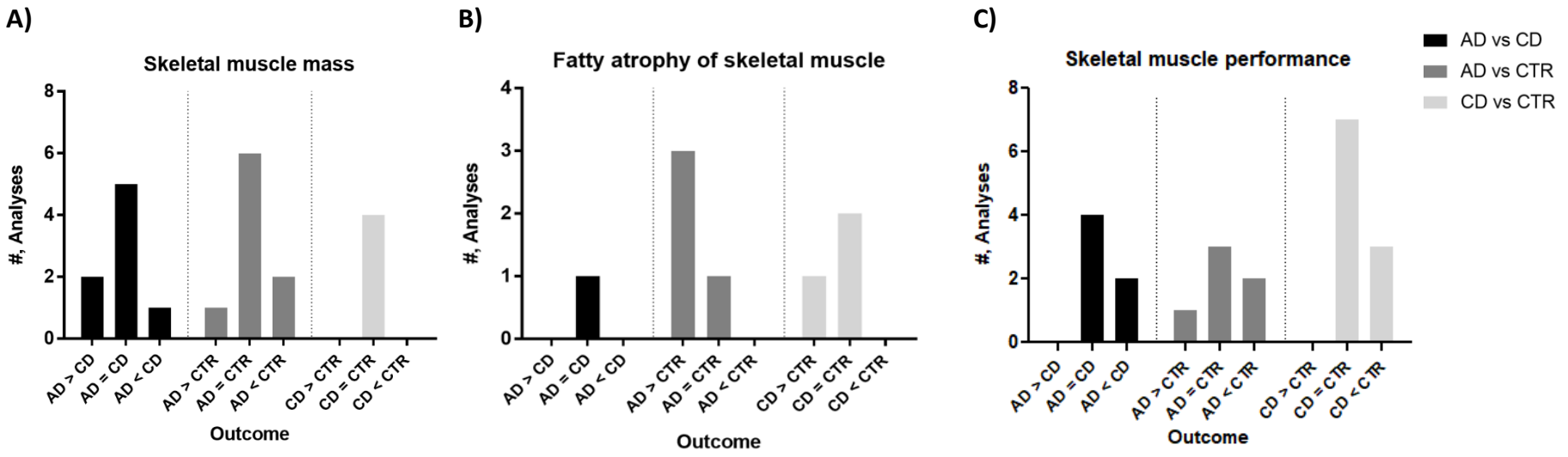

Figure 3. Analyses performed on skeletal muscle mass (A), fatty atrophy (B), and performance (C). Each thick line on the y-axis represents one analysis defined as a comparison of two different groups with a given technique. Of note, some studies performed more than one analysis because they compared two groups with more than one technique or compared more than two groups with each other.

Legend. AD: active disease group; CD: controlled disease group; CTR: control group.

Table 1 | Skeletal muscle mass evaluation.

				Acromegaly patients				
Study	Design	Technique	Body district	Total	Active Disease (AD)	Controlled disease (CD)	Control group (CTR)	Outcome
Eroğlu I, 2023	Cross-sectional	DXA	Appendicular	33	16	17	19	AD = CD AD = CTR CD = CTR
		MRI	Abdominal	33	16	17	19	AD = CD AD = CTR CD = CTR
Lopes AA, 2022	Cross-sectional	DXA	Appendicular	28	13	15	-	AD = CD
		BIA	Total body	28	13	15	-	AD = CD
Kuker AP, 2021	Cross-sectional/ Prospective[a]	MRI	Total body	16	16	-	n.a.[b]	AD < CTR
Ozturk Gokce B, 2020	Cross-sectional	US	Thigh and calf[c]	39	22	17	39	AD < CTR AD = CD CD = CTR
Guo X, 2018	Cross-sectional/ Prospective	BIA	Total body	36	36	-	37[d]	AD > CTR
				-	16[e]	16[e]	-	AD < CD
Bredella MA, 2017	Cross-sectional/ Prospective	MRI	Thigh	20	20[e]	16[e]	20	AD > CD AD = CTR CD = CTR
Reyes-Vidal CM, 2015	Cross-sectional/ Prospective[a]	MRI	Total body	23	23	-	n.a.[f]	AD = CTR
Reid TJ, 2015	Cross-sectional	DXA	Total body	138[g]	77	61	-	AD > CD
Freda PU, 2009	Cross-sectional	MRI	Total body	27	27	-	n.a.[h]	AD = CTR
		DXA	Total body	25	25	-	n.a.[h]	AD = CTR
				360	250	142		

Legend. DXA: dual-energy X-ray absorptiometry. BIA: bioelectrical impedance analysis. US: ultrasound. MRI: magnetic resonance imaging. AD: active disease. CD: controlled disease. CTR: control group. n.a.: not available.

[a] Prospective data were not included since AD and CD subjects were pooled together.

[b] A prediction equation was developed for skeletal muscle (SM) accounting for gender, age, height, weight, and race using generalized linear models from data obtained from 315 non-acromegalic patients.

[c] Vastus medial, vastus intermedius, vastus lateralis and gastrocnemius medial head have been evaluated.

[d] In this study, subjects affected by a non-functioning pituitary adenoma were considered as control group.

[e] The AD and CD groups included the same acromegaly subjects before and after the disease control.

[f] The observed acromegaly values were compared with predicted values calculated using a previously derived prediction equation described by generalized linear models that account for age, weight, race, height, and gender [25].

[g] Of the 138 patients included, 22 did not undergo DXA assessment. However, it is not possible to determine how many of these individuals were active and how many were in the control group.

[h] The control group was selected from a larger group of 315 non-acromegalic patients in a 3-4:1 ratio to acromegalic patients and matched for gender, weight, and age.

The total number of patients included is shown in the last row.

Table 2 | Fatty atrophy of skeletal muscle.

					Acromegaly patients				
Study	Design	Technique	Body district	Evaluation	Total	Active Disease (AD)	Controlled Disease (CD)	Control group (CTR)	Outcome
Kuker AP, 2021	Cross-sectional/ Prospective[a]	MRI	Total body	IMAT	16	16	-	n.a.[b]	AD > CTR
Martel-Duguech L, 2021	Cross-sectional	MRI	Thigh	Fat Fraction	36	-	36	36	CD > CTR
Bredella MA, 2017	Cross-sectional/ Prospective	HMRS	Soleus muscle	IMCL	20	20[c]	16[c]	20	AD = CD AD = CTR CD = CTR
Reyes-Vidal CM, 2015	Cross-sectional/ Prospective[a]	MRI	Total body	IMAT	23	23	-	n.a.[d]	AD > CTR
Szendroedi J, 2008	Cross-sectional	HMRS	Soleus and tibialis anterior muscles	IMCL	7	-	7	7	CD = CTR
Freda PU, 2008	Cross-sectional	MRI	Total body	IMAT	16	16	-	n.a.[b]	AD > CTR
					118	75	59		

Legend. MRI: magnetic resonance imaging. HMRS: proton magnetic resonance spectroscopy. IMAT: intermuscular adipose tissue. IMCL: intramyocellular lipids. AD: active disease. CD: controlled disease. CTR: control group. n.a.: not available

[a] Prospective data were not included since AD and CD subjects were pooled together.

[b] A prediction equation was developed for IMAT accounting for gender, age, height, weight, and race using generalized linear models from data obtained from 315 non-acromegalic patients.

[c] The AD and CD groups included the same acromegaly subjects before and after the disease control.

[d] The observed acromegaly values were compared with predicted values calculated using a previously derived prediction equation described by generalized linear models that account for age, weight, race, height, and gender [41].

The total number of unique patients included is shown in the last row.

Table 3 | Skeletal muscle performance.

			Acromegaly patients				
Study	Design	Test	Total	Active Disease (AD)	Controlled Disease (CD)	Control group (CTR)	Outcome
Eroğlu I, 2023	Cross-sectional	Hand grip	33	16	17	19	AD = CD
							AD = CTR
							CD = CTR
		Thigh extension	33	16	17	19	AD = CD
							AD = CTR
							CD = CTR
		Gait speed	33	16	17	19	AD = CD
							AD = CTR
							CD = CTR
Martel-Duguech L, 2021	Cross-sectional	Gait speed	36	36	-	36	CD < CTR
		30s chair stand	36	36	-	36	CD < CTR
		Timed up and go	36	36	-	36	CD < CTR
		Hand grip	36	36	-	36	CD = CTR
Ozturk Gokce B, 2020	Cross-sectional	Pennation angle[a]	39	22	17	39	AD = CD
							AD < CTR
							CD = CTR
Füchtbauer L, 2017	Cross-sectional/ Prospective	Thigh flex and extension	48	48	-	n.a.[b]	AD > CTR
			-	-	23	n.a.[b]	CD = CTR
		Hand grip	48	48	-	n.a.[b]	AD < CTR
			-	23[c]	23[c]	-	AD < CD
			-	-	23	n.a.[b]	CD = CTR
Lopes AJ, 2016	Cross-sectional	Thigh extension	53	23	30	-	AD < CD
			209	145	87		

Legend. [a] Vastus medial, vastus intermedius, vastus lateralis and gastrocnemius medial head have been evaluated by ultrasonography.
[b] The control group was selected from a larger group of 144 non-acromegalic patients in a 6-27:1 ratio to acromegalic patients, matched for age and gender.

[c] The AD and CD groups included the same acromegaly subjects before and after the disease control.
The total number of unique patients included is shown in the last row.
A detailed explanation of the different methods used to assess muscle performance is reported in the text of the related reference.

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Temporal and masseter muscle evaluation by MRI provides information on muscle mass and quality in patients with acromegaly

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Introduction

Acromegaly is a rare and severe endocrine disorder due to the prolonged exposure to high levels of GH and IGF-1, caused in more than 95% of cases by the presence of a GH-secreting pituitary adenoma. Patients with acromegaly are burdened by several comorbidities and, if untreated, show an increased mortality risk compared to the general population [1]. As concerns skeletal muscle, a shift of amino-acid metabolism toward protein synthesis has been demonstrated in these patients [2]. Differently from the general population, active acromegaly constitutes a unique and paradoxical combination of insulin resistance and a lean body composition [3]. Overall, skeletal muscle of active acromegaly patients is characterized by decreased protein breakdown and increased protein synthesis.

However, despite an increase in lean mass and total body water, acromegaly is characterized by the presence of myopathy with weakness and pain and reduced muscular endurance [4, 5]. The increase in skeletal muscle mass does not necessarily translate into an increased strength and function, probably due to direct changes on muscle fibers [6, 7]. In addition, an increase in intramuscular fat content, due to increased lipolysis in extra-muscular deposits and increased muscle uptake of lipids during the active phase of the disease, has been shown [8, 9]. Whole-body magnetic resonance imaging (WB-MRI), dual-energy X-ray absorptiometry (DXA), computed tomography (CT) and bioelectrical impedance analysis (BIA) techniques have been used to carry out a qualitative/quantitative assessment of muscle mass in patients with acromegaly, with varying results [10-13].

Recent studies have shown that the morphometric analysis of the temporalis muscle, mainly performed measuring temporal muscle thickness (TMT) using both CT and MRI techniques, strongly correlates with the measure of psoas muscle, as well as with the patient functional status/prognosis, thus representing a reliable measure of skeletal muscle mass in various clinical contexts (e.g. melanoma brain metastases, glioblastoma) [14-17].

The primary aim of our study was to investigate whether the measure of two craniofacial muscles, namely temporal and masseter muscle thickness, was consistent among the evaluated subjects, and whether it correlated with demographic characteristics known to be associated with skeletal muscle mass, such as gender, age, weight and height, in patients with acromegaly. We decided to focus on temporal and masseter muscles since they are easily visible at the brain/sella turcica MRIs which are routinely performed by patients with acromegaly, thus not requiring any additional investigation or costs. Given the peculiar study population, we also investigated the potential correlations between disease activity (GH and IGF-1 levels), time from diagnosis, and muscle thickness.

Patients and Methods

Study design and patients

Single center retrospective longitudinal study carried out at the Endocrinology Unit of the IRCCS Ospedale Policlinico San Martino in Genoa (Italy), a referral center for the management of pituitary diseases.

Sixty-nine patients with an established diagnosis of acromegaly were included in the study.

Diagnosis of acromegaly was made based on clinical features, biochemical evidence of GH hypersecretion (lack of suppression of GH to <1 μg/L after a 2-hour oral glucose tolerance test), IGF-1 levels above the age-adjusted upper limit of normality (ULN) range (>1 xULN), and the presence of a pituitary adenoma on MRI [18]. GH and IGF-1 assays used in this study have been recently described in detail [18].

Inclusion criteria were: i) availability of at least of one MRI of the brain/sella turcica, performed to investigate the sellar region, in which the measurement of temporal and/or masseter muscle thickness was feasible; ii) presence of data about demographics and general characteristics of the patients, information about time from diagnosis, as well as treatment path at the time of the first available MRI. No additional exclusion criteria were applied, based on study design and set endpoints.

A total of 182 brain/sella turcica MRIs were collected. Indeed, 48 out of 69 patients had more than one MRI available for evaluation. In detail, 1 MRI: 69 patients, 2 MRIs: 48/69, 3 MRIs: 32/69, 4 MRIs: 16/69, 5 MRIs: 9/69, 6 MRIs: 6/69, 7 MRIs: 2/69.

For each available MRI, data collected on temporal and masseter muscle thickness were correlated with biochemical values (GH levels, absolute IGF-1 values, and sex- and age-adjusted IGF-1 values), as well as with the presence of hypogonadism. This latter was defined as the presence of low total testosterone levels and associated symptoms in men, or low levels of estradiol accompanied by the absence of menstrual cycles in women. Women with menopause were included in the hypogonadal group [19]. A maximum interval of one month between MRI and hormone evaluation (before/after) was considered acceptable for the study purpose.

Additional parameters were collected for each patient at the time of first available MRI (e.g. anthropometric data, time since diagnosis, treatment modalities, presence of hyperprolactinemia or other pituitary hormone deficits than hypogonadism, glycated hemoglobin levels, diagnosis of diabetes mellitus, clinical history of cancers; see Table 1) and, therefore, additional analyses were carried out in this setting.

Due to the retrospective study design, not all information was available for all patients.

Definition of biochemical control

In line with previous studies and the suggestion of recent consensus statements, we considered as reaching biochemical control those subjects with age-adjusted IGF-1 values ≤1 the upper limit of normality (ULN), defined as controlled patients [20, 21].

Single fasting GH measurements were evaluated in patients not treated with pegvisomant (PEGV). Data obtained were used to investigate the possible correlations observed between GH values, clinical variables and muscle thickness, but did not contribute to the definition of biochemical control (based solely on age-adjusted IGF-1 values, as above stated).

Analyses of temporal (TMT) and masseter muscle thickness (MMT) and quality

TMT and MMT were calculated on patient MRI available on the Hospital Picture Archiving and Communications System (PACS). Muscular thickness was calculated with electronic calipers on axial and coronal planes of isovoxel (1 × 1 × 1 mm) T1-weighted MR images as follows: i) at the level of the orbital roof, perpendicular to the long axis at the point of maximal depth for the temporal muscle (as already done in literature [18]), and ii) at the middle of mandibular ramus, perpendicular

to the muscle belly for the masseter muscle (Figure 1). In all patients, TMT and MMT on both the left and the right side were determined separately, and the mean value considered for analysis. Muscles were evaluated for fatty infiltration and eventually atrophy using a modified Goutallier classification as semi-quantitative score: grade 0, normal appearance and early moth-eaten appearance, with scattered small areas of fat comprising less than 30% of the volume of the muscles; grade 1, late moth-eaten appearance, with numerous discrete areas of fat with beginning confluence, comprising 30–60% of the volume of the muscles; and washed-out appearance, fuzzy appearance due to confluent areas of fat with muscle still present at the periphery. All measurements on MRI were done by one musculoskeletal radiologists with more than 10 years of experience in MR imaging (A.S.T.) in consensus with a second musculoskeletal radiologists with 7 years of experience in MR imaging, blinded to the results of the first radiologist and to clinical features (B.B.).

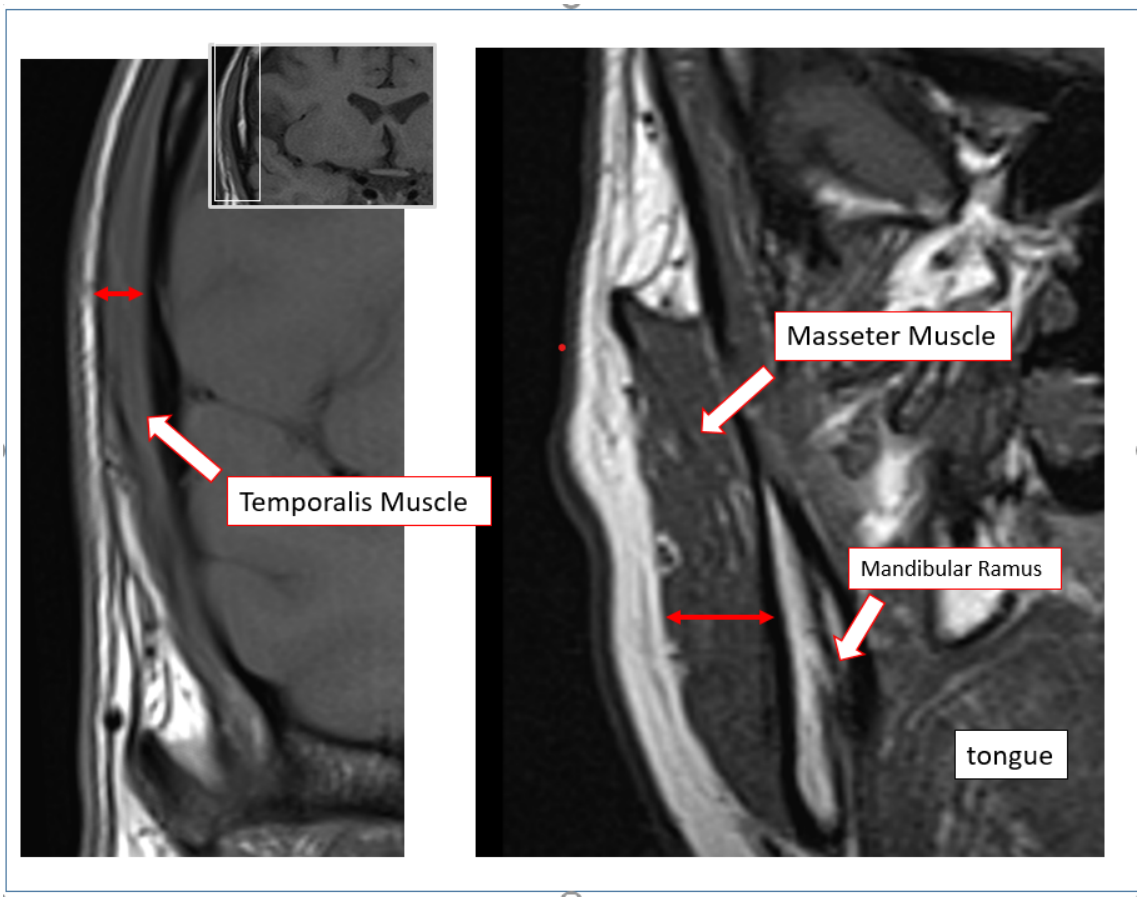

Figure 1. Examples of temporalis muscle and masseter muscle thickness measurements in two different patients on coronal T1 weighted sequences (double headed arrow).

Statistical analysis

Statistical analysis was conducted using both the SPSS 27.0 software (SPSS, Chicago, IL, USA) and the GraphPad Prism software v. 7.0 (GraphPad Software, San Diego, CA, USA) for Windows. Continuous variables are expressed as median and interquartile range (IQR), whereas dichotomous variables are reported as frequency and percentage. Comparisons between continuous variables were performed using nonparametric tests (Mann-Whitney U test and Kruskal-Wallis test). Correlation coefficients were calculated using the Spearman rank order R. Chi square and K tests were used for the analyses between two dichotomous variables. The potential predictors of temporal and masseter muscle thickness were identified performing summary statistics and the above described tests. Afterwards, the selected variables were included in the different prediction models. Based on the dependent variables (categorical or continuous), univariable and multivariable logistic regression and linear regression analyses were performed, respectively. Significance was accepted for a p-value <0.05.

Results

Patient characteristics at the time of first available MRI

Sixty-nine patients were included in the study, 44 females (64%) and 25 males (36%), with a median age of 54 years (IQR 45-64) at the time of first available (and evaluated) MRI. In 19 patients, the first available MRI was performed at the time of diagnosis, while in the remain 50 patients it referred to a follow-up investigation (median time from diagnosis to first analyzed MRI: 21.5 months).

Median BMI was 27.3 Kg/m^2 (IQR 23.9-30.6), with a median weight of 80 Kg (IQR 68.3-90), and a median height of 1.70 m (IQR 1.63-1.77).

Thirty-one patients (44.9%) were considered has having biochemical control (IGF-1 ≤1 xULN), and median age-adjusted IGF-1 value was 1.1 xULN (IQR 0.8-1.9). Median GH level was 2.9 µg/L (IQR 1.1-9.0); as expected, GH values showed a strong and direct correlation with both absolute IGF-1 (rho: 0.722, p<0.001) and age-adjusted IGF-1 values (rho: 0.681, p<0.001). Noteworthy, total testosterone levels (available in 20 male patients) were inversely correlated with both absolute and age-adjusted IGF-1 values (rho: -0.794 and rho: -0.787, respectively; p<0.001). Accordingly, patients who achieved biochemical control had significantly higher total testosterone levels (median 330 ng/dl, IQR 251-456) compared to active disease subjects (169 ng/dl, 101-255; p=0.002).

Fifteen patients had hyperprolactinemia (21.7%), which was mild in most cases, thus requiring treatment with dopamine-agonists (cabergoline, CAB) only in 5 individuals. Median PRL value in

patients with hyperprolactinemia (including individuals treated with CAB) was 31 µg/L (IQR 7.0-49.3) vs 8.4 µg/L (IQR 5.8-14.2) in the remaining patients; p=0.004. Seven patients (7.2%) had hypocortisolism and 10 patients (14.5%) had hypothyroidism; they were adequately treated with proper hormone replacement therapy (glucocorticoids and levothyroxine, respectively) in all cases. No patient had arginine vasopressin deficiency.

The presence of diabetes mellitus was reported in 14 out of 69 patients (20.3%), with 10/14 individuals undergoing antidiabetic treatment (i.e. n=5, metformin monotherapy; n=1, metformin plus sulfonylurea; n=1, metformin plus SGLT-2 inhibitor; n=1, metformin plus DPP-4 inhibitor; n=1, metformin plus SGLT2 inhibitor (daily) plus GLP-1 receptor agonist (weekly); n=1, insulin).

Thirteen patients had a clinical history of cancer (13/69, 18.8%; n=3, prostate cancer; n=2, papillary thyroid carcinoma; n=1, parotid gland adenocarcinoma; n=1, rectum adenocarcinoma; n=1, renal cancer and nasopharynx cancer; n=1, basocellular skin carcinoma; n=1, melanoma; n=1, lung cancer; n=1, endometrial cancer; n=1, gastric cancer). However, 9 out of 13 patients had a complete removal of the primary (localized) lesion and were considered in remission, while 4 patients had a persistent disease at the time of the evaluation.

Thirty-three patients (47.8%) already had neurosurgery at the time of first available MRI, while 35 subjects (50.7%) were undergoing medical therapy at that time (the majority being treated with first-generation somatostatin receptor ligands; i.e. fg-SRL therapy n=24, 70.6%).

Patients' characteristics are summarized in Table 1.

Temporal and masseter muscle thickness at first available MRI

Median temporal muscle thickness (TMT) was 6.1 mm (IQR 5-7), being significantly higher in male (6.9 mm, IQR 5.7-7.5) compared to female patients (6.0 mm, IQR 4.5-6.5; p=0.001). Similarly, median masseter muscle thickness (MMT) was 15.1 mm (IQR 12.9-17.7), with significantly higher values in males (17.6 mm, IQR 13.7-20.5) than in females (14.9 mm, IQR 12.5-16.5; p=0.016) (Figure 2 A-B).

Overall, TMT showed a strong and significant direct correlation with MMT (rho: 0.497, p<0.001; n=60). This correlation was maintained when analyzing the subgroup of female patients (rho: 0.529, p<0.001; n=40), while statistical significance was lost analyzing the smaller subgroup of male patients (rho: 0.278, p=0.234; n=20). At univariable regression analysis, TMT and MMT were strongly and directly associated (adjusted R^2 0.245, B 0.935, β 0.508; p<0.001).

Age of patients at time of first available MRI did not correlate with either TMT (rho: 0.066, p=0.595) or MMT (rho: -0.046, p=0.720).

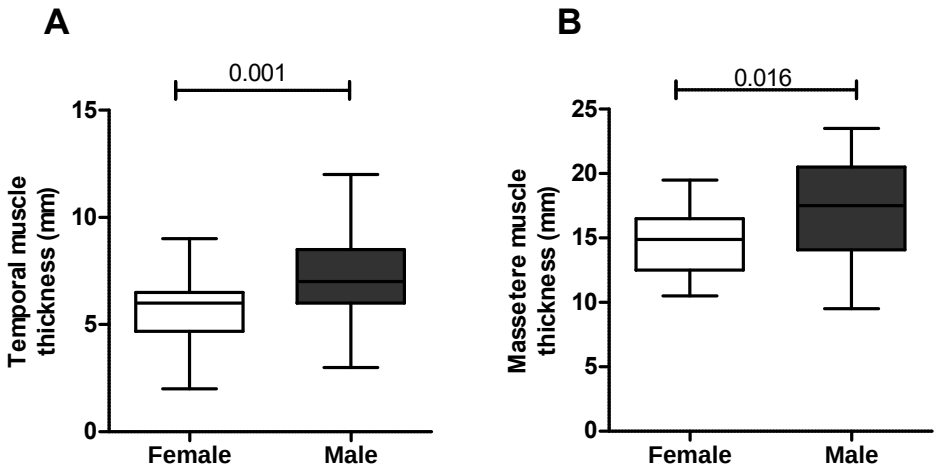

Figure 2. TMT (A) and MMT (B) in female and male patients

Although patients with cancers can present with reduced muscle mass (e.g. sarcopenia), TMT and MMT were not significantly different in patients with a clinical history of cancer compared to those individuals with no cancer reported in clinical charts (p=0.491 and p=0.767; respectively). Furthermore, the same findings were observed when comparing patients with active cancers, individuals with cancers in remission, and those with a negative clinical history for malignancies (TMT: p=0.565; MMT: p=0.752).

TMT and patients' characteristics at first MRI

At first available MRI, TMT showed a trend for a direct correlation, although without reaching statistical significance, with both patients' weight (rho: 0.228, p=0.064) and height (rho: 0.243, p=0.058). No correlation was found between TMT and BMI (rho: 0.086, p=0.509).

Of note, TMT showed a significant direct correlation with age-adjusted IGF-1 values (rho: 0.249, p=0.047), while patients with active disease at time of first available MRI had numerically higher TMT values compared to controlled subjects (median 6.15 mm, IQR 4.95-7.4 vs 6.0 mm, IQR 4.5-6.63), although this latter difference was not statistically significant (p=0.088) (Figure 3 A). No significant correlation was found between TMT, absolute IGF-1 levels (rho: 0.227, p=0.070), and GH levels (rho: 0.216, p=0.124), as well as between TMT and total testosterone values (rho: -0.288, p=0.247). In the whole cohort, the presence of hypogonadism did not have a significant impact on TMT (hypogonadal patients 6.0 [4.7-7.0] mm, vs eugonadal patients 6.0 [4.9-6.7] mm; p=0.855).

TMT was higher in patients who had a first MRI closer to the time of diagnosis (i.e. TMT vs time from diagnosis to first MRI: rho -0.315, p=0.010).

The presence of hyperprolactinemia, treated hypocortisolism and hypothyroidism, or diabetes mellitus did not significantly affected TMT (all p values >0.05). Glycated hemoglobin levels showed not statistically significant correlation with TMT (rho: -0.020, p=0.875).

Identification of patients at risk of sarcopenia

Previous studies reported sex-specific TMT cut-offs to identify patients at risk of sarcopenia (males, TMT ≤ 6.3 mm; females, TMT ≤ 5.2 mm) [22]. Therefore, at the time of first MRI 19/44 (43%) of female patients and 8/23 (35%) of male patients could be considered at risk of sarcopenia (overall, 27/67 [40%] of patients with an available TMT measurement at MRI 1). No clear correlation emerged between this patient stratification and the different clinico-demographic variables evaluated (e.g. age, weight, height, BMI, time from diagnosis, GH and IGF-1 values, hypogonadism, other pituitary deficits, etc).

Considering all MRIs, in 95/180 (53%) radiological evaluations the patients were classified as at risk of sarcopenia. In this setting, age-adjusted IGF-1 values were significantly lower in the presence of risk of sarcopenia, compared to the condition of normal muscle status (p=0.016).

MMT and patients' characteristics at first MRI

MMT showed a direct and significant correlation with both patients' weight (rho: 0.307, p=0.015) and height (rho: 0.346, p=0.006), while no correlation was found with BMI (rho: 0.200, p=0.139).

Patients with active disease at time of first available MRI had significantly higher MMT values compared to controlled subjects (median 16.2 [14.4-19.0] mm vs 13.8 [12.4-17.4] mm; p=0.044; Figure 3 B), and MMT showed a significant direct correlation with both absolute IGF-1 (rho: 0.393, p=0.002) and age-adjusted IGF-1 values (rho: 0.413, p=0.001). Of note, sex distribution among controlled and active disease patients was almost superimposable (Chi square test, p=0.376). MMT was higher in patients who had a first MRI closer to the time of diagnosis (i.e. MMT vs time from diagnosis to first MRI: rho -0.368, p=0.004). Furthermore, MMT showed a significant direct correlation with GH values (rho: 0.432, p=0.002), as well as a trend for an inverse correlation with total testosterone levels (rho: -0.451, p=0.053). Overall, the presence of hypogonadism did not significantly affect MMT (hypogonadal patients 15.2 [12.8-17.5] mm vs eugonadal patients 16.5 [12.5-18.3] mm; p=0.766). Similarly, hyperprolactinemia, treated hypocortisolism and hypothyroidism, as well as diabetes mellitus did not have a significant impact on MMT (all p values

>0.05). Glycated hemoglobin levels showed not statistically significant correlation with MMT (rho: -0.175, p=0.189).

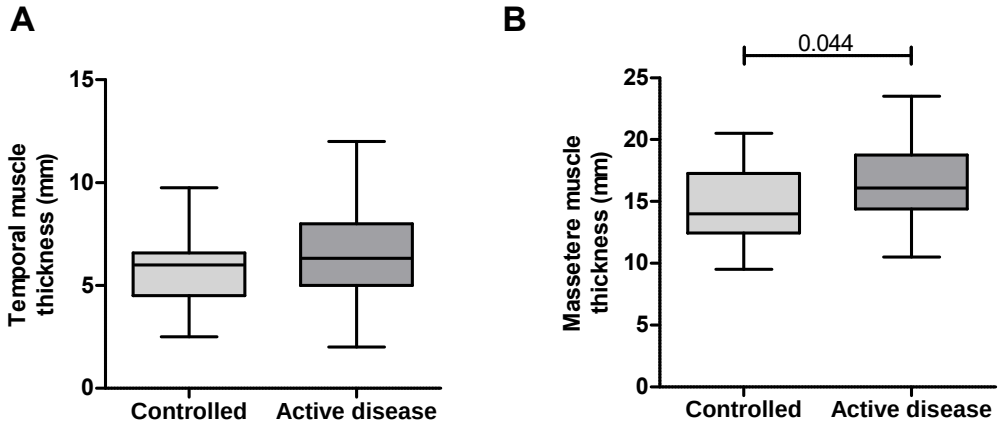

Figure 3. TMT (A) and MMT (B) in controlled subjects and in patients with active disease at time of first available MRI

Impact of surgical and medical treatments

At time of first available MRI, 23 patients were naïve to treatment (33%), 11 (16%) had undergone neurosurgery alone, 22 (32%) had previous neurosurgery and ongoing medical therapy, while 13 (19%) received first-line medical treatment alone. Performing a comparison among these four treatment groups, we found a statistically significant difference in both TMT and MMT values (p=0.031 and p=0.043, respectively). Particularly, treatment naïve patients had higher TMT and MMT values compared to patients which underwent neurosurgery alone (TMT: p=0.005; MMT: p=0.041), and to patients treated with neurosurgery plus medical therapy (TMT: p=0.052 [borderline significant]; MMT: p=0.008). Interestingly, at pairwise comparison, the difference in TMT and MMT values between treatment naïve patients and individuals treated with medical therapy alone was not statistically significant (TMT: p=0.191; MMT: p=0.128). However, age-adjusted IGF-1 values were significantly higher in treatment naïve patients compared to individuals treated with both neurosurgery alone (p<0.001), medical therapy alone (p=0.011), and neurosurgery plus medical therapy (p<0.001). Similar results were found for both absolute IGF-1 and GH levels (data not shown).

As concerns the use of different medical therapies (grouped as: fg-SRL monotherapy [n=24] vs other therapies [n=10]; see Table 1), we did not find statistically significant differences when evaluating TMT, MMT, absolute and age-adjusted IGF-1, as well as GH levels.

Analysis of all available MRIs

Considering all the MRIs analyzed (n=182), a proper measurement of TMT and MMT was possible in 180 and 158 images, respectively. In line with the observations obtained from the first available MRI, TMT and MMT confirmed a strong and direct significant correlation (rho: 0.526, p<0.001, n=156) in the longitudinal evaluation. This correlation was maintained when analyzing both the subgroup of female patients (rho: 0.516, p<0.001, n=102), and male subjects (rho: 0.345, p=0.011, n=54) (Figure 4). At univariable regression analysis, TMT and MMT were strongly and directly associated (adjusted R^2 0.287, B 1.018, β 0.540; p<0.001).

As expected, considering all MRIs median TMT and MMT values were higher in males compared to females (TMT: 6.5 [5.5-7.5] mm vs 5.0 [4.5-6.6] mm, p<0.001; MMT: 17.5 [14.5-20.5] mm vs 14.0 [12.5-16.0] mm, p<0.001). Age at the time of each MRI did not correlate with either TMT or MMT (rho: -0.008, p=0.915 and rho: -0.079, p=0.330; respectively).

TMT showed a significant direct correlation with absolute IGF-1 values (rho: 0.182, p=0.015), age-adjusted IGF-1 values (rho: 0.193, p=0.010), while no significant correlation was found with GH levels (rho: 0.104, p=0.226). In line with the findings reported for the first available MRI, also when considering all MRIs, no significant correlation was found between TMT and total testosterone values in males (rho: -0.061, p=0.650), and the presence of hypogonadism did not have a significant impact on TMT (hypogonadal patients 5.8 [4.5-6.7] mm vs eugonadal patients 5.5 [4.7-6.9] mm; p=0.599).

As concerns MMT, we observed a significant direct correlation with absolute IGF-1 values (rho: 0.273, p=0.001), age-adjusted IGF-1 values (rho: 0.269, p=0.001), as well as GH levels (rho: 0.213, p=0.019). In line with the findings reported for TMT, no significant correlation was found between MMT and total testosterone values in males (rho: -0.190, p=0.195), and the presence of hypogonadism did not have a significant impact on MMT (hypogonadal patients 14.8 [12.7-17.0] mm vs eugonadal patients 15.0 [12.7-17.9] mm; p=0.519).

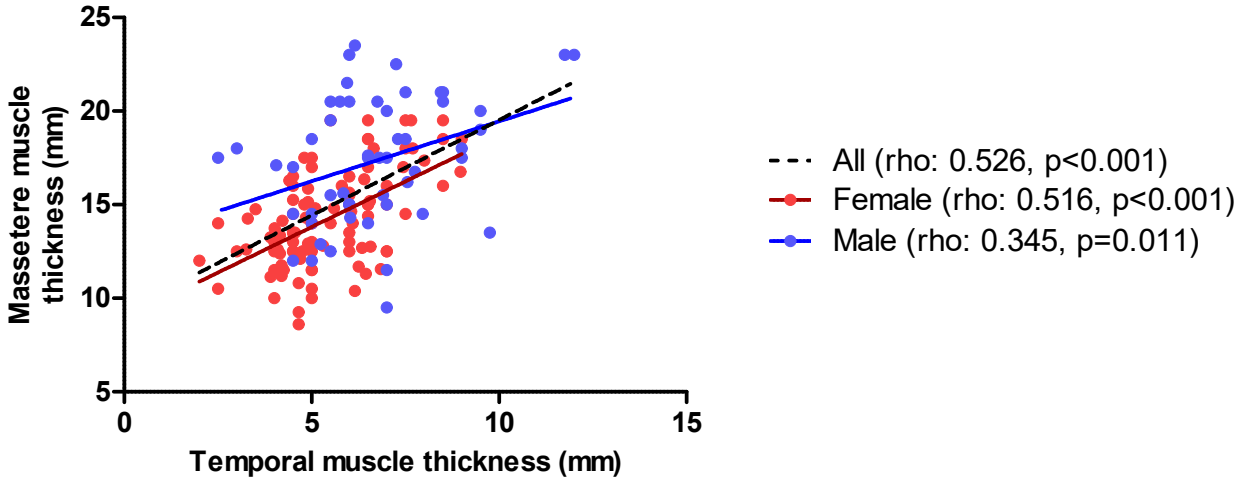

Figure 4. Correlation between TMT and MMT in the longitudinal evaluation

Longitudinal evaluation of TMT and MMT

As previously described (see Patients and Methods), we managed to evaluate more than one MRI over time in a subgroup of patients (e.g. two MRIs in 48 out of 69 patients, three MRIs in 32 out of 69, etc.).

In patients with at least two MRI evaluations, median time from first to last available MRI was 49 months (IQR 19.0-70.7). When considering this specific patient group, we observed a trend towards lower median TMT and MMT values from the first MRI to the following ones; however, these differences were not statistically significant (p=0.157 and p=0.472; respectively). Detailed measures are reported in Table 2.

Of note, considering the subgroup of patient with at least two MRI evaluations and active disease (IGF-1 >1 xULN) at the time of first MRI (MRI 1), we observed a statistically significant decrease of MMT values during the repeated MRIs (MMT baseline 16.9 [14.5-19.5] mm vs nadir 12.5 [11.5-14.8] mm, this latter reached at MRI 5; p=0.044, Table 2 and Figure 5 A). Of note, we found a consensual significant reduction of age-adjusted IGF-1 levels over time (baseline 2.3 [1.3-3.3] xULN vs nadir 0.76 xULN at MRI 6; p<0.001, Figure 5 A). As concerns TMT, a numerical decrease was observed over time, with a median baseline value of 6.5 [6.1-8.0] mm and a nadir of 3.0 [3.0-5.5] mm at MRI 6, although this difference did not reach statistical significance; p=0.111 (Table 2, Figure 5 B).

Interestingly, the paired evaluation of patients that had three consecutive MRIs available for MMT evaluation (n=13), showed a significant reduction in muscle thickness at MRI 3 (14.0

[12.5-15.9] mm) compared to MRI 1 (16.9 [14.5-19.5] mm; p=0.031), which was associated with a concordant IGF-1 decrease (Figure 5 C). The same analysis performed for TMT showed no significant reduction on muscle diameter (p=0.655; Figure 5 D).

As previously mentioned, a proper measurement of both TMT and MMT was not possible in all MRIs, according to the described Methods.

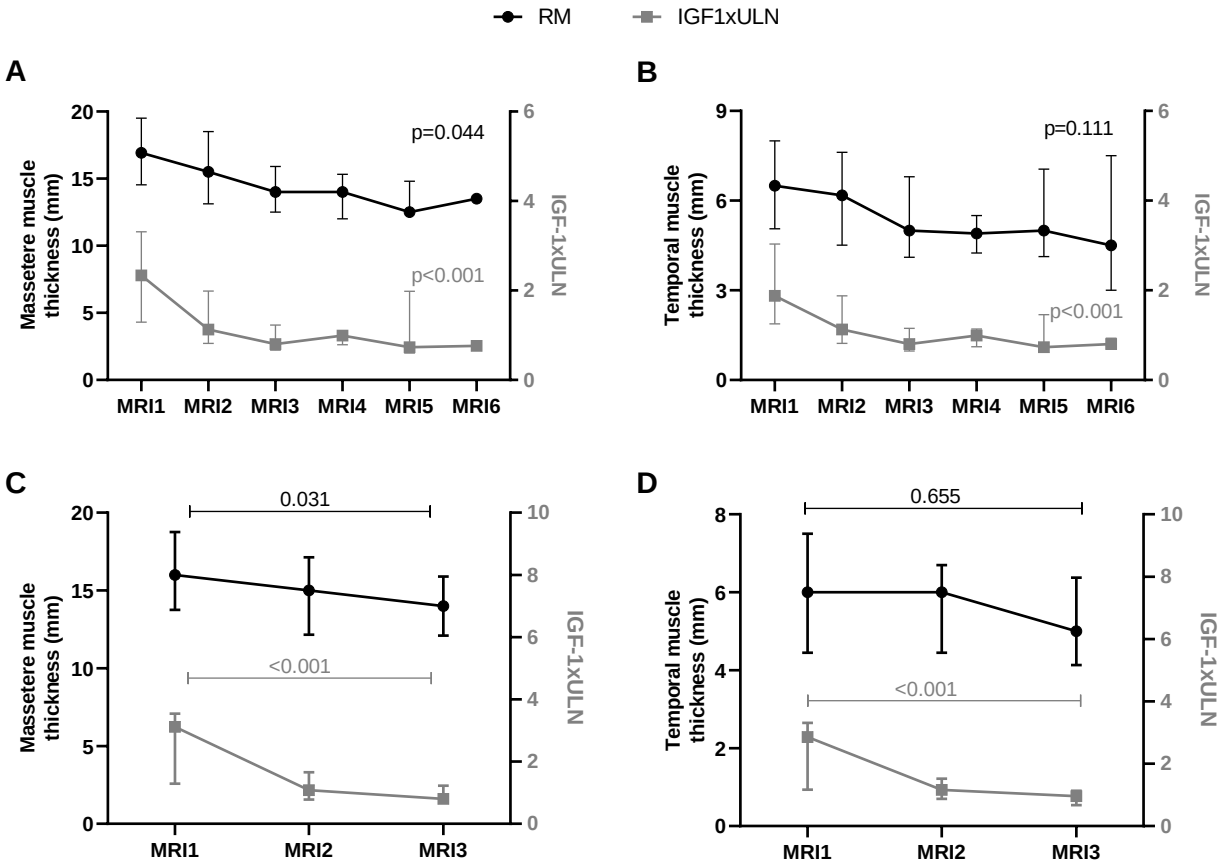

Figure 5. Longitudinal evaluation of MMT (A) and TMT (B) (black lines) and corresponding IGF-1 values (grey lines) on all available data considering all patients. Longitudinal, paired analysis of the patients in which biochemical and radiological data were available ot the time of MRI 1,2 and 3, and the corresponding IGF-1 in MMT (C) and TMT (D). Legend. IGF-1, insulin-like growth factor 1; MRI, magnetic resonance images

Determinants of TMT and MMT values

Based on the correlation analyses performed, gender and age-adjusted IGF-1 values emerged as the main determinants of TMT and MMT, among the different variables evaluated in our study. Univariable regression analysis to predict TMT found a negative association with female sex (β -0.349, p<0.001), while a positive association was observed with age-adjusted IGF-1 values (β 0.282,

p=0.018) (Table 3). A similar pattern was observed for MMT, with both female sex (β -0.451, p<0.001) and age-adjusted IGF-1 (β 0.376, p<0.001) identified as significant independent predictors. These two variables were still significant predictors of TMT and MMT at multivariable analysis, showing an overall adjusted R^2 of 0.189 for TMT (p<0.001), and 0.312 for MMT (p<0.001, Table 3).

Temporal and masseter muscle quality

The evaluation of fatty infiltration on both temporal and masseter muscle was performed using a modified Goutallier classification as described in the Material and Methods section.

Considering the first-available MRI, we observed that 43/66 patients (65%) with a temporal muscle evaluation at MRI 1 had a grade 0 (normal muscle appearance), while 23 patients (35%) had grade 1 (remarkable fatty infiltration). The same pattern was observed for masseter muscle, since 36/61 patients (59%) had grade 0, while 25 individuals (4%) has grade 1.

Patients showing lower fatty infiltration (grade 0) at the temporal muscle had significantly higher absolute and age-adjusted IGF-1 values compared to patients scored as grade 1 (p=0.043 and p=0.039, respectively). No statistically significant differences were observed when evaluating age, sex, height, weight, BMI, GH levels, hyperprolactinemia, hypogonadism, total testosterone values in males, hypocortisolism, as well as muscle thickness. A trend for a longer time from diagnosis to first MRI was observed in patients scored as 1 compared to those individuals scored as 0 (p=0.059). The ten patients with (treated) hypothyroidism had a numerically higher prevalence of fatty infiltration compared to other patients (60% vs 30%, p=0.070).

As concerns masseter muscle, patients with fatty infiltration (grade 1) had a longer time from diagnosis to first MRI compared to those subjects showing better muscle quality (grade 0) (p=0.032). Furthermore, patients with hypothyroidism had a significantly higher prevalence of grade 1 muscles compared to the other ones (78% vs 35%, p=0.025). As above reported for temporal muscle, none of the other evaluated variables significantly affected muscle quality.

We then considered all available MRIs. We observed that temporal muscle was considered as having a normal appearance (score 0) in 115/175 available MRIs (66%), while only 60 images (34%) were scored as 1 (fatty infiltration). Similarly, 96/153 available MRIs for masseter muscle were scored as 0 (63%, normal quality), and 57 images (37%) were scored as 1 (fatty infiltration). Of note, the measurement of muscle quality showed a high concordance between temporal and masseter muscle (K coefficient: 0.929; p<0.001).

When fatty infiltration was found at MRI images, patients were significantly older compared to individuals with normal muscle quality at radiological examination; this finding was observed for both temporal (57 years [52-64.5] vs 51 years [42-61.8], p=0.005) and masseter muscles (56 years [50.8-64] vs 51 years [41-58], p=0.009) (Figure 6 A-B). Other variables, such as sex, height, weight, BMI, IGF-1 values (absolute and age-adjusted), GH, total testosterone in males, and muscle thickness were not significantly different between MRI images classified as having normal quality (grade 0) or fatty infiltration (grade 1). At univariable logistic regression analysis, age was a determinant of fatty infiltration for both temporal (OR 1.037, 95% CI 1.009-1.065; p=0.008) and masseter muscle (OR 1.037, 95% CI 1.008-1.067; p=0.013). Of note, when age was stratified into tertiles, the prediction model for was stronger (temporal muscle: OR 1.665, 95% CI 1.112-2.493; p=0.013; masseter muscle: OR 1.793, 95% CI 1.158-2.777; p=0.009).

Finally, performing the longitudinal evaluation of the different MRIs (from MRI 1 to MRI 7), the prevalence of images scored as grade 0 and grade 1 did not change significantly during repeated measurements over time, for both temporal and masseter muscle (Chi square test: p=0.823 and p=0.712, respectively; Figure 6 C-D).

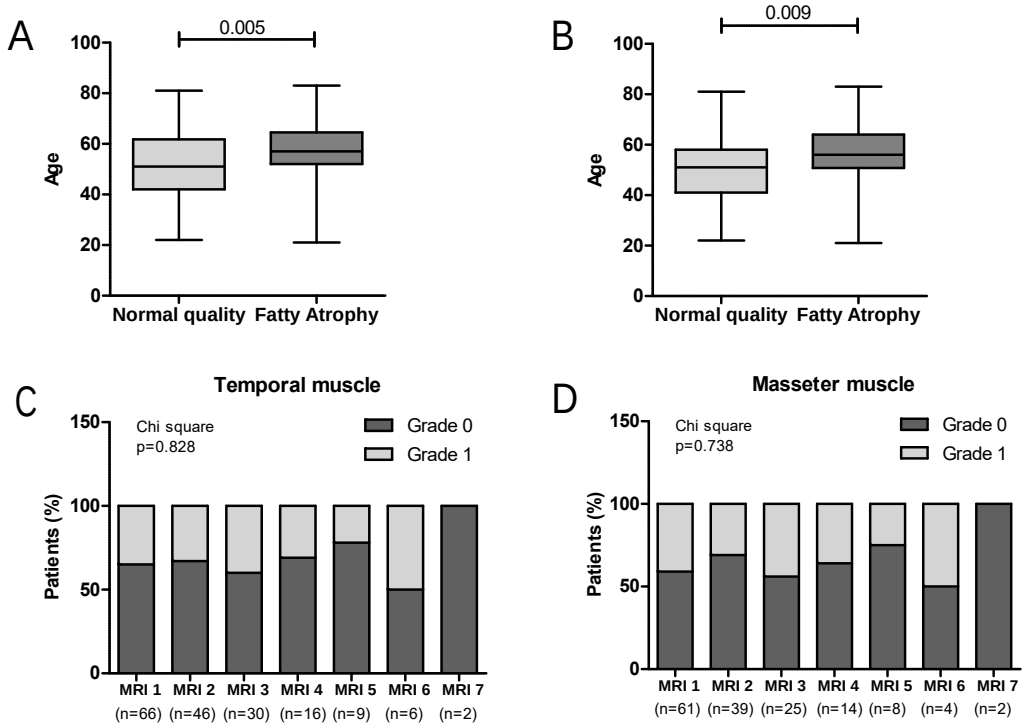

Figure 6. Impact of age on muscle quality in temporal (A) and masseter (B) muscle. Longitudinal evaluation of muscle quality in temporal (C) and masseter (D) muscle. Legend. MRI, magnetic resonance imaging

Discussion

To our knowledge, this is the first study investigating temporal and masseter muscle thickness, as well as quality, in patients with acromegaly, performing a correlation analysis with clinical and demographic characteristics.

Temporal muscle thickness (TMT) is nowadays considered a reliable measure of skeletal muscle mass, as well as a useful prognostic marker in patients with different diseases, including various cancers and neurological complaints [15, 16, 23]. This is mainly due to the strong correlation found between TMT and the skeletal muscles cross-sectional area (CSA) at the level of the third lumbar vertebra (L3), as well as the psoas muscle CSA, evaluated by use of both CT and MRI scans [14, 24]. Indeed, these measures and the derived skeletal muscle index (SMI, computed as CSA/patient height in m^2) represent the current gold-standard for the evaluation of sarcopenia [25].

Similarly to TMT, masseter muscle thickness (MMT) has been recently proposed as an additional independent measure to evaluate skeletal muscle mass [26, 27]. Trying to acquire information about patient skeletal muscle mass (and quality) throughout the evaluation of two craniofacial muscles in the context of a pituitary disease, such as acromegaly, provides some clear advantages compared to the investigation of abdominal muscles. All patients with acromegaly perform a brain or sella turcica MRI during their clinical history; first at diagnosis, to identify the presence of a pituitary adenoma, and then during follow-up to monitor the effects of the different treatment approaches performed [28]. Therefore, our study is based on a "opportunistic" analysis of available images, to retrieve additional information from radiological examinations originally performed for another scope, with no additional costs and burden for the patient as well as the healthcare system.

Temporal muscle has been chosen in early studies since it can be depicted in its full extent on almost all cranial imaging routinely performed (including brain and sella turcica MRIs), thus limiting the impact of circumstances which could influence muscle thickness (e.g. muscle edema or atrophy) [15, 16, 24]. Since oral and dental diseases could affect TMT and MMT, in line with previous studies the mean of TMT and MMT of both sides (left and right) was used [16]. Craniectomy and radiotherapy can also affect TMT and MMT; however, all the patients included in our cohort had transsphenoidal surgery (if operated), while only one patient underwent conventional radiotherapy. Therefore, these cannot be considered as confounders of the herein reported results.

In line with previous studies, we found that TMT and MMT are strongly and directly correlated each other also in patients with acromegaly [26]. Patient sex, height and weight are general characteristics that have been widely demonstrated to impact on TMT, MMT and, more in general,

on skeletal muscle mass [26]. Accordingly, in our study we found that male patients had significantly higher TMT and MMT values compared to females. At first MRI, patient height and weight were also directly and significantly correlated with MMT, while showing a consensual trend with TMT (although not statistically significant). The lack of correlation between TMT, MMT and BMI that we observed in our cohort has been previously reported in other populations, although not unanimously [14, 16, 26, 29]. This is mainly due to the fact that BMI does not provide a direct estimate of body composition, particularly in patients with acromegaly, in which changes on body composition occur during the different phases of the disease (i.e. decrease in % lean mass and increase in % fat mass after achieving biochemical control) [12-14, 16].

Temporal muscle is relatively small in its maximum diameter, requiring high accuracy in the measurement and strict adherence to predefined landmarks. Masseter muscle is thicker, and therefore differences between patients (and within the same individual during longitudinal evaluations) can be detected easier. This difference may, at least partially, explain our findings showing stronger correlations/associations between MMT and the different variables evaluated, compared to TMT.

Overall, we found that TMT and MMT values were higher in patients with active disease, with the strongest direct correlations observed between MMT and both GH and IGF-1 levels. Of note, when considering all available MRIs, we observed that age-adjusted IGF-1 values (xULN) were strong predictors of muscle thickness (TMT and MMT), independent of patients' sex at multivariable regression analysis. Indeed, sex was identified as the other strong independent determinant of both TMT and MMT values (males > females). These findings are in line with some previous reports, particularly with the largest study published so far evaluating the body composition in 138 patients with acromegaly, by use of DXA [12]. In their manuscript, Reid and colleagues found that both male sex and IGF-1 xULN were positively and significantly associated with the estimated skeletal muscle mass in their cohort, while nor age neither the presence of hypogonadism had a significant impact on it [12]. Accordingly, in our cohort we observed that TMT and MMT did not significantly correlate with patients' age and the presence of hypogonadism. Previous studies evaluating TMT and MMT in healthy subjects and other diseases than acromegaly mostly reported that elderly patients have thinner muscle measures [22, 26]; however, in the above mentioned studies the age range is usually wider than the one described in our cohort, including more patients >60 year-old, when muscle mass is known to decrease more consistently. As concerns hypogonadism, although gonadal steroids are important modulators of body composition, different authors did not find a significant

association between changes in this parameter and skeletal muscle mass in patients with acromegaly [12, 30]. This could be due to the strong impact of GH/IGF-1 axis on gonadal function, and the significant inverse correlation observed between IGF-1 and total testosterone levels in males, that we also found in our cohort.

The association between IGF-1 xULN and skeletal muscle mass is strengthened by the longitudinal evaluation of our patients, since the decrease in IGF-1 levels observed during follow-up was mirrored by a related decrease in MMT and, at less extent, TMT (irrespective of patient age at the time of the repeated MRIs). In this light, we hypothesized that craniofacial muscle thickness could provide us information about patient disease activity. However, performing ROC curve analyses after proper stratification for sex, we did not manage to find reliable cut-offs for both MMT and TMT to proper predict disease control in our cohort (data not shown). This is likely due to the relatively small sample size, dealing with a rare disease.

However, since sex-specific cut-off values are available for TMT to classify patients as “at risk of sarcopenia” or “with normal muscle status” [22], we found that about 40% of patients had low muscle mass at first MRI, while this percentage raised up to about 50% when considering all available images in the longitudinal evaluation.

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Competing interests Diego Ferone has been a speaker for and participated on advisory boards and received research grants from Novartis AAA, Ipsen, Camurus and Recordati. Diego Ferone is a member of the Editorial Board of Journal of Endocrinological Investigation. The other Authors have no conflicts of interest to declare.

Data	*Number (%)*
Patients	69
Sex	F, 44 (64)
Age (median, IQR)	54, 45-64
Body weight (Kg; median, IQR)	80, 68.3-90
Height (m; median, IQR)	1.70, 1.63-1.77
BMI (Kg/m²; median, IQR)	27.3, 23.9-30.6
Time from diagnosis to first MRI (months; median, IQR)	21.5, 1-100.8
Biochemical values	
GH (µg/L; median, IQR)	2.9, 1.1-9.0
IGF-1 (µg/L; median, IQR)	264, 171-453
IGF-1 (ULN; median, IQR)	1.1, 0.8-1.9
Disease control (IGF-1 ≤1 xULN)	31 (44.9)
Hyperprolactinemia *(yes, %)*	15 (21.7)
- CAB treatment	5/15
PRL (µg/L; median, IQR)	9.0, 5.8-17.3
Hypogonadism *(yes, %)*	41 (59.4)
- Males/females	14/27
- TR therapy	2/14
Hypocortisolism *(yes, %)*	7 (7.2)
- HR therapy	7/7
Hypothyroidism *(yes, %)*	10 (14.5)
- HR therapy	10/10
AVP deficiency *(yes, %)*	0 (0)
Glucose metabolism	
Diabetes mellitus (yes, %)	14 (20.3)
- AD therapy	10/14
HbA1c (%; median, IQR)	5.8, 5.5-6.4
Concomitant malignancies*	
Remission	9 (13.0)
Active disease	4 (5.8)
Overall (remission or active disease)	13 (18.8)
Treatment modalities	
Neurosurgery (n, %)	33 (47.8)
Radiotherapy (n, %)	1 (1.4)
Medical therapies (n, %):	35 (50.7)

- fg-SRLs	24 (70.6)
- PAS	2 (5.9)
- PEGV	1 (2.9)
- fg-SRL + PEGV	2 (5.9)
- fg-SRL + CAB	5 (14.7)
Skeletal muscle evaluation	
Temporal muscle thickness (mm; median, IQR)	6.1, 5-7
Masseter muscle thickness (mm; median, IQR)	15.1, 12.9-17.7

Table 1. Demographics, clinical and biochemical characteristics of the patients included in the study, together with skeletal muscle evaluation at the time of first MRI

Legend. CAB, cabergoline; TR, testosterone replacement; HR, hormone replacement; AVP, arginine vasopressin; AD, antidiabetic; HbA1c, glycated hemoglobin; fg-SRLs, first-generation somatostatin receptor ligands; PAS, pasireotide; PEGV, pegvisomant;

Muscle measurement	MRI evaluation							
All patients	MRI 1	MRI 2	MRI 3	MRI 4	MRI 5	MRI 6	MRI 7	p value
TMT median (IQR); mm	6.1 (4.9-7.5)	5.9 (4.6-7.5)	5.3 (4.2-7.0)	4.9 (4.4-5.6)	5.0 (4.4-5.8)	4.75 (3.7-6.0)	6.3 (6.0-6.5)	0.157
Pt. number[a]	n=47	n=46[b]	n=29	n=17	n=13	n=6	n=2	
MMT median (IQR); mm	16.0 (13.0-18.5)	15.0 (12.9-19.3)	14.0 (12.6-16.7)	14.2 (12.6-17.0)	13.4 (12.5-15.0)	14.0 (11.7-15.3)	16.3 (15.0-17.5)	0.472
Pt. number[a]	n=42	n=37[c]	n=24	n=15	n=7	n=4	n=2	
Active disease at MRI 1	MRI 1	MRI 2	MRI 3	MRI 4	MRI 5	MRI 6	MRI 7	p value
TMT median (IQR); mm	6.5 (5.1-8.0)	6.2 (3.5-4.5)	5.0 (2.5-4.1)	4.3 (4.1-4.9)	4.1 (3.5-5.0)	3.0 (3.0-4.5)	n.a	0.111
Pt. number[a]	n=28	n=28	n=16	n=11	n=5	n=3		
MMT median (IQR); mm	16.9 (14.5-19.5)	15.5 (13.1-18.5)	14.0 (12.5-15.9)	14.0 (12.0-15.3)	12.5 (11.5-14.8)	13.5	n.a.	0.044
Pt. number[a]	n=24	n=24	n=13	n=9	n=3	n=1		

Table 2. Longitudinal evaluation of TMT and MMT over time, in patients with at least two MRI evaluations. Legend. TMT, temporal muscle thickness; MMT, masseter muscle thickness; MRI, magnetic resonance imaging; Pt., patient; IQR, interquartile range; n.a., not available.

[a]As previously mentioned, a proper measurement of both TMT and MMT, according to the described Methods, was not possible in all MRIs. [b]One patient had TMT measurement available at MRI 1 and MRI 4. [c]Five patients had MMT measurements available at MRI 1 and the following ones at MRI 3 (four cases) and MRI 4 (one case).

Univariable linear regression analyses						
Dependent Variables	Independent Variables (IVs)	Adjusted R^2	B	B 95% CI	β	p value
TMT (mm)	Sex, F	0.117	-1.235	-1.726, -0.744	-0.349	<0.001
	IGF-1 xULN	0.074	0.579	0.284, 0.874	0.282	<0.001
MMT (mm)	Sex, F	0.196	-2.990	-3.926, -2.053	-0.451	<0.001
	IGF-1 xULN	0.136	1.295	0.783, 1.806	0.376	<0.001
Multivariable linear regression analyses						
Dependent Variables	Independent Variables (IVs)	Adjusted R^2	B	B 95% CI	β	p value
TMT (mm)	All IVs	0.189	-		-	<0.001
	Sex, F	-	-1.232	-1.712, -0.751	-0.345	<0.001
	IGF-1 xULN	-	0.528	0.251, 0.805	0.257	<0.001
MMT (mm)	All IVs	0.312	-		-	<0.001
	Sex, F	-	-2.836	-3.724, -1.949	-0.426	<0.001
	IGF-1 xULN	-	1.129	0.670, 1.588	0.328	<0.001

Table 3. Univariable and multivariable linear regression analyses for the prediction of TMT and MMT (all available MRIs) Legend. TMT, temporal muscle thickness; MMT, masseter muscle thickness; MRI, magnetic resonance imaging.

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New approaches in treatment management

Pasireotide improves biochemical control in acromegaly patients previously treated with combination therapies or unconventional dosages of somatostatin analogs

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Abstract

Purpose To evaluate the impact of pasireotide (PAS) therapy in patients with acromegaly previously treated with combination medical therapies or unconventional dosages of first-generation somatostatin receptor ligands (fg-SRLs).

Methods Retrospective study carried out in two referral centers for pituitary diseases. Twenty-one acromegalic patients were switched to PAS irrespective of disease status (12 had biochemical control, 9 were uncontrolled). Data were collected after 3- and 6-months PAS treatment, and at the last available visit (median 35 months).

Results After switching to PAS therapy, a significant reduction in IGF-1 values was observed [median 39%; 0.79 xULN (IQR 0.5-1.01) vs 1.29 xULN (IQR 1.06-1.83); p=0.009]. IGF-1 reduction was statistically significant in the 9 patients previously uncontrolled (61%, p=0.016), and in the 12 controlled subjects (33%, p=0.037). At last follow-up, the number of patients reaching an acceptable biochemical control (IGF-1 <1.3 xULN) raised from 57% to 90% (p=0.032). Mean HbA1c levels increased from 5.7% (5.5-5.9) to 6.0% (5.9-7) (p=0.002), and the percentage of diabetic patients raised from 14% (3/21) to 67% (14/21) (p=0.004). At the last evaluation HbA1c was ≥7.0% in 5 patients (24%). Antidiabetic drugs were initiated in 9 new patients, and in 7 out of 9 metformin alone was effective. Younger age and male sex were predictors for the maintenance of glucose homeostasis.

Conclusion PAS monotherapy can be effective in acromegalic patients previously treated with combination medical therapies or unconventional dosages of fg-SRLs. Glucose imbalance can be managed in the vast majority of cases by the use of lifestyle interventions and metformin.

Introduction

Acromegaly is a systemic endocrine disease due to prolonged exposure to high levels of growth hormone (GH) and its peripheral mediator, insulin-like growth factor-1 (IGF-1) [1, 2]. In more than 95% of cases it is due to the presence of a GH-secreting pituitary adenoma [3].

Comorbidities and mortality risk in patients with acromegaly are tightly linked to long-term exposure to GH and IGF-1 excess. However, reaching biochemical control may result in a significant reduction of patient mortality rate, as well as in the restoration of normal life expectancy [4-6].

The objectives of acromegaly management include reaching biochemical control (namely, glucose-suppressed GH levels <0.4 µg/L and normal sex and age-adjusted IGF-1 values), controlling the tumor size and preserving the remaining pituitary function, adequately treating all the disease-related comorbidities, as well as improving patient symptoms and quality of life (QoL) [7-10].

Nowadays, pituitary surgery represents the first-line treatment of acromegaly. In referral centers, the success rate of surgery (cured disease) is about 65%, although it drops to 40-50% in patients harboring a macroadenoma [11].

The use of first-generation somatostatin receptor ligands (fg-SRLs) is recommended in patients showing persistence of the disease after surgery, and in those subjects not eligible for surgical intervention, as well as in patients who refuse surgery, and in selected cases at risk for poor surgical success. According to the most recent data, about 40% of patients treated with the commercially available fg-SRLs, octreotide (OCT) and lanreotide (LAN), achieve biochemical control [12-15].

The latest treatment algorithms, proposed by consensus statements and guidelines, suggest different strategies for those subjects partially responsive or even resistant to fg-SRL therapy, based on the peculiar patient characteristics [7, 16].

Besides disease control, the individualized medical treatment may lead to an improved compliance to therapies, as well as to a reduction of disease-related direct costs [5, 17].

As a first step, in case of partial response, it is suggested to increase the dosage of OCT or LAN as far as allowed (e.g. 40 mg/4 weeks for OCT), or to reduce the interval between drug administrations (e.g. LAN 120 mg/3 weeks) [7, 18-20]. On the other hand, in patients with mild disease activity, the dopamine agonist (DA) cabergoline (CAB) can be successfully added to fg-SRL therapy [21, 22].

The combination of fg-SRLs with the GH receptor antagonist (GHRA) pegvisomant (PEG), or the switch to PEG monotherapy is, instead, strongly suggested in patients with impaired glucose metabolism [23, 24].

In case of persistent disease, the switch to pasireotide (PAS) is currently suggested mainly in patients with normal glucose metabolism, since early studies described a remarkable rate of drug-induced hyperglycemia and diabetes mellitus associated with PAS therapy, besides the higher efficacy of the compound compared to fg-SRLs [25, 26]. However, data from a recent clinical trial focused on PAS adverse events, clearly show that, when hyperglycemia occurs, most patients are successfully managed with first-line antidiabetic medications (e.g. metformin), while reaching target glycated hemoglobin levels (HbA1c <7%) with no need for additional treatments [27]. Real-life data confirm that the degree of PAS-associated hyperglycemia is dependent on the glycemic control at baseline, although an impaired glucose metabolism is unlikely to represent an absolute contraindication to treatment, since PAS effects are quickly reversible, and few patients are reported to discontinue treatment due to this issue (reviewed in [28]).

Besides the evaluation of glycemic status, PAS therapy is suggested as second-line medical treatment when a (residual) pituitary tumor mass can represent a concern for the patient, or in case of headache unresponsive to fg-SRLs [7, 29].

From a pharmacological perspective, PAS is a second-generation somatostatin receptor ligand, showing a higher binding affinity for SST_5 and a slightly lower affinity to SST_2, compared to OCT [30]. Furthermore, when bound to SST_2, PAS may exhibit different functional properties compared to both fg-SRLs and naïve somatostatin [31-34]. In the last 20 years, PAS has been shown to be superior to OCT and LAN in the inhibition of hormone secretion and cell growth in preclinical studies, as well as in reaching biochemical control and tumor volume reduction in patients with acromegaly naïve to medical treatment, or not adequately controlled with fg-SRLs [26, 35-38].

Since 2018, few studies have been published describing in a real-life setting the efficacy and the effects of PAS treatment on glucose metabolism [39-42]. By definition, these studies included different patient populations, with various treatment paths before starting PAS therapy. Moving from the experience of the PAPE clinical trial [43], Lasolle and colleagues switched to PAS monotherapy a group of patients previously treated with combination therapy (fg-SRLs + PEG or fg-SRL + CAB), showing that PAS monotherapy successfully maintained the biochemical control (with even a slight improvement compared to the previous treatments), although facing with a higher incidence of hyperglycemia [39].

In the present study, we retrospectively evaluated the biochemical outcome, together with the glycometabolic profile, in a cohort of acromegalic patients previously treated with combination medical therapies or unconventional dosages of fg-SRLs, then switched to PAS therapy. Noteworthy,

the switch to PAS was performed both in patients showing uncompleted response to the previous therapies, as well as in biochemically controlled patients, aiming to simplify the ongoing treatment schedule.

Patients and Methods

Study design and patients

A two-center retrospective study carried out at the Endocrinology Units of the IRCCS Ospedale Policlinico San Martino (Genoa, Italy) and the University Hospital "Federico II" (Naples, Italy).

Twenty-one acromegalic patients previously treated with combination medical therapies (fg-SRL + PEG, fg-SRL + CAB, fg-SRL + PEG + CAB) or unconventional dosages of fg-SRLs were included in the analysis. The definition of unconventional fg-SRL dosages meant the use of OCT 40 mg/4 weeks (requiring two i.m. injections each administration), or the high-frequency schedule of OCT and LAN (30 mg/3 weeks and 120 mg/3 weeks, respectively). A minimum of 6-month combination/unconventional treatment before switching to PAS, as well as at least 3 months of PAS therapy were considered additional inclusion criteria.

Patients who initiated PAS de novo (e.g. in the context of a clinical trial), or patients directly switched to PAS from fg-SRL monotherapy (at standard maximum dosage: OCT 30 mg/4 weeks or LAN 120/4 weeks) were excluded from the evaluation. No other exclusion criteria were applied (e.g. previous neurosurgery and/or radiotherapy).

All the 21 patients were switched to PAS monotherapy. Data were collected and recorded after 3- and 6-months PAS treatment, as well as at the last available visit in those subjects treated with PAS for more than 6 months (median 45.5 months, IQR 18.5- 49.5).

Data about the last follow-up were available at: 3 months in 1 patient, 6 months in 4 patients and >6 months in the remaining 16 patients (total: 21 patients treated with PAS for at least 3 months). Overall, patients were treated with PAS for a median of 35 months (IQR 7.5-47), with a starting dose of 40 mg/4 weeks in most cases (20 out of 21 patients). In one patient a starting dose of 20 mg/4 weeks has been prescribed, due to concerns related to the presence of overt diabetes mellitus. However, it was increased to 40 mg/4 weeks after 12 months, with no substantial changes in the glycemic profile.

Glucose metabolism impairment was defined as impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or overt diabetes mellitus according to the Standards of Medical Care in Diabetes 2022 - American Diabetes Association [44]. The study was conducted in accordance with the

recommendations of the Declaration of Helsinki and all patients gave written informed consent to use clinical data for research purpose.

Definition of biochemical control

Based on the study design, in line with previous studies evaluating the same outcome (as well as other recent clinical studies and consensus statements), we considered as having an acceptable biochemical control (defined as controlled) those subjects with sex- and age-adjusted IGF-1 values <1.3 the upper limit of normality (ULN) [39, 45-48]. Accordingly, we defined patients with IGF-1 levels ≥1.3 xULN as uncontrolled. Of note, the more stringent criteria of IGF-1 ≤1 xULN (normal IGF-1) was also used when analyzing the results of the efficacy of PAS treatment.

Single fasting GH measurements were evaluated in patients not treated with PEG (9 out of 21 patients). Data obtained were used to investigate GH changes after the switch to PAS in this patient subgroup (e.g. significant GH reduction), but did not contribute to the definition of biochemical control (based solely on age- and sex- adjusted IGF-1 values, as above mentioned).

Tumor volume reduction

Changes in tumor volume after switching patients to PAS were evaluated. Due to the retrospective design of the study, imaging data immediately before the switch to PAS and at the last follow-up were available in 19 out of 21 patients (complete information was missing in two patients). According to previous studies, significant tumor shrinkage was defined when a tumor volume reduction ≥20% was observed [25].

Assays

At the IRCCS Ospedale Policlinico San Martino (Genoa, Italy), GH levels were determined using a two-site chemiluminescent immunometric assay (Immulite 2000, Siemens Healthcare Diagnostics Products), calibrated to the World Health Organization (WHO) 98/574 International Standard (IS). The lower detection limit of the assay is 0.05 μg/L, while analytical sensitivity is 0.01 μg/L. The intra-assay and interassay coefficients of variation (CVs) are 2.9% to 4.6% and 4.2% to 6.6%, respectively. IGF-1 values were measured with a chemiluminescent immunometric assay (Immulite 2000, Siemens Healthcare Diagnostics Products), calibrated to the WHO 87/518 IS. The assay has a detection range from 20 to 1600 μg/L, and an analytical sensitivity of 20 μg/L. The intra-assay and inter-assay CVs are 2.3% to 3.9% and 3.7% to 8.1%, respectively.

At the University Hospital Federico II (Naples, Italy), GH levels were determined using a chemiluminescent immunometric assay (Liaison XL Analyzer, Diasorin), calibrated to the WHO 98/574 IS. The lower detection limit is 0 µg/L, while analytical sensitivity is 0.095-0.1 µg/L. The intra-assay and interassay CVs are 1.93% to 4.73% and 3.76% to 6.25%, respectively.

IGF-1 values were measured with a chemiluminescent immunometric assay (Liaison XL Analyzer, Diasorin), calibrated to the WHO 02/254 IS. The assay has a detection range from 0 to 1500 µg/L, and an analytical sensitivity of 3 µg/L. The intra-assay and inter-assay CVs are 3% to 5.1% and 5.6% to 9.6%, respectively.

Statistical analysis

Statistical analysis was conducted using the GraphPad Prism software v. 5.02. Continuous variables are expressed as median and interquartile range (IQR), whereas dichotomous variables are reported as frequency and percentage. Comparisons between continuous variables were performed using nonparametric tests (Mann-Whitney U test and Kruskal-Wallis test). Fisher's exact test was used for the analysis between two dichotomous variables. The potential predictors of glucose imbalance were identified performing summary statistics and the above described tests. Afterwards, the selected

variables were included in the different prediction models. Indeed, based on the dependent variables (categorical or continuous), univariable and multivariable logistic regression and linear regression analyses were performed, respectively. Significance was accepted for a p-value <0.05.

Results

Patient characteristics

Twenty-one patients were included in the study, 13 females (62%) and 8 males (38%), with a median age at diagnosis of 37 years (IQR 31-52). At time of data collection, median age was 56 years (IQR 42-64). Acromegaly was due to the presence of a pituitary macroadenoma in the vast majority of cases (19/21, 91%), with only two patients (2/21, 9%) having a microadenoma at diagnosis.

Fourteen patients (14/21, 67%) were on combined medical therapy before starting PAS (10/21 fg-SRL + PEG, 2/21 fg-SRL + DA, 2/21 fg-SRL + PEG + DA), while 7 patients were undergoing unconventional dosages of fg-SRLs (3/21 OCT 40 mg/4 weeks, 3/21 LAN 120 mg/3 weeks, 1/21 OCT 30 mg/3 weeks). Median PEG dosage before the switch to PAS was 105 mg/week (IQR 45-170), while median CAB dosage was 0.75 mg/week (IQR 0.5-2.87).

Thirteen patients (62%) underwent surgery during their clinical history, and in 3 cases (14%) radiotherapy was performed. In detail, radiotherapy was performed before the switch to PAS in 2 patients (after 5 and 2 years, respectively), while one patient was referred to radiotherapy after 18 months of PAS withdrawal (the drug was discontinued 7 months after the switch to PAS due to the worsening of glycemic control and the lack of substantial treatment benefit in terms of IGF-1 reduction).

Patients' characteristics are summarized in Table 1.

Data	Number (%)
Patients	21
Sex	M, 8 (38)
Tumor size	Macro, 19 (91)
Age at diagnosis (years; median, IQR)	37, 31-52
Age at time of data collection (years; median, IQR)	56, 42-64
Biochemical values at diagnosis	
GH (µg/L; median, IQR)	18.4, 8.42-52.1
IGF-1 (µg/L; median, IQR)	815, 513.8-1157
IGF-1 (ULN; median, IQR)	3.1, 2.32-4.08
Pre-PAS treatment modalities	
Neurosurgery	13 (62)
Radiotherapy	2 (10)
Medical therapy	
Unconventional doses of fg-SRLs	7 (33)
Combination therapy (fg-SRLs +/- PEG +/- CAB)	14 (67)
Pre-PAS biochemical values	
GH (µg/L; median, IQR)	3.38, 2.08-7.70
IGF-1 (µg/L, median, IQR)	318, 218-512.5
IGF-1 (ULN; median, IQR)	1.29, 1.06-1.83
PAS treatment duration (months; median, IQR)	35, 7.5-47

Table 1. Main demographics, clinical and biochemical characteristics of patients at diagnosis and before switching to pasireotide (PAS)

Legend. M: male, macro: macroadenoma, IQR: interquartile range, GH: growth hormone, IGF-1: insulin-like growth factor-1, ULN: upper limit of normal, PAS: pasireotide, fg-SRLs: first-generation somatostatin receptor ligands.

Based on the definition of biochemical control above described, before the switch to PAS treatment 12/21 (57%) patients were considered as having biochemical control (7 on combination therapy, 5 on unconventional dosages of fg-SRLs), while 9/21 (43%) patients were uncontrolled (7 on combination therapy, 2 on unconventional dosages of fg-SRLs).

Patient comorbidities before starting PAS were also recorded. Comorbidities were defined according to the definitions reported in Supplementary Table 1, and collected in patient clinical charts, according to current recommendations [49]. In detail, 5 (24%) patients had arterial hypertension, 2 (9.5%) left ventricular hypertrophy, 7 (33%) obstructive sleep apnea syndrome, 2 (9.5%) osteoporosis, 10 (48%) were diagnosed with thyroid nodules (including 1 case of thyroid carcinoma), and 5 (24%) had evidence of colonic polyps. As concerns the remaining pituitary function, central hypogonadism was detected in 4 (19%) patients, central hypocortisolism in 5 (24%), and central hypothyroidism in 4 (19%) patients. Four patients had more than one pituitary deficit (one patient with central hypothyroidism and central hypogonadism, while three patients had central hypothyroidism and central hypocortisolism).

Glucose metabolism was carefully monitored and recorded before switching patients to PAS. In detail, the median value of glycated hemoglobin (HbA1c) was 5.7% (IQR 5.5-5.9), while median fasting plasma glucose was 93 mg/dl (IQR 86.5-115). Out of 21 patients, 8 (38%) had normal glucose metabolism, 10 (48%) had pre-diabetes [IFG and/or IGT], while 3 (14%) had overt diabetes mellitus. Lipid profile showed a median total cholesterol of 205 mg/dl (IQR 170-238.5), median low-density lipoprotein (LDL) cholesterol of 132 mg/dl (IQR 95-159), and median triglycerides of 83 mg/dl (IQR 73-108). Median BMI before the first PAS administration was 26.2 Kg/m^2 (IQR 23.9-30).

Biochemical response to pasireotide

Overall, after starting PAS therapy a significant reduction in IGF-1 values [39%; median IGF-1 before PAS 1.29 xULN (1.06-1.83) vs 0.79 xULN (0.5-1.01) was observed at the last available follow-up; p=0.009] (Fig. 1a). The observed reduction in IGF-1 values was statistically significant both in the 9 patients previously uncontrolled (IGF-1 reduction: 61%, p=0.016), as well as in the 12 subjects of the controlled group (IGF-1 reduction: 33%, p=0.037). In detail, IGF-1 values dropped from 2.03 xULN (1.44-2.35) to 0.79 xULN (0.60-1.34) in the uncontrolled patients, while they decreased from 1.1 xULN (0.71-1.21) to 0.74 xULN (0.43-0.97) in the controlled ones (Fig. 1b-c).

At the last available follow-up, the number of patients reaching an acceptable biochemical control (IGF-1 <1.3 xULN) raised from 12/21 to 19/21 (57 vs 90%; p=0.032). Considering a narrower cut-off

to define biochemical control (IGF-1 ≤1 xULN), the number of controlled patients raised from 4/21 (19%) before PAS to 17/21 (81%) at last follow-up (p<0.001). In detail, after switching to PAS treatment 7/9 (78%) previously uncontrolled patients normalized IGF-1.

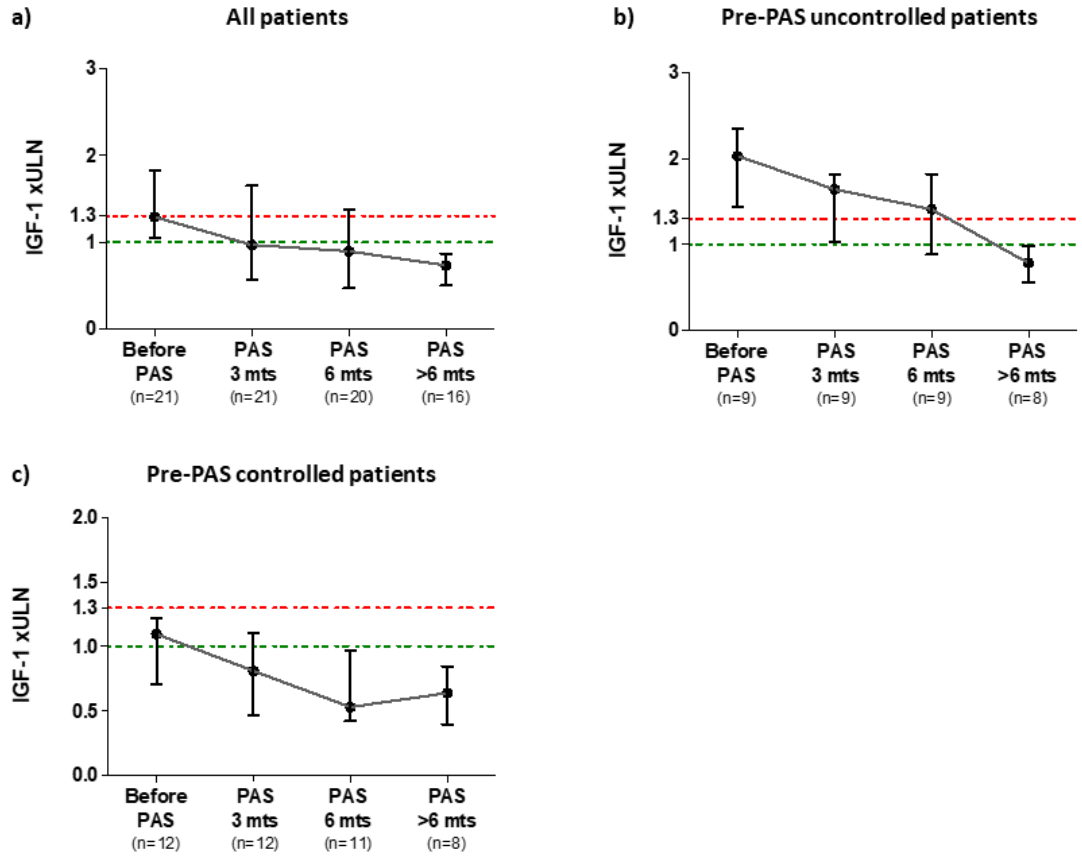

Fig.1 Biochemical response (IGF-1 values) after switching patients to pasireotide therapy. Median IGF-1 xULN values before and during PAS therapy in the full patient cohort (panel a), in the subgroup of patients not controlled with previous therapies (panel b), and in those subjects who achieved biochemical control before the switch to PAS (panel c). The red dotted line indicates the less stringent cut-off set for biochemical control (sex- and age-adjusted IGF-1 values <1.3 xULN), while the green dotted line indicates the more stringent IGF-1 cut-off (≤1 xULN, normal IGF-1). Abbreviations: ULN, upper limit of normal; PAS, pasireotide; mts, months; n, number

GH values were evaluated only in patients not treated with PEG. A decrease in median GH values from 3.38 µg/L (2.08-7.7) to 1.59 µg/L (0.55-2.44) was observed, although this difference was not statistically significant due to the large variability observed among patients (p=0.13).

Considering "resistant" to fg-SRLs those patients needing combination treatments or being uncontrolled using unconventional dosages of fg-SRLs (n=16), and defining "partial responders"

those subjects achieving an acceptable biochemical control (IGF-1 <1.3 xULN) with unconventional dosages of fg-SRLs (n=5), we did not find any statistically significant difference in terms of biochemical control between the two groups after switching to PAS (IGF-1 levels at last follow-up, p=0.354; patients with IGF-1 <1.3 xULN, p=1.000; patients with normalized IGF-1, p=0.228).

Table 2 details the median GH and IGF-1 xULN values just before starting PAS, and at 3-months, 6-months and >6-months follow-up.

	Before PAS	3 mts follow-up	6 mts follow-up	>6 mts follow-up
GH (µg/L; median, IQR)	3.38 (2.08-7.70)	2.71 (0.79-5.03)	1.70 (0.70-3.65)	1.07 (0.41-2.25)
IGF-1 (ULN; median, IQR)	1.29 (1.06-1.83)	0.97 (0.57-1.65)	0.90 (0.47-1.38)	0.74 (0.50-0.87)
IGF-1 controlled patients* (ULN; median, IQR)	1.10 (0.71-1.22)	0.81 (0.46-1.10)	0.53 (0.42-0.97)	0.64 (0.40-0.85)
IGF-1 uncontrolled patients* (ULN; median, IQR)	2.03 (1.44-2.35)	1.64 (1.03-1.81)	1.41 (0.89-1.82)	0.79 (0.56-0.98)

Table 2. Biochemical follow-up during observation in the total group

Legend. PAS: pasireotide, mts: months, GH: growth hormone, IQR: interquartile range, IGF-1: insulin-like growth factor-1, ULN, upper limit of normality. *Patients were stratified into controlled and uncontrolled based on the IGF-1 values (xULN) recorded before switching to PAS therapy (namely, controlled patients: IGF-1 <1.3 xULN before PAS; uncontrolled patients: IGF-1 ≥1.3 xULN before PAS)

The two patients that received radiotherapy before switching to PAS (5 years and 2 years, respectively), did not show significantly different IGF-1 values at last follow-up, compared to patients that did not undergo radiation therapy [median IGF-1 xULN 0.76 vs 0.79 (0.51-1.00), p=0.952)]. Similarly, previous neurosurgery did not significantly impact on biochemical control after switching to PAS (neurosurgery vs medical therapy alone; median IGF-1 xULN 0.78 (0.50-0.94) vs 0.85 (0.55-1.08); p=0.500).

In 5/21 (24%) patients, after a median of 5 months (IQR 4.5-9 months) treatment with PAS 40 mg, it was decided to increase the dose up to 60 mg/4 weeks, due to the lack of biochemical control. On the other hand, in one patient it was possible to reduce the dose from 40 mg to 20 mg/4 weeks after 36 months of continued therapy with PAS, while maintaining a satisfying biochemical control.

In 3 patients PAS therapy was discontinued. Particularly, one patient withdrew PAS after 4 months for gastrointestinal intolerance, while two patients stopped PAS treatment for a significant

worsening of glycemic control, not associated with a substantial therapeutic benefit in terms of IGF-1 reduction (after 3 and 7 months, respectively). Of note, in the latter two patients, a normal glucose profile was restored within 3 months of PAS discontinuation. No other side effects potentially related to PAS therapy (e.g. cholelithiasis, QT interval prolongation, liver function impairment) were observed.

Tumor volume response to pasireotide

After switching patients to PAS, we observed a significant reduction (≥20%) in tumor volume in 6 out of 19 patients (32%); 3 patients with previous neurosurgery and 3 patients treated with medical therapy alone. In 13 subjects imaging data did not change during follow-up (9 patients with previous neurosurgery, and 4 patients treated with medical therapy alone). In 2 out of 13 patients no residual tumor was visible at the time of PAS initiation, and MRI remained negative during follow-up. None of the evaluated patients showed an increase in tumor volume during the study period.

As previously mentioned ("Patients and Methods" section), complete imaging data could not be retrieved in two patients.

Changes in glucose and lipid metabolism after switching to pasireotide

Mean HbA1c levels, although considering appropriate therapeutic interventions (e.g. lifestyle changes and antidiabetic drugs), increased from 5.7% (5.5-5.9) before starting PAS to 6% (5.9-7) at the time of the last follow-up (p=0.002) (Fig. 2a). Three patients had a diagnosis of diabetes mellitus before starting PAS (3/21, 14%), while during the study period 14 out of 21 patients met the criteria for diagnosis (67%; p=0.004). Out of 11 new diabetic patients, 4 had normal glucose metabolism at diagnosis, while 7 had pre-diabetes.

However, due to a proactive management of hyperglycemia, at the last evaluation HbA1c values were ≥7.0% in 3 new patients, and in 5 out of 21 patients overall (24%). Antidiabetic drugs were initiated in 9 new patients, and in 7 out of 9 subjects the administration of metformin alone, combined with lifestyle interventions, was effective.

A detailed description of HbA1c levels during the follow-up, as well as a report of all the treatment strategies used to control the glucose imbalance possibly encountered during PAS therapy (e.g. lifestyle interventions, antidiabetic drugs), are reported in Table 3. Focusing on the 3 patients with diabetes mellitus before switching to PAS, we had different approaches: one patient required basal insulin in addition to metformin (patient n.13, Table 3), in another patient (patient n.14) a GLP-1

receptor agonist was added to metformin, but PAS was then withdrawn due to the lack of substantial treatment benefit in terms of IGF-1 reduction, while in the third patient previous antidiabetic therapy was maintained unchanged (metformin + DPP-4 inhibitor, patient n. 17).

Median HbA1c levels raised from 5.7% (5.4-5.8) to 6.0% (5.9-6.6) in the subgroup of pre-PAS controlled patients (p=0.006), while in the uncontrolled ones median HbA1c values increased from 5.9% (5.5-6) to 6.3% (5.6-7.4) (p=0.3) (Fig. 2b-c).

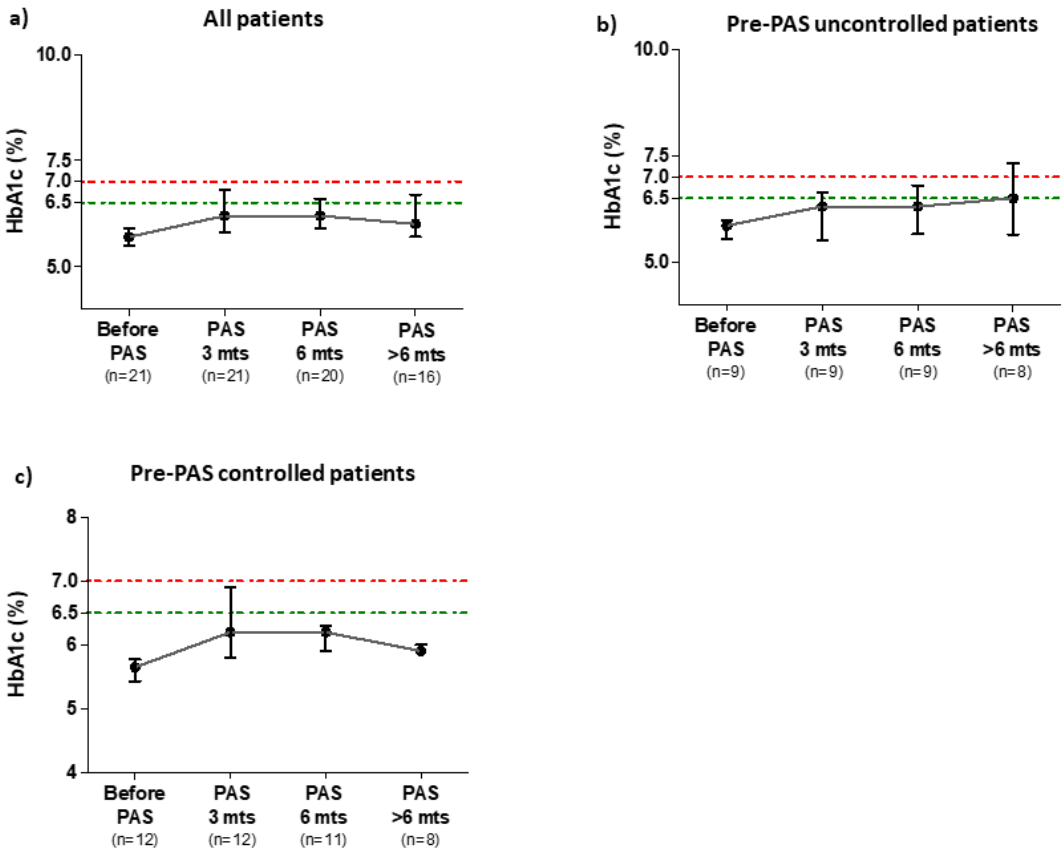

Fig.2 Changes in glycated hemoglobin (HbA1c) values after switching patients to pasireotide therapy. Median HbA1c values before and during PAS therapy in the entire patient cohort (panel a), in the subgroup of patients not controlled with previous therapies (panel b), and in those subjects who achieved biochemical control before the switch to PAS (panel c). The red dotted line indicates the HbA1c value (≤ 7.0%) defined as acceptable for the diabetic patients included in the study. The green dotted line represents the diagnostic HbA1c value for diabetes mellitus (≥ 6.5%). Abbreviations: HbA1c, glycated hemoglobin; PAS, pasireotide; mts, months; n, number

As concerns fasting plasma glucose, a statistically significant increase was observed in the whole cohort [median 124 mg/dl at the last available follow-up (104-143) vs 93 mg/dl before the first

administration of PAS (87-115); p=0.003], as well as in the subgroup of pre-PAS uncontrolled patients [median 128 mg/dl at the last available follow-up (104-156) vs 87 mg/dl before the first administration of PAS (72-113); p=0.045]. In the pre-PAS controlled group, the increase was not statistically significant [median 119 mg/dl at the last available follow-up (103-133) vs 97 mg/dl before the first administration of PAS (91-117); p=0.08] (Fig. 3a-c).

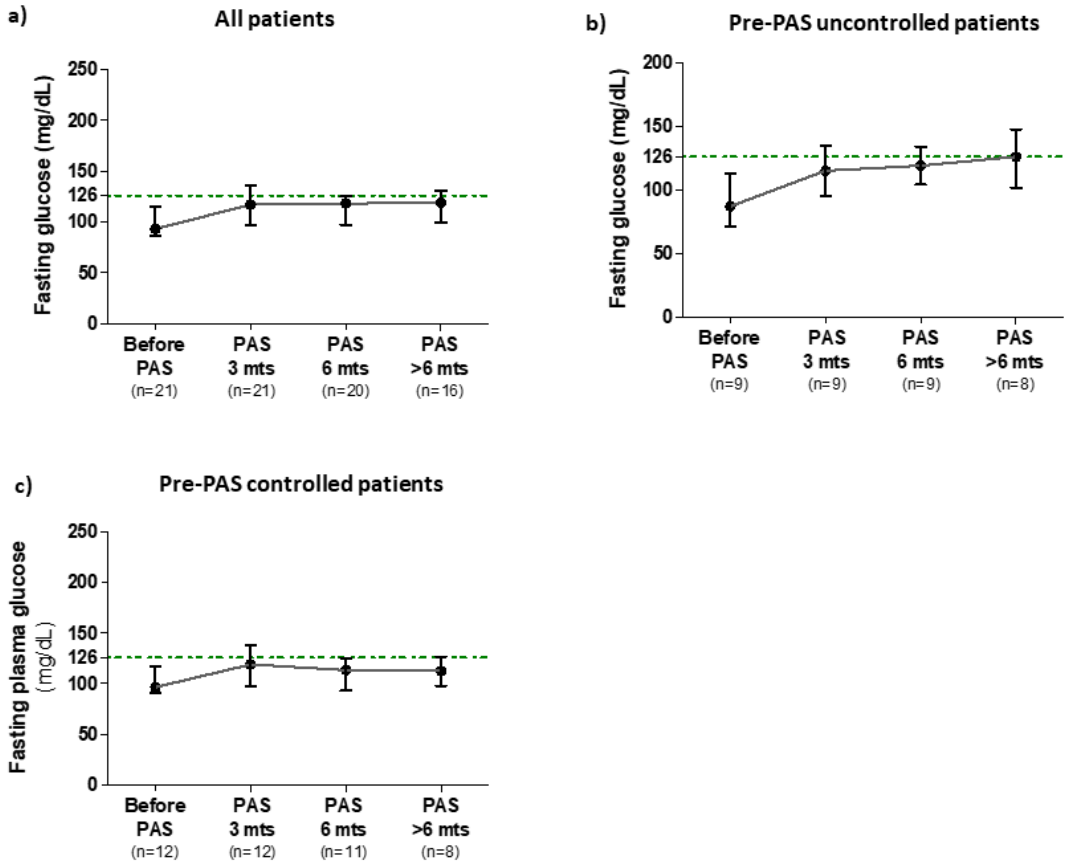

Fig.3 Changes in fasting plasma glucose values after switching patients to pasireotide therapy. Median fasting plasma glucose values before and during PAS therapy in the entire patient cohort (panel a), in the subgroup of patients not controlled with previous therapies (panel b), and in those subjects who achieved biochemical control before the switch to PAS (panel c). The green dotted line indicates the fasting plasma glucose values diagnostic for diabetes mellitus (≥126 mg/dl). Abbreviations: PAS, pasireotide; mts, months; n, number

No significant changes in lipid profile were observed after switching patients to PAS therapy [median total cholesterol at the last available follow-up 201 mg/dl (158-220) vs 205 mg/dl (170-239) before the first administration of PAS, p=0.94; HDL cholesterol 57 mg/dl (45-75) vs 57.5 mg/dl (45-73), p=0.91; LDL cholesterol 124 mg/dl (83-141) vs 132 mg/dl (95-159), p=0.64; triglycerides 95 mg/dl

(65-139) vs 83 mg/dl (73-108), p=0.78]. Accordingly, BMI values did not change significantly after switching to PAS therapy [median 25 Kg/m^2 (23.6-27) vs 26.2 Kg/m^2 (23.9-30), p=0.59] (Fig. 4a-e).

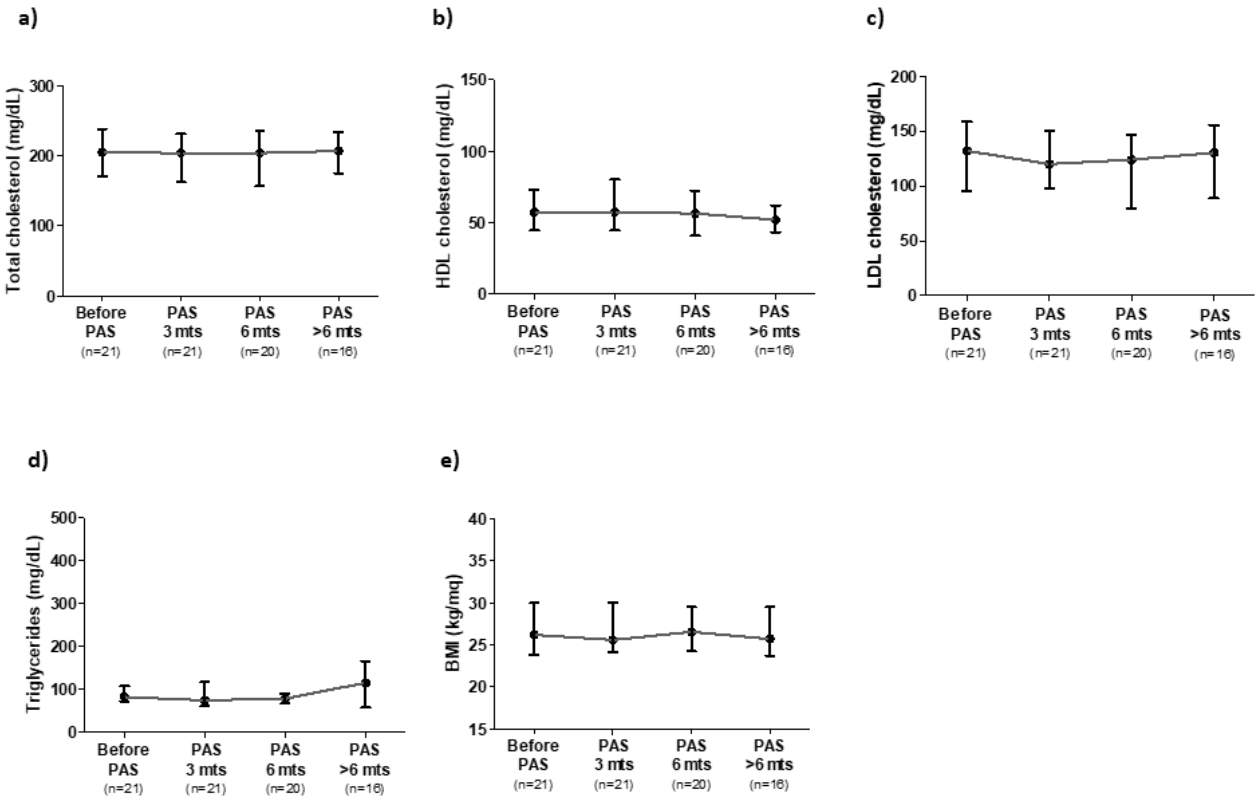

Fig.4 Changes in lipid metabolism (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides) and body mass index (BMI) after switching patients to pasireotide therapy. Median values of total cholesterol (panel a), HDL cholesterol (panel b), LDL cholesterol (panel c), triglycerides (panel d) and BMI (panel e) before and during PAS therapy in the entire patient cohort. Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; BMI, body mass index; PAS, pasireotide; mts, months; n, number

Correlations between patient characteristics and changes in glucose metabolism after pasireotide treatment

We investigated whether peculiar patient characteristics correlated with a worse outcome in terms of glucose imbalance after switching to PAS treatment. Patient age at the time of PAS initiation was directly correlated with fasting plasma glucose levels at the last follow-up (rho: 0.438, p=0.047).

At linear regression analysis, both age at diagnosis and age at the time of PAS initiation were significant predictors of fasting plasma glucose levels at last follow-up (β 0.476, B 0.208, p=0.029 and β 0.460, B 0.186, p=0.036; respectively).

Previous medical therapies before switching to PAS did not significantly impact on glucose metabolism changes. Patients previously treated with PEG (in combination with fg-SRLs or fg-SRLs + CAB), did not experienced a worse glucose metabolism impairment compared to patients undergoing unconventional dosages of SRLs before switching to PAS, both in terms of HbA1c levels (p=0.219) and fasting plasma glucose (p=0.754) at last follow-up. In line with these findings, the prevalence of diabetes mellitus at last follow-up did not significantly differ between patients previously treated with combination therapies with PEG vs unconventional dosages of SRLs (p=1.000).

Focusing on the impact of disease activity, no significant direct correlations were found between IGF-1 levels before starting PAS and both HbA1c and fasting plasma glucose at last follow-up (rho: 0.153, p=0.508 and rho: 0.099, p=0.669; respectively).

As concerns the diagnosis of diabetes mellitus after PAS treatment, patients with diabetes at the last follow-up had older age at diagnosis (p=0.016) and at the time of PAS start (p=0.046). A trend towards higher IGF-1 and higher HbA1c values pre PAS in patients with diabetes mellitus at last follow-up was observed, although these data did not reach statistical significance (p=0.067 and p=0.076; respectively). Of note, only one patient of the controlled group developed diabetes mellitus (1 out of 9, 11%), while 6 patients (6 out of 16, 50%) of the uncontrolled group did. However, this difference did not reach statistical significance at both Chi square (p=0.061) and Fisher's exact tests (p=0.16). Finally, in our cohort female patients had a significantly higher likelihood to develop diabetes mellitus during PAS treatment compared to males (OR 66, 95% CI 2.69-481.212; p=0.007). Of note, 9 out of 14 (64%) female patients were in menopause at the time of diagnosis.

Biochemical response to pasireotide in patients on previous combination medical therapy

In the subgroup of 14 patients previously treated with combination medical therapy, a statistically significant reduction in IGF-1 levels after switching to PAS treatment was observed [median IGF-1 reduction: 44%, p=0.045; median IGF-1 0.73 xULN (0.5-0.91) vs 1.31 xULN (1.04-2.05)] (Fig. 5a, Supplementary Table 2).

In this peculiar patient subgroup, the percentage of patients achieving IGF-1 levels <1.3 xULN increased after switching to PAS, although the difference was not statistically significant [IGF-1 xULN <1.3 xULN: 12/14 (86%) vs 7/14 (50%); p=0.10]. Of note, the percentage of patients reaching normal age-adjusted IGF-1 values (IGF-1 ≤1 xULN) significantly increased after moving to PAS therapy [12/14 (86%) vs 3/14 (21%); p=0.002].

Changes in glucose and lipid metabolism in patients on previous combination medical therapy

HbA1c values recorded at the last follow-up showed a moderate, although statistically significant, increase compared to pre-PAS levels [median 6% (5.9-6.6) vs 5.7% (5.5-5.9); p=0.025]. Similarly, fasting plasma glucose levels raised from 92 mg/dl (84-114) up to 121 mg/dl (100-139), (p=0.031) (Fig. 5b-c).

In line with the findings observed in the whole cohort, no significant changes were recorded in lipid profile after PAS initiation [median total cholesterol at the last available follow-up 194 mg/dl (154-225) vs 203 mg/dl (164-236) before the first administration of PAS, p=0.91; HDL cholesterol 54 mg/dl (46-69) vs 56 mg/dl (46-66), p=0.99; LDL cholesterol 119 mg/dl (80-146) vs 129 mg/dl (87-147), p=0.78; triglycerides 93 mg/dl (63-169) vs 88 mg/dl (74-127), p=0.89]. Accordingly, BMI values did not change significantly after switching to PAS therapy [median 25 Kg/m^2 (23.6-27.6) vs 27.4 Kg/m^2 (24.7-30.1), p=0.59] (Fig. 5d).

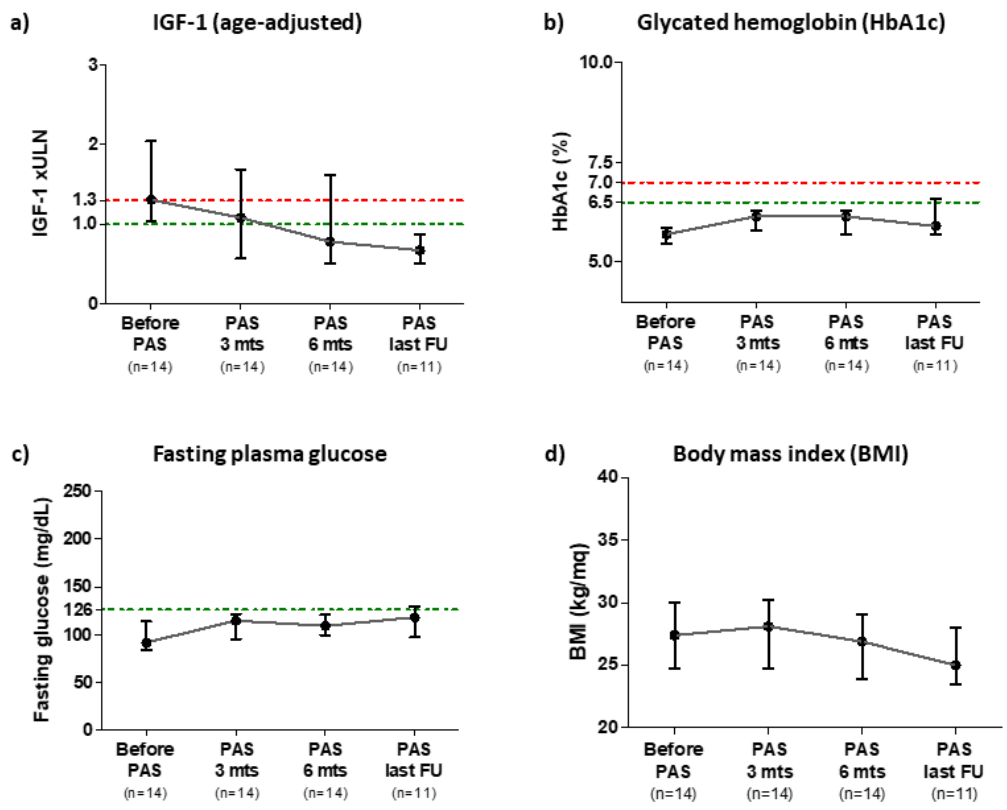

Fig.5 Changes in age-adjusted IGF-1, glycated hemoglobin, fasting plasma glucose and body mass index in 16 patients previously treated with combined medical therapy, then switched to pasireotide. Median values of IGF-1 xULN (panel a), HbA1c (panel b), fasting plasma glucose (panel c) and BMI (panel d) before and during PAS therapy in the subgroup of patients previously treated with combined medical therapy. Panel a: The red dotted line indicates the less stringent cut-off set for biochemical control (sex- and age-adjusted IGF-1 values

<1.3 xULN), while the green dotted line indicates the more stringent IGF-1 cut-off (≤1 xULN, normal IGF-1). Panel b: The red dotted line indicates the HbA1c value (≤ 7.0%) defined as acceptable for the diabetic patients included in the study. The green dotted line represents the diagnostic HbA1c value for diabetes mellitus (≥ 6.5%). Panel c: The green dotted line indicates the fasting plasma glucose values diagnostic for diabetes mellitus (≥ 126 mg/dl). Abbreviations: ULN, upper limit of normal; PAS, pasireotide; mts, months; n, number; HbA1c, glycated hemoglobin; BMI, body mass index

Discussion

The need for a personalized approach in the management of patients with acromegaly is definitely a hot topic of current research in pituitary diseases [50]. In this scenario, real-life studies can play a pivotal role towards a better understanding of the correct positioning of the different treatment strategies currently available in the algorithm of acromegaly management, including PAS.

In the current study the potential role of PAS was investigated in a peculiar group of patients, including only subjects previously treated with combination medical therapies or unconventional dosages of fg-SRLs. This represents a challenging cohort of patients to be treated, due to the high burden of medical therapy at the time of study start.

Noteworthy, the switch to PAS monotherapy resulted in a significant decrease in age-adjusted IGF-1 levels, as well as in a higher percentage of patients reaching biochemical control (defined as <1.3 xULN) and full IGF-1 normalization (defined as IGF-1 ≤1 xULN) compared to baseline. At the last follow-up, 90% of patients (19/21) had IGF-1 <1.3 xULN. None of the pre-PAS controlled patients lost biochemical control during follow-up and 7/9 (78%) pre-PAS uncontrolled patients reached normal IGF-1 values at the last follow-up. However, in line with a recent study from Gadelha and colleagues, we did not identify any clinical parameter able to strongly predict biochemical control/IGF-1 normalization following PAS treatment [51].

As concerns the analysis of PAS efficacy in the biochemical control in our cohort, the observed results are superior to previous findings reported in the literature, particularly those described in clinical trials. Indeed, the PAOLA study, a multicenter, randomized, phase III trial published in 2014, showed that “only” 25% of patients uncontrolled after fg-SRL therapy reached normal age-adjusted IGF-1 levels 6 months after switching to PAS [26].

Later on in 2018, the first real-life study conducted by Shimon and colleagues showed that PAS treatment normalized IGF-1 values in 17 out of 30 (56.6%) patients uncontrolled with previous medical therapies (mainly fg-SRL monotherapy and fg-SRL + PEG) [41]. In line with this finding, a

recent real-world experience, including 26 patients with active acromegaly following fg-SRL treatment, reported IGF-1 values ≤1.3 xULN in 53.8% of patients after switching to PAS therapy [42]. Taken together, the current results and the experience of previous real-life clinical studies suggest that the efficacy of PAS therapy in reducing IGF-1 levels in patients partially or fully resistant to fg-SRLs is higher than that demonstrated in early clinical trials.

Notably, a recent single-center study from Lasolle and colleagues investigated the efficacy and the safety of switching to PAS monotherapy patients on previous combination therapy (fg-SRLs + PEG in 11 subjects, fg-SRLs + CAB in 5) [39]. The authors found that PAS treatment led to the maintenance and/or the achievement of acceptable biochemical control (IGF-1 ≤1.3 xULN) in 8 out of 15 (53.3%) patients, during a long-term follow-up (median 29 months). While 7 of 15 patients discontinued PAS therapy, due to a lack of efficacy or severe hyperglycemia despite intensification of anti-diabetic treatment, 6 out of 8 patients controlled with PAS monotherapy did not need antidiabetic medications. Therefore, the authors concluded that PAS monotherapy can be a suitable alternative to combination therapies, for the control of IGF-1 in a subset of acromegaly patients (partially) resistant to fg-SRLs.

In the current cohort, 14 out of 21 (67%) patients were previously treated with combination therapy. In line with the report of Lasolle and colleagues, PAS monotherapy was a good alternative to combination therapy, with an observed improvement of IGF-1 values in a subset of patients and, even more importantly, the maintenance of acceptable biochemical control using an easier and less expensive treatment schedule in the other subjects evaluated, thus potentially leading to an increased patient compliance.

Overall, we are aware that a tight comparison between our results and the previously mentioned studies is challenging, mainly due to the heterogeneity in the study populations and the study designs. Particularly, we included in our analysis both controlled and uncontrolled patients before PAS; patients treated with combination therapies and unconventional dosages of fg-SRLs; as well as we considered in the efficacy analysis all the patients with at least 3 months of PAS treatment. However, we believe that these peculiar characteristics of the study can be useful to provide further insights on the effects of PAS in a real-word setting.

This is also the case for the tumor volume reduction observed in our cohort (32% of cases), which is in line with the data reported in a recent meta-analysis (37.7%, 95% CI 18.7%-61.5%), although showing high heterogeneity among studies [52].

The development of glucose imbalance in patients with acromegaly, resulting in some cases in overt diabetes mellitus, is a well-known adverse event correlated with PAS treatment. This is due to multiple factors, including a direct effect of PAS in reducing insulin secretion by pancreatic β-cells, as well as in the inhibition of incretin secretion [53]. Based on the peculiar expression of SSTs on endocrine pancreatic cells, the decrease in insulin levels is not counteracted by a powerful inhibition of glucagon on α-cells, thus resulting in glucose imbalance. As concerns the data on hyperglycemia-related adverse events during PAS treatment, clinical trials and real-life studies are quite consistent, although glucose imbalance is described using heterogeneous classifications in the different studies (e.g. hyperglycemia, overt diabetes mellitus, pre-diabetes, glucose deterioration). In this light, while the PAOLA study described the presence of hyperglycemia in 67% and 61% of patients treated with pasireotide LAR (40 mg and 60 mg every 4 weeks, respectively), Shimon and colleagues reported glucose control deterioration in 63% of patients [26, 41]. Another recent study carried out in patients with active acromegaly on fg-SRLs showed that, after a 12-month treatment with PAS, 42% of patients were diabetic, and almost all the other study subjects had impaired glucose tolerance [42]. Interestingly, a detailed analysis of these results highlights that the mean HbA1c levels raised from 5.9% at baseline to 6.5% at month 12, with the great majority of patients (77%) being treated with metformin alone. These data are in line with those observed by Shimon and colleagues, with a mean HbA1c increase from 6.1% at baseline to 6.7% at study end [41]. Overall, these findings fit with the results of the current study, since in our cohort HbA1c levels increased from 5.7% before starting PAS to 6.0% at the time of the last follow-up. As previously reported, metformin alone (combined with lifestyle interventions) proved to be effective in 7 out of 9 subjects who needed to initiate antidiabetic drugs after PAS treatment. These safety data are in line with those reported in a recent phase IV study, aimed to investigate the management of PAS-associated hyperglycemia in patients with acromegaly or Cushing's disease not controlled with metformin or other oral antidiabetic drugs [27]. Surprisingly, only 33% of the enrolled patients (81/249) entered the study and were randomized to incretin-based therapy vs insulin. Indeed, 41% (103/249) did not develop hyperglycemia after PAS treatment, 18% (46/249) had hyperglycemia effectively managed on metformin (n=43) and/or other antidiabetic oral drugs (n=3), and 8% (19/249) were already receiving insulin at baseline, before initiating PAS [27]. Considering only patients with acromegaly, the percentage of randomized patients was even lower (56/190, 29%). Therefore, it is clear that the majority of acromegalic patients treated with PAS (about 60%) do not develop severe hyperglycemia (considered as HbA1c >7.0%), or are adequately controlled by the use of metformin alone.

In our patients with diabetes mellitus before starting PAS, we added basal insulin in one case, another one did not require changes in the pre-PAS antidiabetic treatment (metformin plus DPP-4 inhibitor), while a third patient, undergoing metformin plus a GLP-1 receptor agonist, was withdrawn. This is in line with available data from the literature, suggesting to use incretin-based therapies as the first option when metformin alone does not reach the therapeutic target, and to consider basal insulin as a valuable alternative in selected patients [54]. Of course, drug withdrawal has to be taken into account in patients with an unfavorable risk/benefit ratio in terms of impairment of glucose tolerance and acromegaly biochemical control.

In this context, a recent study highlighted some important characteristics of PAS-related hyperglycemia: its reversibility and the existence of predictive factors [55]. Indeed, the post-hoc analysis of Phase III SOM230C2305 (C2305) and SOM230C2402 (C2402; PAOLA) studies showed that the occurrence of hyperglycemia during PAS treatment was less frequent in patients with lower age (<40 years, C2402; <30 years, C2305), BMI <25 Kg/m^2 (C2402), normal glucose tolerance, and no history of hypertension or dyslipidemia at baseline [55]. In line with these findings, the results of the current study demonstrated that younger patients had lower levels of fasting plasma glucose after PAS treatment, compared to elderly patients. Furthermore, a significant impact of sex was observed, since female patients had a higher likelihood to develop diabetes mellitus, compared to males. Although females evaluated in the present cohort had slightly higher fasting plasma glucose and HbA1c pre-PAS than males, the difference observed in the prevalence of diabetes mellitus at the last follow-up was still present after proper adjustment. Female patients maintained a significantly higher risk to develop diabetes mellitus even after adjustment for age at the time of PAS initiation, while this regression analysis lost statistical significance when adjusted for the age at diagnosis (higher in females). In this light, a recent study highlighted some interesting gender difference in patients with controlled acromegaly, with females showing a worse metabolic health and well-being than males [56].

The role of disease activity in the impairment of glucose metabolism during PAS treatment is controversial. Indeed, while the analysis of phase III clinical trials did not find any predictive value of baseline GH and IGF-1 values in the development of hyperglycemia, a recent single-center study (including 50 patients) showed that baseline IGF-1 values were significantly correlated with HbA1c and fasting plasma glucose levels during PAS treatment [25, 26, 55]. In this context, our findings do not allow us to make strong conclusions, claiming for further research on this issue.

Since the hypersecretion of GH and IGF-1 has anabolic and lipolytic effects, the presence of hypertriglyceridemia is reported in 33-40% of patients with acromegaly (three times more than in the general population), and low HDL cholesterol levels are found in 39-47% of cases [57]. Alterations in the lipid profile have a major role in the cardio-metabolic risk of patients with acromegaly, as they promote the development of vascular damage and atherosclerosis. In this light, biochemical control has been proved to have a beneficial effect on lipid alterations [58-60]. The efficacy of PAS in reducing cholesterol and triglyceride levels has been investigated in patients with Cushing's disease and in healthy subjects [61-63]. However, no data are currently available on the impact of this drug in patients with acromegaly. In our study, we did not find any significant change in lipid profile (as well as in BMI values) during the observation time, despite a significant reduction in IGF-1 levels.

Finally, we acknowledge that the lack of detailed information about the impact of PAS therapy on tumor size in our peculiar cohort represents a limitation of the study.

In conclusion, the results of the current study demonstrated that PAS monotherapy can be effective in reaching biochemical control in acromegaly patients previously treated with combination medical therapies or unconventional dosages of fg-SRLs in a real-life setting. In selected patients, the efficacy of the drug is higher than previously reported in clinical trials, allowing a simplification of the treatment schedule, which is well accepted by the patients and might, therefore, result in an increased compliance. The observed (and expected) increase in fasting plasma glucose and HbA1c can be managed in the vast majority of cases by the use of lifestyle interventions and metformin as single antidiabetic agent. Younger age and male sex emerged as favorable predictors for the maintenance of glucose homeostasis, highlighting the importance of a careful patient selection to tailor the most appropriate therapeutic regimen in acromegaly.

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Competing interests Diego Ferone has been a speaker for and participated on advisory boards and received research grants from Novartis AAA, Ipsen, Camurus and Recordati. Diego Ferone is a member of the Editorial Board of Journal of Endocrinological Investigation. Annamaria Colao received research grants from Novo Nordisk and Ipsen. Rosario Pivonello has been the Principal Investigator of Clinical and/or Translational Research Studies for Novartis, HRA Pharma, Ipsen, Shire, Corcept Therapeutics, Cortendo AB, Janssen Cilag, Camurus, Strongbridge, and Pfizer; Co-investigator of Research Studies for Pfizer; received research grants from Novartis, Pfizer, Ipsen, HRA Pharma, Shire, IBSA, Strongbridge Biopharma; has been an occasional consultant for Novartis, Ipsen, Pfizer, Shire, HRA Pharma, Cortendo AB, Ferring, Strongbridge Biopharma, Recordati, Corcept Therapeutics, Crinetics Pharmaceuticals, ARH Healthcare, Biohealth Italia; and has received fees and honoraria for presentations from Novartis, Shire, Pfizer and Recordati beyond the confines of this work. The other Authors have no conflicts of interest to declare.

Informed consent Informed consent was obtained from all individual participants included in the study.

		Before PAS			3-month follow-up			6-month follow-up			>6-month follow-up		
#	Sex	DM	HbA1c (%)	Ongoing AD therapy	DM	HbA1c (%)	Therapeutic adjustments	DM	HbA1c (%)	Therapeutic adjustments	DM	HbA1c (%)	Therapeutic adjustments
1	M	No	5.3	--	No	5.9	--	No	6.2	--	No	6.0	--
2	M	No	6.0	--	No	6.4	--	Yes	6.7	Metformin 1000 mg/d	Yes	6.3	Metformin 1000 mg/d
3	F	No	5.6	--	No	5.7	--	No	6.0	--	No	5.6	--
4	F	No	5.6	--	Yes	6.8	PAS withdrawn (lack of efficacy, hyperglicaemia)	No	6.0	--	No	5.6	--
5	M	No	5.5	--	No	5.8	--	No	6.3	--	Yes	6.7	Diet
6	M	No	5.5	--	No	5.8	--	No	5.7	--	No	5.9	--
7	F	No	5.9	--	No	6.3	--	Yes	6.2	Linagliptin 5 mg/d (intolerance to metformin and sitagliptin, started at month 4)	Yes	7.7	Repaglinide 2 mg/d (intolerance to linagliptin)
8	M	No	5.4	--	No	6.1	--	No	6.3	--	No	5.9	--
9	F	No	6.0	--	Yes	6.7	Diet	Yes	6.9	Diet	Yes	7.2	Metformin 1000 mg/d
10	F	No	5.7	--	Yes	6.9	Metformin SR 750 mg/d	Yes	5.9	PAS withdrawn at month 4 (GI side effects)	--	--	--
11	F	No	5.1	--	Yes	7.0	Metformin SR 1000 mg/d	Yes	7.1	Metformin SR 1500 mg/d (poorly tolerated)	Yes	7.1	Metformin SR 1000 mg/d
12	M	No	5.7	--	No	6.2	--	No	6.1	--	--	--	--
13	F	Yes	7.0	Metformin 2000 mg/d	Yes	11.4	Metformin 2000 mg/d Insulin glargine 21 U/d	Yes	8.6	Metformin 2000 mg/d Insulin glargine 21 U/d	--	--	--

Table 3. Glycemic status and therapeutic changes before and after switching to PAS

14	F	Yes	5.9	Metformin 1000 mg/d	Yes	9.2	Metformin 3000 mg/d	Yes	8.0	Metformin 3000 mg/d Liraglutide 1.2 mg/d	n.a.	n.a.	PAS withdrawn (after 7 months)
15	F	No	5.6	--	n.a.	n.a.	--	No	6.0	--	Yes	6.6	Metformin 1000 mg/d
16	M	No	5.7	--	No	5.6	--	No	5.9	--	No	n.a.	--
17	F	Yes	5.8	Metformin 1000 mg/d Sitagliptin 100 mg/d	Yes	n.a.	Metformin 1000 mg/d Sitagliptin 100 mg/d	Yes	5.7	Metformin 1000 mg/d Sitagliptin 100 mg/d	Yes	6	Metformin 1000 mg/d Sitagliptin 100 mg/d
18	F	No	5.4	--	No	5.3	--	No	5.3	--	Yes	5.2	Metformin 500mg/d (started at month 9 for HbA1c >6.5%)
19	F	No	5.8	--	Yes	6.3	Metformin 750 mg/d (started at month 1 for HbA1c >6.5%)	Yes	6.3	Metformin 1000 mg/d	Yes	5.7	Metformin 1000 mg/d
20	M	No	n.a.	--	No	5.4	--	No	5.3	--	No	5.5	--
21	M	No	6.1	--	No	6.2	--	Yes	6.2	Metformin 1000 mg/d (started at month 5 for HbA1c >6.5%)	Yes	5.9	Metformin 1000 mg/d

Legend. PAS: pasireotide, DM: diabetes mellitus, HbA1c: glycated haemoglobin, AD: anti-diabetic, M: male, D: day, F: female, SR: slow release, GI: gastrointestinal, n.a.: not avaiable

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Conclusion

Acromegaly still remains a challenging endocrine disease to recognise, manage and treat. Patients often require multimodal treatment to optimally achieve therapeutic goals, reduce excess morbidity and improve quality of life. Recommendations for the management of acromegaly have changed considerably since the advent of pasireotide. Patients now have more treatment options and are more likely to achieve biochemical control. An optimal strategy must aim to adapt therapeutic approaches by considering the entire clinical spectrum of the disease. In addition, effective management of comorbidities should lead to further reductions in morbidity and mortality and improvements in QoL. Further studies will help to better define the patient populations most likely to benefit from each treatment strategy and to tailor acromegaly treatments to the needs of individual patients.