SAPIENZA
UNIVERSITÀ DI ROMA

DOTTORATO DI RICERCA IN SCIENZE ENDOCRINOLOGICHE
XXXII CICLO

Facoltà di Medicina e Odontoiatria
Dipartimento di Medicina Sperimentale

Direttore: Prof. Andrea Lenzi

Tesi di Dottorato

The heart in rare endocrine diseases: Cardiometabolic studies in Cushing's syndrome and Acromegaly

Relatore
Prof. Andrea M. Isidori

Candidata
Dott.ssa Alessia Cozzolino
Matr. N.1749357

A.A. 2019-2020

Abstract

Introduction: Cushing's syndrome (CS) and acromegaly are two rare endocrine diseases associated to increased morbidity and mortality, mainly for cardiovascular events. Cardiac magnetic resonance (CMR) is the established non-invasive gold standard method for measuring (left ventricle) LV volume, cardiac function and LV mass (LVM) due to its higher accuracy and reproducibility and lower variability in comparison with echocardiography.

Aim: The aim of the current study was to evaluate the metabolic profile and to perform a cardiological study through CMR in patients with CS and acromegaly.

Materials and methods: This was a prospective multicentric case-control study. Consecutive patients with CS and acromegaly, both cured and with active disease, entered the study. The control group included patients with non-functioning adrenal incidentaloma matched with patients for sex, age and BMI. Metabolic and clinical parameters and CMR parameters have been compared between patients and controls.

Results: Sixteen patients with CS, 20 patients with acromegaly and 18 controls entered the study. Fasting glucose levels were significantly lower in CS patients than controls (p=0.003), whereas they were significantly higher in acromegaly patients than controls (p=0.033). No significant differences were found neither in lipid levels nor in systolic and diastolic blood pressure levels between patients and controls. No significant differences in the prevalence of cardiometabolic complications were found between patients and controls. LV end-systolic volume (LV-ESV) and LV-ESV indexed with respect to the body surface (LV-ESVi) were significantly higher in CS patients than controls (p=0.041; p=0.030). Right ventricle end-diastolic volume (RV-EDV), RV-EDVi, RV-ESV and RV-ESVi were significantly higher in CS patients than controls (p=0.025; p=0.033; p=0.004; p=0.008). LV-EDV, LV-EDVi, LV-ESV and LV-ESV were significantly and markedly higher in acromegaly patients than controls (p=0.001; p=0.003; p=0.001; p=0.001). LVM was significantly higher in acromegaly patients

than controls (p=0.002). RV-EDV, RV-EDVi, RV-ESV and RV-ESVi were significantly and markedly higher in acromegaly patients than controls (p=0.000; p=0.001; p=0.000; p=0.000). Consequently, acromegaly patients had a significantly lower RV-EF than controls (p=0.002). Moreover, a significant correlation was found between IGF-1 levels and cardiac parameters at CMR in acromegaly patients.

Conclusions: CS and acromegaly have been demonstrated to have biventricular cardiac structural and functional impairment at CMR, which seem to have a multifactorial pathogenesis: the presence of disease-related cardiovascular risk factors and a direct effect of hormone excess. The results of the current studies suggest that CMR may have a place in the cardiac work-up of selected patients with rare endocrine diseases, such as CS and acromegaly.

Summary

1. Introduction

1.1 Cushing's syndrome

Epidemiology and clinical picture

Cushing's syndrome (CS), or chronic endogenous hypercortisolism, is a serious endocrine disease caused by chronic and autonomous hypersecretion of cortisol from the adrenal glands, with an estimated prevalence of around 40 cases per million and an estimated incidence of 0.7–2.4 cases per million per year, although the worldwide epidemiology has not been fully determined (1-4). CS is at least three times more prevalent in women than in men, and although it can occur at any age, is more frequent during the fourth to sixth decades of life (1-4). In the great majority of cases (around 70%), CS is caused by a pituitary tumour secreting adrenocorticotropic hormone (ACTH) that stimulates excessive cortisol secretion from the adrenal cortex, which is defined as pituitary-dependent CS or Cushing's disease (CD). ACTH-independent adrenal production of cortisol by an adrenal tumour or bilateral adrenal hyperplasia is responsible for around 20% of cases of CS. An extrapituitary tumour secreting ACTH or, very rarely, corticotropin-releasing hormone, causes ectopic CS in the remaining 10% of cases (1-5). CS can also be caused by excessive exposure to exogenous glucocorticoids, which is defined as exogenous CS (1-3).

The clinical picture of CS is characterized by weight gain with central obesity, fatigue with proximal myopathy, skin thinning with purplish *striae* and easy bruising. Several comorbidities are associated with CS (1-3,5) and are responsible for an impairment of quality of life and an increase in mortality (1-3,5-7). The diagnosis and determination of the origin of CS can be challenging and time-consuming, requiring different laboratory tests and imaging procedures (8-10). Prompt and effective treatment of CS is crucial for the reversal of comorbidities, prevention of serious acute and chronic complications, and protection from the increased mortality risk (3,5,11). Notably, the increased mortality and

morbidity that affect patients with CS during the active phase of the disease might not completely revert after disease remission. The reasons why surely morbidity and possibly mortality remain increased after remission of CS remain unclear (3,5,12,13). CS is associated with increased mortality, mainly due to cardiovascular or infectious diseases, and their systemic consequences, mainly myocardial infarction, stroke, and sepsis (3,5,14). The excess mortality usually concerns patients with no initial surgical remission, whereas in patients with postoperative hormonal control, mortality was described to be either increased or similar to that in the general population. Cardiovascular disease is the major cause of death in patients with CD, either during active disease or after remission. Infectious diseases and sepsis represent frequent causes of death and suicide associated with psychiatric disorders has also been described in patients with CD (3,5,6,14,15). The main predictive factors for mortality have been identified as older age at diagnosis, the presence and duration of active disease and the presence of comorbidities, mainly hypertension and diabetes (3,5).

Glucocorticoids are involved in metabolism regulation and chronic hypercortisolism can lead to a specific form of the metabolic syndrome (3). Glucocorticoid excess affects a range of metabolic pathways determining the different manifestations of this metabolic syndrome. Glucocorticoids stimulate key enzymes involved in liver gluconeogenesis, increasing glucose output and circulating glucose levels and cause hepatic and peripheral insulin resistance by direct and indirect mechanisms (3,16). In adipose tissue and skeletal muscle, glucocorticoids reduce amino acid uptake and increase lipid oxidation and lipolysis, whereas in the liver, they promote lipoprotein secretion and stimulate enzymes involved in fatty acid synthesis, contributing to the development of liver steatosis and impairing insulin sensitivity (3). These processes all contribute to glucocorticoid-induced insulin resistance, a major feature of the metabolic syndrome (3).

Weight excess, as documented by the pathological increase in BMI, is among the most common features of CS; indeed, weight excess is seen in 57–100% of patients (3). The obesity associated with CS is abdominal rather than generalised weight gain, with preferential visceral rather than subcutaneous accumulation of fat tissue (3).

An impairment of glucose metabolism has been described in 27–87% of patients with CS (3). Glucose and insulin levels have been described to be higher in patients with CD compared with sex and age-matched controls and compared with BMI-matched controls after glucose loading, suggesting that some effects are independent of weight (3,17). Remission from hypercortisolism can improve, but does not always normalise the impairment in glucose homeostasis (3,13).

Dyslipidaemia has not been extensively investigated, but it is described in 12–72% of patients with CS (3). Dyslipidaemia in CS is commonly characterised by increased total and LDL cholesterol and triglyceride levels and reduced HDL cholesterol levels (3,17,18). After short-term (1-year) or long-term (5-year) remission, total cholesterol and LDL cholesterol levels have been demonstrated to be higher than in sex-matched and age-matched, but not BMI-matched controls, suggesting that the persistence of obesity might contribute to the persistence of abnormal lipid profile (3,12,17).

Cardiovascular disease

Cardiovascular disease is commonly reported as the main cause of death in patients with CS. Indeed, the increased mortality has traditionally been attributed to chronic damage from hypertension, in particular vascular atherosclerosis and cardiac remodelling and dysfunction (3,19-21). A range of changes in metabolic, haemodynamic, and coagulatory pathways induced by glucocorticoid excess are responsible for hypertension as well as vascular and cardiac disease and thrombosis diathesis (3).

Hypertension is a very common clinical feature of CS, occurring in 25–93% of patients (3,19-21). Most studies showed that systolic and diastolic blood pressure was raised to a similar extent in these patients, with loss of the physiological nocturnal decrease being an early feature (non-dipper blood pressure profile) (3,20,22). The main mechanisms involved in the pathogenesis of hypertension in CS include the modification induced by glucocorticoid excess in the renin-angiotensin system, the mineralocorticoid activity, the sympathetic nervous system and the vasoregulatory system (3,20). Remission from hypercortisolism can improve hypertension but it does not always normalise. In fact, the presence of hypertension has been reported in 25–54% of patients in remission from CS (3,12,17).

CS is associated with an increased risk for myocardial infarction and cardiac failure (3,23). Concentric left ventricle hypertrophy, together with a decrease in systolic strain and impairment in diastolic filling caused by an abnormal relaxation pattern, has been described in CS (3,24-26). Patients with CS develop a more pronounced left ventricle hypertrophy than do hypertensive controls, suggesting that hypertension is not the only factor determining cardiac hypertrophy and consequent dysfunction (3,24). Increased myocardial fibrosis, caused by an enhanced responsiveness to angiotensin II and activation of the mineralocorticoid receptor in direct response to cortisol excess, has been proposed as an underlying cause of the cardiac damage (3).

Vascular atherosclerosis is a common feature of CS. An increased prevalence of well-defined vascular wall plaques has been reported in patients with CS (3,17). The intima-media thickness of both carotid and aortic arteries can be increased in CS (3,17). A major role of insulin resistance in the development of vascular damage has been suggested, but different factors such as glucocorticoid-induced endothelial dysfunction, enhancement of arterial stiffness, thrombosis diathesis, increase in homocysteine and decrease in taurine

levels could also have a role (3,27,28). The vascular damage is probably the cause of the increased risk of stroke associated with CS (3,23). The described cardiovascular changes are only partly reversible after successful treatment. Myocardial fibrosis and cardiac abnormalities showed a partial improvement after successful treatment of CS (29), whereas vascular intima media thickness remained increased compared with controls for up to 5 years (3,12,17).

CS is associated with a more than ten-fold increased risk of venous thromboembolism compared with general population (3,12,17,30). Thromboembolic events have been reported in 6–20% of patients with CS, particularly in the early postoperative period (3). The increased cardiovascular mortality in CS is also attributed to an increased thrombotic risk (3,30,31). Many alterations of coagulation and fibrinolysis occur in CS (3,31). Increased activity of endogenous coagulation inhibitors has also been reported, probably as a compensatory mechanism for the increased coagulatory factors (3,31). Haemostatic abnormalities seem to improve 1 year after remission, although they do not fully normalise (3,30). Successful pharmacological treatment does not seem to improve the hypercoagulable state and this might be partly explained by persistence of the metabolic syndrome (3,31).

Prompt and effective treatment of cortisol excess is crucial for the reversal of comorbidities, prevention of serious acute and chronic complications and protection from the increased mortality risk associated with CS (3,5). Surgical resection of the causal lesion is generally the first-line approach. The choice of second-line treatments, including medical therapies, bilateral adrenalectomy and radiation therapy (for pituitary corticotrope tumors), must be individualized to each patient (5,11,32). Treatment specific to the various comorbidities should be provided in parallel with therapy targeting cortisol excess to accelerate their resolution or improvement and reduction of mortality risk

(3,5,11). Nevertheless, comorbidities can persist in a subgroup of patients even after remission, necessitating continuing management (3).

1.2 Acromegaly

Epidemiology and clinical picture

Acromegaly is a slowly progressive disease characterized by increased release of growth hormone (GH) and, consequently, insulin-like growth factor I (IGF1), which in the majority of cases is induced by a GH-secreting pituitary tumour and more rarely by pituitary hyperplasia or ectopic secretion of GH or GH-releasing hormone (33). Prolonged exposure to excess hormone leads to progressive somatic disfigurement and a wide range of systemic complications that are associated with increased mortality (33). Although considered a rare disease, recent studies have reported an increased incidence of acromegaly owing to better disease awareness, improved diagnostic tools and perhaps a real increase in prevalence (33). At the time of diagnosis, patients usually present with acral overgrowth, including exaggerated growth of the hands and feet; facial overgrowth, including prognatism (a protruding jaw); and soft-tissue hypertrophy (33-35). Other possible symptoms include hyperhidrosis (excessive sweating), goitre, osteoarthritis, carpal tunnel syndrome, fatigue, colon polyps, sleep apnoea, reproductive disorders, metabolic disturbances and cardiovascular disease, which most commonly includes cardiac hypertrophy, hypertension and arrhythmias, although congestive heart failure also occurs rarely (33,35-37).

Studies published in the 1980s and 1990s reported a global annual incidence of acromegaly of 0.28–0.4 cases per 100,000 individuals (33,38,39), whereas the global annual incidence reported in more recent studies is much higher, up to 1.1 cases per 100,000 individuals (40,41).

A median age at diagnosis of 40–50 years has been consistently reported (33,41,42). Interestingly, on the basis of the duration of symptoms until diagnosis, modern epidemiological studies seem to show a decrease in the delay until diagnosis (median estimated interval ≤5 years), although in some cases much longer intervals (up to 25 years) have been reported (33). This estimate is based mainly on patient recollection rather than on medical records. As the duration of active disease is the main determinant of the severity of most acromegaly complications, including the larger tumour volume at diagnosis (33,34), these data emphasize that further education and improved awareness of the disease among clinicians and patients is important (33,43).

The most common complications associated with acromegaly include cardiovascular, respiratory and metabolic comorbidities that are among the main clinical conditions responsible for the increase of mortality associated with the disease (35,36). Cardiovascular disease represents the most prevalent comorbidity, accounting for up to 80% of complications and has historically reported to contribute to nearly 50% of deaths (36,44). However, according to a recent 20-year follow-up study (36,45), causes of death in patients with acromegaly progressively shifted from 44% cardiovascular and 28% neoplastic during the first decade, to 23% cardiovascular and 35% neoplastic during the second decade.

Cardiovascular disease associated with acromegaly is mainly characterized by a cardiac damage, represented by a typical cardiomyopathy, which progressively develops during the disease course, with a minor impact of vascular damage, which may worsen the cardiac disease and increase the cardiovascular risk associated with the disease (36).

Acromegaly is frequently associated with deterioration of glucose and lipid metabolism. The insulin resistance represents the main pathogenetic mechanism for glucose intolerance, diabetes mellitus (DM) and dyslipidemia, which are common features of

acromegaly and represent risk factors for the increased cardiovascular morbidity and mortality (36).

The disorders of glucose metabolism associated with acromegaly include impaired glucose tolerance (IGT), impaired fasting glucose (IFG) and DM. The prevalence DM in acromegaly differs greatly among studies, ranging from 16% to 56% and this variability is explained by heterogeneity of the study populations and differences in the criteria used for the diagnosis (35,36). The direct action of GH is mainly diabetogenic by increasing lipolysis and inducing insulin resistance (36,46), whereas the indirect actions of GH, via increased IGF-I, may in turn facilitate insulin action (35,36).

Medical treatment of acromegaly may variably influence glucose metabolism. The effect of the different medical therapies of acromegaly on a complete panel of metabolic outcomes was recently investigated in two meta-analyses: the first evaluated the effect of somatostatin analogs (SSAs) and the second the effect of pegvisomant (PEG), both in monotherapy and in combination with SSAs (47,48). SSAs were found to reduce insulin levels and increase HbA1c and after-load glucose while improving disease control, without affecting FPG (47). In contrast, PEG in monotherapy or combined with SSAs improved glucose metabolism, reducing FPG, HbA1c, FPI and HOMA-I; this effect was independent of disease control (48).

Surgical treatment appears to improve both glucose tolerance and DM prevalence (36).

The disorders of lipid metabolism associated with acromegaly mainly include hypertriglyceridemia and decrease of HDL-cholesterol (35,36). The prevalence of hypertriglyceridemia in acromegaly is three-times higher than that of the general population and ranges from 33% and 40% of patients, whereas the prevalence of low HDL-cholesterol ranges from 39% to 47% (35,36). Moreover, acromegaly is also associated with alteration of the lipoprotein metabolism, particularly with an increase of

circulating levels of Lp-a, Apo A-I, and Apo E, involved in the transport of triglycerides and cholesterol, as well as small dense LDL particles, possibly as a consequence of insulin resistance, so contributing to the development of vascular damage (36,49).

Control of acromegaly, induced by either pituitary surgery or medical therapy improves dyslipidemia (35,36).

Cardiovascular disease

Acromegaly is associated with a typical cardiomyopathy, characterized by biventricular hypertrophy, mainly involving the LV in 80% and consequent diastolic dysfunction, in 44% of patients with active disease (33,35-37); hypertension, valvulopathies, arrhythmias and vascular endothelial dysfunction represent additional relevant cardiovascular complications, which, together with the respiratory and metabolic complications, contribute to the development of cardiac disease and the increase in cardiovascular risk in acromegaly (33,35,36).

The pathogenesis of acromegalic cardiomyopathy includes either a direct action of GH and IGF-I excess on the heart, or indirect mechanisms by which GH and IGF-I excess induces hypertension and disorders of glucose and lipid metabolism, resulting in cardiac glucotoxicity and lipotoxicity and cardiac remodelling and hypertrophy (36,50).

The pathogenesis of acromegalic cardiomyopathy has been proposed to develop after three steps (35,36). The early phase, which is reversible, is characterized by initial cardiac hypertrophy, with increase of heart rate and systolic output, altogether configuring the hyperkinetic syndrome (35,36). In the middle phase of untreated or uncontrolled disease, cardiac hypertrophy becomes more evident and signs of diastolic dysfunction with the appearance of systolic dysfunction on effort (35,36). In the end-stage of untreated or uncontrolled disease, cardiac damage may include systolic dysfunction at rest and heart

failure until the development of dilative cardiomyopathy, which is not reversible even with the treatment of the disease (35,36).

At diagnosis, cardiac hypertrophy is a common feature in patients with acromegaly, mainly in those with a long disease history (36,51). Acromegalic heart has thickened walls but rarely enlarged chambers (36,52). At histology, the most relevant abnormalities are interstitial fibrosis, increased extra-cellular collagen deposition, myofibrillar derangement, and areas of monocyte necrosis and lympho-mononuclear infiltration, gradually impairing the whole organ architecture (36).

Coexistence of cardiovascular comorbidities, including hypertension, valvulopathy, arrhythmias, together with vascular endothelial dysfunction and disorders of glucose and lipid metabolism, may worsen cardiomyopathy (36,53).

Hypertension is one of the most common cardiovascular comorbidities in acromegaly, with an average prevalence of approximately 35%, ranging from 18% to 60% (36,54). Pathogenic mechanisms responsible for hypertension in acromegaly are yet to be fully elucidated, but the chronic exposure to GH and IGF-I excess has been suggested to act directly in the kidneys by exerting a potential antidiuretic and antinatriuretic effect (36), or indirectly by leading to the expansion of plasma volume and increasing responsiveness to angiotensin action and consequently increasing peripheral resistance (36,54).

Arrhythmias frequently characterize the acromegalic cardiomyopathy. Cardiac arrhythmias affects up to nearly 90% of patients with active disease (36,55). This complication is consequence of the interstitial fibrosis, myofibrillar derangement and cardiac hypertrophy, contributing to induce anatomical changes in cardiomyocytes possibly leading to abnormalities in cardiac conduction (36,55).

The most striking functional disturbance in acromegalic cardiomyopathy is the diastolic dysfunction, manifested by inadequate ventricular filling capacity, as mainly demonstrated

by the decrease of early (E) to late or atrial (A) peak velocities ratio (E/A) and the prolongation of the isovolumetric relaxation time (IVRT) (35,36,50). LV hypertrophy rarely leads to systolic dysfunction until heart failure in approximately 10% of acromegalic patients (35,36), particularly in the end-stage of acromegalic cardiomyopathy in patients with untreated or uncontrolled disease (35,36). The relative risk to develop systolic dysfunction has been demonstrated to be higher in patients with acromegaly compared with control subjects (36,56).

Disease duration plays a pivotal role in the determination of acromegalic cardiomyopathy, since it is correlated with the prevalence of hypertension, DM and cardiac complications, including valvulopathy, arrhythmias, diastolic and systolic dysfunction, suggesting a potential cumulative effect of the exposure to chronic hormone excess (36).

Vascular disease has been described in acromegaly, although it seems to exert a relatively minor role in the determination of cardiovascular disease. The most common vascular damage described in acromegaly is the vascular endothelial dysfunction; although endothelial dysfunction, together with oxidative stress, represents the main underlying mechanism of atherosclerosis, a clear-cut vascular atherosclerosis represents a controversial finding in acromegaly (36).

Control of acromegaly, induced by either pituitary surgery or medical therapy with conventional SSAs, or PEG improves cardiac structure and performance (35,36,57), as well vascular damage (36,58).

2. Materials and methods

2.1 Study design and population

This was a prospective multicentric case-control study. Consecutive patients with CS and acromegaly, both cured and with active disease, were recruited from the outpatient endocrinology clinic of the Department of Experimental Medicine at "Sapienza" University of Rome and from the outpatient endocrinology clinic of the Department of Clinical Medicine and Surgery at "Federico II" University of Naples from September 2014 to January 2020. Diagnosis of CS and acromegaly was made according to current criteria (10,59). Inclusion criteria were: patients older than age 18 years with CS or acromegaly. Exclusion criteria were: contraindications to cardiac magnetic resonance (CMR). The control group included patients with non-functioning adrenal incidentaloma, diagnosed according to current criteria (60), undergoing the follow-up imaging for the adrenal lesion, matched with patients for sex, age and BMI. Sixteen patients with CS, 20 patients with acromegaly and 18 controls entered the study. All patients and controls provided written informed consent after full explanation of the purpose and nature of all procedures used. The study has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

2.2 Study procedures

Clinical and laboratory assessment

All patients and controls underwent an accurate history, including drugs use, comorbidities (hypertension, glucose metabolism impairment, dyslipidemia) and cardiovascular risk factors (eg. smoking habit). Diagnostic procedures included: physical examination with measurement of anthropometric parameters (weight, waist circumference, hip circumference) and vital signs (blood pressure, heart rate); blood

sampling for assessing glucose and lipid metabolism, liver, renal, hematopoietic and coagulative function, thyroid and androgen hormones, serum cortisol, ACTH, urinary free cortisol (UFC), GH and IGF-1. Homeostatic model assessment of insulin resistance (HOMA-I) has been calculated according the formula: [fasting plasma glucose (in mg/100ml) x fasting insulin (in μUI/L)]/405. Clinical and laboratory findings and the prevalence of cardiometabolic complications have been compared between patients and controls (CS vs controls and acromegaly vs controls) and between cured and active patients (Cured CS vs active CS and cured acromegaly vs active acromegaly).

Cardiac evaluation

All patients and controls underwent cardiac evaluation with CMR imaging. CMR imaging studies were performed with a 1.5-T clinical magnetic resonance imaging system (Avanto, Siemens, Healthcare Solutions, Erlangen, Germany). During the examination an ECG device was used for cardiac gating and all acquisitions were made in apnea at the end of inspiration. The following have been carried out:

- Acquisitions along the short axis with balanced technique "steady state free precession (SSFP)", covering the entire left ventricle (LV) and right ventricle (RV) for the evaluation of the ventricular volumes. The end-diastolic volume (EDV), end-systolic volume (ESV), cardiac output, ejection fraction (EF) and the mass of LV (LVM) were quantified and indexed with respect to the body surface;
- "Inversion-recovery segmented gradient-echo" of T1-weighted sequences 10-15 minutes after the intravenous injection of paramagnetic contrast medium, to exclude ischemic cardiomyopathy with a semi-quantitative method;
- “Shortened Modified Look-Locker Inversion recovery sequence (ShMOLLI)” technique for T1-mapping for the subsequent detection of diffuse fibrosis.

T1-mapping for the evaluation of fibrosis

The T1-mapping technique has been used to non-invasively quantify the degree of myocardial fibrosis. The measured extracellular volume fraction (ECV) is highly sensitive and is used as an indicator of diffuse myocardial fibrosis. T1-mapping is automatically calculated as the average of the intensity of the individual pixels with and without contrast medium in T1 and expressed in msec (CMR 42 SW). The calculation of the ECV is performed using the mathematical formula using the hematocrit value [DR1 myocardium: (1/T1 myocardial-post) - (1/T1 myocardial-pre); DR1 blood: (1/T1 blood-post) - (1/T1 blood-pre); Myocardial partition coefficient (λ) = (DR1 myocardial/DR1 blood); ECV = (1 - hematocrit) x (λ)].

CMR findings have been compared between patients and controls (CS vs controls and acromegaly vs controls) and between cured and active patients (Cured CS vs active CS and cured acromegaly vs active acromegaly).

2.3 Statistical analysis

All continuous variables are expressed as their mean and standard deviation (SD) and dichotomous variables as frequencies and percentages when relevant. Student's t test or the Mann-Whitney U test was performed to compare numerical variables between patients and controls and between cured and active patients. Differences between qualitative variables were evaluated by χ^2 statistics. Correlations between numerical variables were analyzed using Pearson's correlation test. The statistical significance was set at $p<0.05$. Statistical analyses were performed using SPSS 20.0 for MacOS (SPSS Inc.).

3. Results

3.1 Cushing's syndrome study

3.1.1 Biochemical and clinical evaluation

Sixteen patients with CS (12 females, 4 males) with a mean age of 48 years (range 20-71 years) entered the study. Twelve patients (75%) had CD and four (25%) had cortisol secreting adrenal adenoma. Eleven patients (69%) were cured and five (31%) had active disease. Among active patients, three were on cabergoline treatment and one was on metyrapone. One patient was naïve. Patients' characteristics are summarized in **Table 1**. Nine patients (56%) had arterial hypertension and were on antihypertensive treatment. Seven patients (44%) had dyslipidemia, five of whom were on lipid-lowering therapy. One patient (6%) had diabetes and was on incretins therapy and five patients (31%) had an impaired fasting glucose. Five patients (31%) were obese (BMI≥30 Kg/m^2).

Biochemical and clinical parameters of patients and controls are compared in **Table 2**. As expected, sex, age, and BMI were similar in the patients and controls. Fasting glucose levels were significantly lower in patients than controls (p=0.003). Conversely, HbA1c, fasting insulin and HOMA-I were not significantly different in the two groups. No significant differences were found neither in lipid levels nor in systolic and diastolic blood pressure levels between patients and controls. No significant differences in the prevalence of cardiometabolic complications (hypertension, dyslipidemia and impairments of glucose metabolism) were found between patients and controls.

The comparison between cured and active patients showed that the latter had significantly higher systolic and diastolic blood pressure levels (p=0.045; p=0.026). No significant differences were found either in the other biochemical parameters or in the prevalence of cardiometabolic complications.

3.1.2 Cardiac evaluation

Cardiac parameters of patients and controls are compared in **Table 3**. LV-ESV and LV-ESV indexed with respect to the body surface (LV-ESVi) were significantly higher in patients than controls (p=0.041; p=0.030). Conversely, LV-EDV and LV-EDVi were not significantly different in patients and controls. RV-EDV and RV-EDVi were significantly higher in patients than controls (p=0.025; p=0.033) and, concomitantly, RV-ESV and RV-ESVi were markedly higher in patients than controls (p=0.004; p=0.008) (**Figure1**). Consequently, patients had a trend toward lower RV-EF than controls (p=0.067). No patient had LV hypertrophy according to conventional CMR thresholds defining LV hypertrophy (61).

Evaluation with T1 mapping technique did not reveal any significant difference between patients and controls. No patient had ECV greater than 30%, indicating the presence of myocardial fibrosis (62).

The comparison between cured and active patients showed no significant differences in cardiac parameters.

3.2 Acromegaly study

3.2.1 Biochemical and clinical evaluation

Twenty patients with acromegaly (7 females, 13 males) with a mean age of 50 years (range 31-75 years) entered the study. Six patients were cured and fourteen had active disease. Among active patients, three were on first generation SSAs, four patients were on combined therapy with first generation SSAs and PEG, two patients were on pasireotide therapy, three patients were on combined therapy with pasireotide and cabergoline and one patient was on combined therapy with first generation SSAs and cabergoline. One patient was naïve. Patients' characteristics are summarized in **Table 4**. Ten patients (50%) had arterial hypertension, nine of whom were on antihypertensive treatment. Nine patients (45%) had dyslipidemia, four of whom were on lipid-lowering therapy. Three

patients (15%) had diabetes, two of whom were on metformin treatment and one was on incretins therapy; one patient (5%) had an impaired glucose tolerance and six patients (30%) had an impaired fasting glucose.

Biochemical and clinical parameters of patients and controls are compared in **Table 5**. As expected, sex, age, and BMI were similar in patients and controls. Fasting glucose levels were significantly higher in patients than controls (p=0.033). Conversely, HbA1c, fasting insulin and HOMA-I were not significantly different in the two groups. No significant differences were found neither in lipid levels nor in systolic and diastolic blood pressure levels between patients and controls. No significant differences in the prevalence of cardiometabolic complications (hypertension, dyslipidemia and impairments of glucose metabolism) were found between patients and controls.

The comparison between cured and active patients showed that the latter had significantly higher fasting glucose and HbA1c levels (p=0.001; p=0.018). No significant differences were found either in the other biochemical parameters or in the prevalence of cardiometabolic complications.

3.2.2 Cardiac evaluation

Cardiac parameters of patients and controls are compared in **Table 6**. LV-EDV and LV-EDVi were significantly and markedly higher in patients than controls (p=0.001; p=0.003). Concomitantly, LV-ESV and LV-ESVi were significantly and markedly higher in patients than controls (p=0.001; p=0.001). LVM was significantly higher in patients than controls and LVMi was a trend toward significantly higher in patients than controls (p=0.002; p=0.056). Consequently, LV stroke volume (LV-SV) was significantly higher in patients than controls (p=0.001). Only one patient had LV hypertrophy according to conventional CMR thresholds defining LV hypertrophy (61). Differences in RV parameters between controls and patients were similar to the differences in LV parameters. RV-EDV

and RV-EDVi were significantly and markedly higher in patients than controls (p=0.000; p=0.001) and, concomitantly, RV-ESV and RV-ESVi were significantly and markedly higher in patients than controls (p=0.000; p=0.000). Consequently, patients had a significantly lower RV-EF than controls (p=0.002) (**Figure 2**). RV-SV was significantly higher in patients than controls (p=0.010).

Evaluation with T1 mapping technique did not reveal any significant difference between patients and controls. Three patients (19%) had ECV greater than 30%, indicating the presence of myocardial fibrosis (62).

A significant correlation was found between IGF-1 levels and cardiac parameters at CMR in acromegaly patients. In particular, IGF-1 was significantly directly correlated to LV-EDV (r=0.55; p=0.013) and to LV-EDVi (r=0.47; p=0.037). Concomitantly, IGF-1 was significantly directly correlated to LVM (r=0.56; p=0.010), LVMi (r=0.45; p=0.044) (**Figure 3**) and LV-SV (r=0.61; p=0.004). As regards the RV, IGF-1 was found to have a significant direct correlation with RV-EDV (r=0.50; p=0.024) and RV-SV (r=0.56; p=0.011). Moreover, HDL levels were found to have a significant direct correlation with RV-EF (r=0.56; p=0.012), whereas, triglycerides levels were found to have a significant inverse correlation with RV-EF (r=-0.48; p=0.038).

The comparison between cured and active patients showed that the latter had significantly higher LVMi (p=0.013).

4. Discussion

4.1 Cushing's syndrome study

The current study shows that patients with CS, compared with sex, age and BMI-matched controls, have a biventricular impairment detected at CMR. CMR is the established non-invasive gold standard method for measuring LV volume, cardiac function and LVM due to its higher accuracy and reproducibility and lower variability in comparison with echocardiography (63). To date, few studies evaluating small cohorts have analyzed patients with CS using CMR (64,65). Kamenicky and coworkers found that, compared with controls, patients with CS had lower LV, RV, and LA ejection fractions and increased end-diastolic LV segmental thickness (64). The current study partially confirmed the results of the previous study. Indeed, we found that patients had higher LV end-systolic volumes and higher RV end-systolic and end-diastolic volumes compared with controls. Moreover, our results showed a trend toward lower RV ejection fractions in patients than controls.

Another study from the same group evaluated ten patients with active CD and performed a CMR study using T1 mapping technique. They found that native myocardial T1 was increased in CD independently from hypertension, supporting the potential role of diffuse fibrosis and increased extracellular water in this disease (65). Conversely, we didn't find any significant difference between patients and controls in T1 mapping evaluation in our cohort and none of our patients had ECV higher than 30%, suggesting the presence of fibrosis.

Several echocardiographic studies have evaluated cardiac structure and function in patients with CS and found LV systolic and diastolic dysfunction (66-70). Cardiac structure and function assessment by echocardiography is more practical on a clinical basis. Nevertheless, CMR permits an evaluation of ventricular mass and volumes free of

cardiac geometric assumption, ensuring a higher accuracy and reproducibility (71). Moreover, since ultrasound measurement of RV volumes is challenging, CS echocardiographic studies mainly focused on the LV (66-70), whereas, the results of the current study suggest an impairment in both LV and RV.

Interestingly, in our cohort we didn't find significant differences either in the metabolic profile or in blood pressure levels between patients and controls. Moreover, no significant differences in the prevalence of the main cardiometabolic complications, such as hypertension, dyslipidemia and impairments of glucose metabolism, were found between the two groups. These results suggest a direct role of hypercortisolism in cardiac impairment. The cortisol excess per se may exert a toxic effect on the heart, mediated directly through glucocorticoid and/or mineralcocorticoid receptors (3,20,72). The mineralocorticoid pathway increases collagen secretion by the activation of fibroblasts (73), which is why diffuse fibrosis is thought to be a potential player in myocardial involvement in CS. In addition, the stimulation of mineralocorticoid receptors decreases myocyte contractility and stimulates mitosis, resulting in myocardial hypertrophy and dysfunction (74).

Anyway, almost 60% of our patients presented hypertension and this could have contributed to cardiac impairment. Moreover, the presence of other CS-related cardiovascular risk factors, such as visceral obesity, glucose intolerance and dyslipidemia, may also have had a role.

Very few studies have evaluated cardiac structure and function in CS using CMR and this is a strong point of the current study, but it has two main limitations. The first is the limited number of patients, although it is a rare disease. The second is the heterogeneity of the study population, since it includes both cured and active patients and this may have underestimated the cardiac impairment. Nevertheless, our assumption was that cardiac

dysfunction persists in CS even after disease remission. However, the comparison between cured and active patients revealed no significant differences either in biochemical and cardiac parameters or in the prevalence of cardio-metabolic complications, except for blood pressure levels that were higher in active patients.

Cardiovascular disease is the major cause of death in patients with CS, either during active disease or after remission. Ischemic heart disease and complications related to cardiac hypertrophy are the main causes of morbidity and mortality (3). Therefore, an early and reliable detection of cardiac structural and functional abnormalities is mandatory in these patients. We propose a novel approach to cardiac evaluation in CS, suggesting the use of CMR, since it is the established non-invasive gold standard method for measuring LV volume, cardiac function and LVM, with higher accuracy and reproducibility and lower variability in comparison with echocardiography.

4.2 Acromegaly study

The current study shows that patients with acromegaly, compared with sex, age and BMI-matched controls, have a biventricular impairment associated with higher LV mass and lower RV systolic performance detected at CMR. CMR is the established non-invasive gold standard method for measuring LV volume, cardiac function and LVM due to its higher accuracy and reproducibility and lower variability in comparison with echocardiography (63). To date, few studies have analyzed patients with acromegaly using CMR (75-80), only one of whom, evaluating a small cohort, has compared patients with healthy matched controls (77). Andreassen and coworkers evaluated eight acromegaly patients with active disease and eight healthy controls, individually matched 1:1 with a patient as concerned age and gender. They found that patients had significantly higher LVMi compared with controls, without any significant differences in LV-EDVi, LV-ESVi and LV-EF. Moreover, they evaluated patients after three months of treatment and found an

increase in LV-EDVi, suggesting a decrease in cardiac function in the initial phase of treatment with dilatation of LV and increased wall tension (77). Conversely, we found that patients had higher LV end-systolic and end-diastolic volumes and higher RV end-systolic and end-diastolic volumes compared with controls. Concomitantly, we found higher LV and RV stroke volumes and lower RV ejection fraction in patients than controls. As regards the LVMi, our results confirm the findings of the previous study. The differences between the current study and the previous one could be explained by the smaller cohort of the latter.

The CMR results for acromegaly patients varied among different studies. Bogazzi and coworkers demonstrated for the first time that the frequency of LVH in acromegaly patients was 72% as evaluated by CMR (75). This frequency was much higher than 36% as detected by echocardiography in the same group. However, recent studies by dos Santos Silva et al and Warszawski et al from the same group have shown that the frequency of LVH in acromegaly patients was 5–8% (78,79). This difference is probably due to the different cut-off used to define LVH by the authors. In our cohort the prevalence of LVH was 5% (1/20 patients), superimposable to that found in the Brazilian studies and lower than that found in the study by Bogazzi, although we used the same cut-off as the latter.

A recent study used CMR to investigate the frequencies of cardiac abnormalities and detailed quantitative cardiac parameters in a large cohort of acromegaly patients and analyzed the correlations between the changes in cardiac structure and function and patients' clinical characteristics and GH and IGF-1 levels (80). They found that age, BMI, disease duration and hypertension but not GH or IGF-1 levels were associated clinical factors. Conversely, in our cohort, we found a significant correlation between IGF-1 levels and cardiac parameters at CMR. In particular, IGF-1 was significantly directly correlated

to LV-EDV and to LV-EDVi. Concomitantly, it was significantly directly correlated to LVM, LVMi and LV-SV. Moreover, as regards the RV, IGF-1 was found to have a significant direct correlation with RV-EDV and RV-SV. These results suggest a direct impact of IGF-1 levels on cardiac structure and function in acromegaly patients, underling the importance of disease control in these patients. Interestingly, in our cohort we didn't find significant differences either in the metabolic profile or in blood pressure levels between patients and controls, except for fasting plasma glucose that was significantly higher in patients than controls (p=0.033). Moreover, no significant differences in the prevalence of the main cardiometabolic complications, such as hypertension, dyslipidemia and impairment of glucose metabolism, were found between the two groups, supporting the hypothesis of a direct role of GH and IGF-1 in the pathogenesis of acromegalic cardiomyopathy.

Evidence collected in animal and human models supported the role of GH and IGF-I in determining direct changes in cardiac muscle. GH and IGF-I receptors are expressed and IGF-I is synthesized directly in cardiomyocytes. In animal models, GH and IGF-I increase myocardial contractility and induce a hypertrophic response of the heart and GH stimulates cardiac myocytes to re-enter the cell cycle, increasing the number of cardiac myocytes (36).

Anyway, the pathogenesis of acromegalic cardiomyopathy includes either a direct action of GH and IGF-I excess on the heart and indirect mechanisms by which GH and IGF-I excess induces hypertension and disorders of glucose and lipid metabolism, resulting in cardiac glucotoxicity and lipotoxicity and cardiac remodelling and hypertrophy. In our cohort, 50% of acromegaly patients presented hypertension and this could have contributed to cardiac impairment. Moreover, the presence of other acromegaly-related cardiovascular risk factors, such as glucose metabolism impairment (45% of patients) and dyslipidemia (45% of patients), may also have had a role. Indeed, we found a

significant correlation between RV-EF and triglycerides and HDL levels, inverse and direct respectively, suggesting a worse cardiac function in patients with a worse lipid profile.

Ventricular hypertrophy and myocardial fibrosis are considered common findings of acromegalic cardiomyopathy. At histology, interstitial fibrosis has been found as one of the most relevant abnormalities (36). Different echocardiographic studies confirmed histological findings, reporting cardiac fibrosis to be common in the acromegalic heart (36). Conversely, recent studies evaluating patients with active acromegaly by CMR imaging found cardiac fibrosis to be totally absent (75) or rare (78,80), ranging from 0% to 15%. In these studies the presence of cardiac fibrosis has been evaluated as the presence or not of late gadolinium enhancement (LGE). Myocardial T1 mapping and ECV are recent techniques in CMR imaging that permit the acquisition of quantitative measurement of myocardial and blood T1. Extra-cellular volume of the myocardium measures the volume fraction of heart tissue that is not taken by cells and reflects interstitial fibrosis or it can be filled by water, so both would potentially increase the ECV (62). Finding ECV greater than 30% is suggestive of fibrosis. In our cohort three patients (19%) had ECV greater than 30%. The proportion of patients with cardiac fibrosis was slightly higher in our patients than the 0–15% reported in previous studies (75,78,80), but lower than that reported in echocardiographic studies (75,78). Nevertheless, evaluation with T1 mapping technique did not reveal any significant difference between patients and controls.

Several echocardiographic studies have evaluated cardiac structure and function in patients with acromegaly and found biventricular hypertrophy, mainly involving the LV and consequent diastolic dysfunction in patients with active disease (35,36).

Cardiac structure and function assessment by echocardiography is more practical on a clinical basis. Nevertheless, CMR permits an evaluation of ventricular mass and volumes free of cardiac geometric assumption, ensuring a higher accuracy and reproducibility (71). Moreover, since ultrasound measurement of RV volumes is challenging, acromegaly echocardiographic studies mainly focused on the LV (35,36).

Few studies have evaluated cardiac structure and function in acromegaly using CMR and the current study is the first, to our knowledge, comparing patients with healthy matched controls in a larger population. This is a strong point of the current study, but it has two main limitations. The first is the limited number of patients, although it is a rare disease. The second is the heterogeneity of the study population, since it includes both cured and active patients on different medical therapies and this may have underestimated the cardiac impairment. Nevertheless, our assumption was that cardiac dysfunction persists in acromegaly even after disease remission. However, the comparison between cured and active patients revealed no significant differences in biochemical and cardiac parameters, except for fasting glucose and HbA1 levels and LVMi that were higher in active patients.

Cardiovascular disease is the most frequent comorbidity and still represents one of the most important causes of death in acromegaly. Moreover, cardiomyopathy can also persist in acromegaly patients after cure.

The pathogenesis of acromegalic cardiomyopathy has been proposed to develop after three steps, the earlier of whom is reversible and is characterized by initial cardiac hypertrophy, with increase of heart rate and systolic output. Therefore, an early and reliable detection of cardiac structural and functional abnormalities is mandatory in these patients. We propose a novel approach to cardiac evaluation in acromegaly, suggesting the use of CMR, since it is the established non-invasive gold standard method for

measuring LV volume, cardiac function and LVM, with higher accuracy and reproducibility and lower variability in comparison with echocardiography.

5. Conclusions

CS and acromegaly are two rare endocrine diseases associated to increased morbidity and mortality, mainly for cardiovascular events. Both have been demonstrated to have biventricular cardiac structural and functional impairment, which seem to have a multifactorial pathogenesis: 1) the presence of disease-related cardiovascular risk factors, such as hypertension, visceral obesity, glucose metabolism impairment and dyslipidemia; 2) a direct effect of hormone excess, determining changes in cardiac muscle.

It is noteworthy that the heart is a target organ for the great majority of endocrine hormones. Therefore, there is a growing interest in studying and characterizing cardiomyopathy in endocrine diseases. CMR is the established non-invasive gold standard method for measuring LV volume, cardiac function and LVM due to its higher accuracy and reproducibility and lower variability in comparison with echocardiography. Nevertheless, the use of CMR may be limited by its cost and its limited access. However, the results of the current studies suggest that CMR may have a place in the cardiac work-up of selected patients with rare endocrine diseases, such as CS and acromegaly.

6. References

1. Pivonello R, De Martino MC, De Leo M, Lombardi G, Colao A. Cushing's syndrome. Endocrinol Metab Clin North Am 2008; 37: 135–49, ix.
2. Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. *Lancet* 2006; 367: 1605–17.
3. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BMK, Colao A. *Lancet Diabetes Endocrinol* 2016; 4: 611-29.
4. Steffensen C, Bak AM, Rubeck KZ, Jørgensen JO. Epidemiology of Cushing's syndrome. *Neuroendocrinology* 2010; 92 (suppl 1): 1–5.
5. Pivonello R, De Leo M, Cozzolino A, Colao A. The treatment of Cushing's disease. *Endocr Rev* 2015; 36: 385–486.
6. Feelders RA, Pulgar SJ, Kempel A, Pereira AM. The burden of Cushing's disease: clinical and health-related quality of life aspects. *Eur J Endocrinol* 2012; 167: 311–26.
7. Colao A, Cozzolino A, Pivonello R. Quality of life in patients with Cushing's disease: a modern approach. *Clin Endocrinol (Oxf)*. 2012; 76(6): 776 –777.
8. Newell-Price J, Trainer P, Besser M, Grossman A. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. *Endocr Rev* 1998; 19: 647–72.
9. Arnaldi G, Angeli A, Atkinson AB, et al. Diagnosis and complications of Cushing's syndrome: a consensus statement. *J Clin Endocrinol Metab* 2003; 88: 5593–602.
10. Nieman LK, Biller BM, Findling JW, et al. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2008; 93: 1526–40.
11. Nieman LK, Biller BM, Findling JW, et al, and the Endocrine Society. Treatment of Cushing's syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2015; 100: 2807–31.
12. Colao A, Pivonello R, Spiezia S, et al. Persistence of increased cardiovascular risk in patients with Cushing's disease after five years of successful cure. *J Clin Endocrinol Metab*. 1999; 84(8): 2664 –2672.
13. Pivonello R, De Martino MC, De Leo M, et al. Cushing's syndrome: aftermath of the cure. *Arq Bras Endocrinol Metabol* 2007; 51: 1381–91.
14. Clayton RN. Mortality in Cushing's disease. *Neuroendocrinology* 2010; 92 (suppl 1):

71–76.

15. Graversen D, Vestergaard P, Stochholm K, Gravholt CH, Jørgensen JO. Mortality in Cushing's syndrome: a systematic review and meta-analysis. *Eur J Intern Med* 2012; 23: 278–82.
16. Pivonello R, De Leo M, Vitale P, Cozzolino A, et al. Pathophysiology of diabetes mellitus in Cushing's syndrome. *Neuroendocrinology* 2010; 92 (suppl 1): 77–81.
17. Faggiano A, Pivonello R, Spiezia S, et al. Cardiovascular risk factors and common carotid artery caliber and stiffness in patients with Cushing's disease during active disease and 1 year after disease remission. *J Clin Endocrinol Metab* 2003; 88: 2527–33.
18. Giordano C, Guarnotta V, Pivonello R, Amato MC, Simeoli C, Ciresi A, Cozzolino A, Colao A. Is diabetes in Cushing's syndrome only a consequence of hypercortisolism? *Eur J Endocrinol* 2014; 170: 311–19.
19. Mancini T, Kola B, Mantero F, Boscaro M, Arnaldi G. High cardiovascular risk in patients with Cushing's syndrome according to 1999 WHO/ISH guidelines. *Clin Endocrinol (Oxf)* 2004; 61: 768–77.
20. Isidori AM, Graziadio C, Paragliola RM, Cozzolino A, et al, and the ABC Study Group. The hypertension of Cushing's syndrome: controversies in the pathophysiology and focus on cardiovascular complications. *J Hypertens* 2015; 33: 44–60.
21. De Leo M, Pivonello R, Auriemma RS, Cozzolino A, et al. Cardiovascular Disease in Cushing's Syndrome: Heart versus Vasculature. *Neuroendocrinology* 2010; 92 (suppl 1): 50–54.
22. Pecori Giraldi F, Toja PM, De Martin M, et al. Circadian blood pressure profile in patients with active Cushing's disease and after long-term cure. *Horm Metab Res* 2007; 39: 908–14.
23. Dekkers OM, Horváth-Puhó E, Jørgensen JO, et al. Multisystem 51 morbidity and mortality in Cushing's syndrome: a cohort study. *J Clin Endocrinol Metab* 2013; 98: 2277–84.
24. Pereira AM, Delgado V, Romijn JA, Smit JW, Bax JJ, Feelders RA. Cardiac dysfunction is reversed upon successful treatment of Cushing's syndrome. *Eur J Endocrinol* 2010; 162: 331–40.
25. Muiesan ML, Lupia M, Salvetti M, et al. Left ventricular structural and functional characteristics in Cushing's syndrome. *J Am Coll Cardiol* 2003; 41: 2275–79.
26. Kamenický P, Redheuil A, Roux C, et al. Cardiac structure and function in Cushing's

syndrome: a cardiac magnetic resonance imaging study. *J Clin Endocrinol Metab* 2014; 99: E2144–53.

27. Pivonello R, Faggiano A, Lombardi G, Colao A. The metabolic syndrome and cardiovascular risk in Cushing's syndrome. *Endocrinol Metab Clin North Am* 2005; 34: 327–39, viii.
28. Faggiano A, Melis D, Alfieri R, et al. Sulfur amino acids in Cushing's disease: insight in homocysteine and taurine levels in patients with active and cured disease. *J Clin Endocrinol Metab* 2005; 90: 6616–22.
29. Yiu KH, Marsan NA, Delgado V, et al. Increased myocardial fibrosis and left ventricular dysfunction in Cushing's syndrome. *Eur J Endocrinol* 2012; 166: 27–34.
30. van der Pas R, Leebeek FW, Hofland LJ, de Herder WW, Feelders RA. Hypercoagulability in Cushing's syndrome: prevalence, pathogenesis and treatment. *Clin Endocrinol (Oxf)* 2013; 78: 481–88.
31. Isidori AM, Minnetti M, Sbardella E, Graziadio C, Grossman AB. Mechanisms in endocrinology: the spectrum of haemostatic abnormalities in glucocorticoid excess and defect. *Eur J Endocrinol* 2015; 173: R101–13.
32. Pivonello R, Arnaldi G, Scaroni C, Giordano C, Cannavò S, Iacuaniello D, Trementino L, Zilio M, Guarnotta V, Albani A, Cozzolino A, Michetti G, Boscaro M, Colao A. The medical treatment with pasireotide in Cushing's disease: an Italian multicentre experience based on "real-world evidence". *Endocrine* 2019; 64: 657–672.
33. Colao A, Grasso LFS, Giustina A, Melmed S, Chanson P, Pereira AM, Pivonello R. Acromegaly. *Nature Rev.* 2019; 5:20.
34. Petrossians P et al. Acromegaly at diagnosis in 3173 patients from the Liege Acromegaly Survey (LAS) Database. *Endocr Relat Cancer* 2017; 24: 505–518.
35. Colao A, Ferone D, Marzullo P. & Lombardi G. Systemic complications of acromegaly: epidemiology, pathogenesis, and management. *Endocr Rev* 2004; 25: 102–152.
36. Pivonello R et al. Complications of acromegaly: cardiovascular, respiratory and metabolic comorbidities. *Pituitary* 2017; 20: 46–62.
37. Lombardi G et al. The cardiovascular system in growth hormone excess and growth hormone deficiency. *J Endocrinol Invest* 2012; 35: 1021–1029.
38. Alexander L, Appleton D, Hall R, Ross WM & Wilkinson R. Epidemiology of acromegaly in the Newcastle region. *Clin Endocrinol* 1980; 12: 71–79.
39. Bengtsson BA, Eden S, Ernest I, Oden A. & Sjogren B. Epidemiology and long-term

survival in acromegaly. A study of 166 cases diagnosed between 1955 and 1984. *Acta Med Scand* 1988: 223: 327–335.

40. Agustsson TT et al. The epidemiology of pituitary adenomas in Iceland, 1955-2012: a nationwide population-based study. *Eur J Endocrinol* 2015; 173: 655–664.
41. Gruppetta M, Mercieca C. & Vassallo J. Prevalence and incidence of pituitary adenomas: a population based study in Malta. *Pituitary* 2013; 16: 545–553.
42. Dal J et al. Acromegaly incidence, prevalence, complications and long-term prognosis: a nationwide cohort study. *Eur J Endocrinol* 2016; 175: 181–190.
43. Zarool-Hassan R, Conaglen HM, Conaglen JV & Elston MS. Symptoms and signs of acromegaly: an ongoing need to raise awareness among healthcare practitioners. *J Prim Health Care* 2016; 8: 157–163.
44. Holdaway IM, Rajasoorya RC, Gamble GD. Factors influencing mortality in acromegaly. *J Clin Endocrinol Metab* 2004; 89(2): 667–674.
45. Ritvonen E, Löyttyniemi E, Jaatinen P, Ebeling T, Moilanen L, Nuutila P, Kauppinen-Mäkelin R, Schalin-Jäntti C. Mortality in acromegaly: a 20-year follow-up study. *Endocr Relat Cancer* 2015; 23(6): 469–480.
46. Møller N, Jørgensen JO. Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. *Endocr Rev* 2009; 30(2): 152–177.
47. Cozzolino A, Feola T, Simonelli I, Puliani G, Pozza C, Giannetta E, Gianfrilli D, Pasqualetti P, Lenzi A, Isidori AM. Somatostatin Analogs and Glucose Metabolism in Acromegaly: A Meta-analysis of Prospective Interventional Studies. *J Clin Endocrinol Metab* 2018; 103: 2089–2099.
48. Feola T, Cozzolino A, Simonelli I, Sbardella E, Pozza C, Giannetta E, Gianfrilli D, Pasqualetti P, Lenzi A, Isidori AM. Pegvisomant Improves Glucose Metabolism in Acromegaly: A Meta-Analysis of Prospective Interventional Studies. The Journal of clinical endocrinology and metabolism 2019; 104: 2892-2902.
49. Wildbrett J, Hanefeld M, Fucker K et al. Anomalies of lipoprotein pattern and brinolysis in acromegalic patients: relation to growth hormone levels and insulin–like growth factor I. *Exp Clin Endocrinol Diabetes* 1997*;* 105: 331–335.
50. Colao A, Marzullo P, Di Somma C, Lombardi G. Growth hormone and the heart. *Clin Endocrinol* 2001; 54: 137–154.
51. Brüel A, Christo ersen TE, Nyengaard JR. Growth hormone increases the proliferation of existing cardiac myocytes and the total number of cardiac myocytes in the rat heart. *Cardiovasc Res* 2007; 76(3): 400–408.

52. Vitale G, Pivonello R, Galderisi M, D'Errico A, Spinelli L, Lupoli G, Lombardi G, Colao A. Cardiovascular complications in acromegaly: methods of assessment. *Pituitary* 2001; 4(4): 251–257.
53. Colao A, Baldelli R, Marzullo P, Ferretti E, Ferone D, Gargiulo P, Petretta M, Tamburrano G, Lombardi G, Liuzzi A. Systemic hypertension and impaired glucose tolerance are independently correlated to the severity of the acromegalic cardiomyopathy. *J Clin Endocrinol Metab* 2000; 85: 193–199.
54. Bondanelli M, Ambrosio MR, degli Uberti EC. Pathogenesis and prevalence of hypertension in acromegaly. *Pituitary* 2001; 4(4): 239–249.
55. Vitale G, Pivonello R, Lombardi G, Colao A. Cardiac abnormalities in acromegaly. Pathophysiology and implications for management. *Treat Endocrinol* 2004; 3(5): 309–318 .
56. Colao A, Pivonello R, Grasso LF, Auriemma RS, Galdiero M, Savastano S, Lombardi G. Determinants of cardiac disease in newly diagnosed patients with acromegaly: results of a 10 year survey study. *Eur J Endocrinol* 2011; 165(5): 713–721.
57. Colao A, Cuocolo A, Marzullo P, Nicolai E, Ferone D, Della Morte AM, Pivonello R, Salvatore M, Lombardi G. Is the acromegalic cardiomyopathy reversible? Effect of 5-year normalization of growth hormone and insulin-like growth factor levels on cardiac performance. *J Clin Endocrinol Metab* 2001; 86: 1551–1557.
58. Akgul E, Tokgozoglu SL, Erbas T, Kabakci G, Aytemir K, Haznedaroglu I, Oto A, Kes SS. Evaluation of the impact of treatment on endothelial function and cardiac performance in acromegaly. *Echocardiography* 2010; 27(8): 990–996.
59. Katznelson L, Laws ER Jr, Melmed S, Molitch ME, Murad MH, Utz A, Wass JA; Endocrine Society. Acromegaly: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2014; 99(11): 3933-51.
60. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, Sahdev A, Tabarin A, Terzolo M, Tsagarakis S, Dekkers OM. Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. *Eur J Endocrinol* 2016; 175(2): G1-G34.
61. Alfakih K, Plein S, Thiele H, Jones T, Ridgway, JP, Sivananthan MU. Normal human left and right ventricular dimensions for MRI as assessed by turbo gradient echo and steady-state free precession imaging sequences. *Journal of Magnetic Resonance Imaging* 2003; 17:323–329.

62. Moon JC, Messroghli DR, Kellman P, et al. Society for Cardiovascular Magnetic Resonance Imaging; Cardiovascular Magnetic Resonance Working Group of the European Society of Cardiology. Myocardial T1 mapping and extracellular volume quantification: a Society for Cardiovascular Magnetic Resonance (SCMR) and CMR Working Group of the European Society of Cardiology consensus statement. *J Cardiovasc Magn Reson.* 2013; 15:92.

63. Grothues F, Smith GC, Moon JC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *Am J Cardiol*. 2002; 90:29 –34.

64. Kamenický P, Redheuil A, Roux C, Salenave S, Kachenoura N, Raissouni Z, Macron L, Guignat L, Jublanc C, Azarine A, Brailly S, Young J, Mousseaux E, Chanson P. Cardiac structure and function in Cushing's syndrome: a cardiac magnetic resonance imaging study. *J Clin Endocrinol Metab* 2014; 99(11):E2144-53.

65. Roux C, Kachenoura N, Raissuni Z, Mousseaux E, Young J, Graves MJ, Jublanc C, Cluzel P, Chanson P, Kamenický P, Redheuil A. Effects of cortisol on the heart: characterization of myocardial involvement in cushing's disease by longitudinal cardiac MRI T1 mapping. *J Magn Reson Imaging* 2017; 45(1):147-156.

66. Muiesan ML, Lupia M, Salvetti M, et al. Left ventricular structural and functional characteristics in Cushing's syndrome. *J Am Coll Cardiol* 2003; 41:2275–2279.

67. Baykan M, Erem C, Gedikli O, et al. Assessment of left ventricular diastolic function and Tei index by tissue Doppler imaging in patients with Cushing's Syndrome. *Echocardiography* 2008; 25:182– 190.

68. Bayram NA, Ersoy R, Aydin C et al. Assessment of left ventricular functions by tissue Doppler echocardiography in patients with Cushing's disease. *J Endocrinol Invest* 2009; 32:248–252.

69. Yiu KH, Marsan NA, Delgado V et al. Increased myocardial fibrosis and left ventricular dysfunction in Cushing's syndrome. *Eur J Endocrinol* 2012;166:27–34.

70. Toja PM, Branzi G, Ciambellotti F, et al. Clinical relevance of cardiac structure and function abnormalities in patients with Cushing's syndrome before and after cure. *Clin Endocrinol (Oxf)* 2012; 76: 332–338.

71. Armstrong AC, Gidding S, Gjesdal O, Wu C, Bluemke DA, Lima JA. LV mass assessed by echocardiography and CMR, cardiovascular outcomes, and medical practice. *JACC Cardiovasc Imaging* 2012; 5(8):837-48.
72. Mihailidou AS, Loan Le TY, Mardini M, Funder JW. Glucocorticoids activate cardiac mineralocorticoid receptors during experimental myocardial infarction. *Hypertension* 2009; 54:1306-1312.
73. Fujisawa G, Dilley R, Fullerton MJ, Funder JW. Experimental cardiac fibrosis: differential time course of responses to mineralocorticoid-salt administration. *Endocrinology* 2001; 142:3625-3631.
74. Feng X, Reini SA, Richards E, et al. Cortisol stimulates proliferation and apoptosis in the late gestation fetal heart: differential effects of mineralocorticoid and glucocorticoid receptors. *AJP Regul Integr Comp Physiol* 2013; 305:R343-R350.
75. Bogazzi F, Lombardi M, Strata E et al. High prevalence of cardiac hypertophy without detectable signs of fibrosis in patients with untreated active acromegaly: an *in vivo* study using magnetic resonance imaging. *Clinical Endocrinology* 2008; 68:361-368.
76. Gouya H, Vignaux O, Le Roux P et al. Rapidly reversible myocardial edema in patients with acromegaly: assessment with ultrafast T2 mapping in a single-breath-hold MRI sequence. *American Journal of Roentgenology* 2008; 190:1576-1582.
77. Andreassen M, Faber J, Kjaer A, Petersen CL, Kristensen LO. Cardiac effects of 3 months treatment of acromegaly evaluated by magnetic resonance imaging and B-type natriuretic peptides. *Pituitary* 2010; 13:329-336.
78. dos Santos Silva CM, Gottlieb I, Volschan I et al. Low frequency of cardiomyopathy using cardiac magnetic resonance imaging in an acromegaly contemporary cohort *Journal of Clinical Endocrinology and Metabolism* 2015; 100:4447-4455.
79. Warszawski L, Kasuki L, Sa R et al. Low frequency of cardniac arrhythmias and lack of structural heart disease in medically-naive acromegaly patients: a prospective study at baseline and after 1 year of somatostatin analogs treatment. *Pituitary* 2016; 19:582-589.
80. Guo X, Cao J, Liu P, Cao Y, Li X, Gao L, Wang Z, Fang L, Jin Z, Wang Y, Xing B. Cardiac Abnormalities in Acromegaly Patients: A Cardiac Magnetic Resonance Study. *Int J Endocrinol* 2020; 2020:2018464.

7. Tables and figures

Table 1: Cushing's syndrome patients' characteristics.

Sex (M/F)	4/12
Age, years (mean±SD)	47.6±12.1
CS origin (pituitary/adrenal)	12/4
Disease status (cured/active)	11/5
Time from diagnosis, years (mean±SD)	8.4±11.3
Neurosurgery, n (%)	9 (75% of pituitary patients)
Hypocortisolism, n (%)	8 (50%)
Hypothyroidism, n (%)	5 (31.2%)
Hypogonadism, n (%)	2 (50% of males)
Menopause, n (%)	5 (41.7% of females)
DA, n (%)	3 (18.7%)
Metyrapone, n (%)	1 (6.2%)

M:males; F:females; CS: Cushing's syndrome; SD: standard deviation; n: number; DA: dopamine agonist

Table 2: Biochemical and clinical parameters in Cushing's syndrome patients and controls.

Parameters	**CS patients n=16**	**Controls n=18**	**P value**
Sex, males n (%)	4 (25)	8 (44.4)	0.317
Age, years	47.6±12.1	57.5±14.4	0.077
BMI, Kg/m^2	30.6±9.1	25.5±3.2	0.055
Weight, Kg	78.6±21.5	70.7±7.6	0.299
Waist circumference, cm	104.3±26.6	94.7±8.9	0.504
Systolic BP, mmHg	117.9±12.5	126.1±16.5	0.175
Diastolic BP, mmHg	76.3±8.9	74.4±5.3	0.573
Heart rate, beats/min	72.6±9.3	69.0±10.7	0.384
Fasting plasma glucose, mg/dL	76.4±7.0	87.4±9.6	**0.003**
HbA1c, %	5.6±0.4	5.5±0.3	0.686
Fasting insulin, µUI/L	12.7±9.8	9.9±6.2	0.455
HOMA-I	2.5±2.0	2.2±1.6	0.725
Triglycerides, mg/dL	106.2±36.5	100.7±21.0	0.679
Total cholesterol, mg/dL	199.6±24.7	198.0±32.8	0.894
HDL cholesterol, mg/dL	66.2±24.7	59.3±8.4	0.428
LDL cholesterol, mg/dL	112.1±30.4	117.5±30.1	0.667
Hypertension, n (%)	9 (56.2)	6 (33.3)	0.271
Diabetes, n (%)	1 (6.2)	0 (0)	0.444
IGT, n (%)	0 (0)	0 (0)	-
IFG, n (%)	5 (31.2)	0 (0)	0.061
Dyslipidemia, n (%)	7 (43.7)	4 (22.2)	0.282
Smoking habit, n (%)	2 (12.5)	6 (33.3)	0.211

Values are presented as mean±SD

CS: Cushing's syndrome; n: number; BMI: body mass index; BP: blood pressure; IGT: impaired glucose tolerance; IFG: impaired fasting glucose

Table 3: Cardiac parameters in Cushing's syndrome patients and controls.

Parameters	CS patients n=16	Controls n=18	P value
LV-EDV (mL)	134±34.4	113±26.8	0.130
LV-EDVi (ml/m²)	71.2±15.1	62.2±12.6	0.140
LV-ESV (mL)	58.7±20.1	42.8±11.7	**0.041**
LV-ESVi (ml/m²)	31.0±8.7	23.4±5.7	**0.030**
LV-SV (mL)	75.5±16.3	70.4±18.7	0.489
LV-SVi (ml/m²)	40.3±8.0	38.7±9.1	0.651
LV-EF (%)	57.1±6.3	61.6±6.4	0.097
LVM (g)	94.6±25.0	85.5±14.5	0.325
LVMi (g/m²)	51.0±11.8	46.9±8.4	0.364
LVH (yes/no)/(%)	0/16 (0%)	0/18 (0%)	-
Concentricity index (g/mL)	0.7±0.1	0.8±0.2	0.255
IVS-thick	10.3±2.5	9.4±1.9	0.378
RV-EDV (mL)	140.3±30.1	113.2±20.5	**0.025**
RV-EDVi (ml/m²)	74.8±14.2	62.4±10.9	**0.033**
RV-ESV (mL)	63.9±16.4	44.8±9.7	**0.004**
RV-ESVi (ml/m²)	34.0±7.7	25.1±6.5	**0.008**
RV-SV (mL)	76.4±17.1	68.4±18.6	0.286
RV-SVi (ml/m²)	40.8±8.7	38.0±9.5	0.454
RV-EF (%)	54.6±5.5	60.0±8.6	0.067
T1-preMean (ms)	1003.4±25.0	997.1±18.7	0.522
T1-postMean (ms)	443.0±60.5	405.4±46.6	0.129
ECV (%)	25.1±2.3	26.0±2.7	0.406
ECV>30% (yes/no)/(%)	0/16 (0%)	2/18 (11.1%)	0.202

Values are presented as mean±SD

CS: Cushing's syndrome; n: number; LV: left ventricle; EDV: end-diastolic volume; EDVi: end-diastolic volume indexed; ESV: end-systolic volume; ESVi: end-systolic volume indexed; SV: stroke volume; SVi: stroke volume indexed; EF: ejection fraction; LVM: left vetricular mass; LVMi: left vetricular mass indexed; LVH: left ventricular hypertrophy; IVS: interventricular sept; RV: right ventricle; ECV: extra-cellular volume

Table 4: Acromegaly patients' characteristics.

Sex (M/F)	13/7
Age, years (mean±SD)	50.0±12.4
Disease status (cured/active)	6/14
Time from diagnosis, years (mean±SD)	11.8±10.3
Neurosurgery, n (%)	18 (90%)
Hypocortisolism, n (%)	6 (30%)
Hypothyroidism, n (%)	10 (50%)
Hypogonadism, n (%)	8 (61.5% of males)
Menopause, n (%)	2 (28.6% of females)
First generation SSAs, n (%)	3 (15%)
First generation SSAs + PEG, n (%)	4 (20%)
Pasireotide, n (%)	2 (10%)
Pasireotide + DA, n (%)	3 (15%)
First generation SSAs + DA, n (%)	1 (5%)

M: males; F: females; SD: standard deviation; n: number; SSAs: somatostatin analogues; PEG: pegvisomant; DA: dopamine agonist

Table 5: Biochemical and clinical parameters in acromegaly patients and controls.

Parameters	**Acromegaly patients n=20**	**Controls n=18**	**P value**
Sex, males n (%)	13 (65)	8 (44.4)	0.298
Age, years	50.0±12.4	57.5±14.4	0.160
BMI, Kg/m^2	28.2±5.1	25.5±3.2	0.165
Weight, Kg	89.2±19.9	70.7±7.6	**0.002**
Waist circumference, cm	98.3±11.6	94.7±8.9	0.585
Systolic BP, mmHg	118.4±15.5	126.1±16.5	0.240
Diastolic BP, mmHg	74.6±10.8	74.4±5.3	0.961
Heart rate, beats/min	62.9±12.1	69.0±10.7	0.212
Fasting plasma glucose, mg/dL	101.6±17.7	87.4±9.6	**0.033**
HbA1c, %	5.7±0.7	5.5±0.3	0.306
Fasting insulin, µUI/L	5.9±4.9	9.9±6.2	0.111
HOMA-I	1.4±1.1	2.2±1.6	0.184
Triglycerides, mg/dL	110.1±50.5	100.7±21.0	0.597
Total cholesterol, mg/dL	187.4±31.5	198.0±32.8	0.415
HDL cholesterol, mg/dL	51.0±14.5	59.3±8.4	0.127
LDL cholesterol, mg/dL	115.7±31.4	117.5±30.1	0.882
Hypertension, n (%)	10 (50)	6 (33.3)	0.404
Diabetes, n (%)	3 (15)	0 (0)	0.220
IGT, n (%)	1 (5)	0 (0)	0.394
IFG, n (%)	6 (30)	0 (0)	0.065
Dyslipidemia, n (%)	9 (45)	4 (22.2)	0.242
Smoking habit, n (%)	3 (15)	6 (33.3)	0.260

Values are presented as mean±SD

n: number; BMI: body mass index; BP: blood pressure; IGT: impaired glucose tolerance; IFG: impaired fasting glucose

Table 6: Cardiac parameters in acromegaly patients and controls.

Parameters	Acromegaly patients n=20	Controls n=18	P value
LV-EDV (mL)	166.8±40.2	113±26.8	**0.001**
LV-EDVi (ml/m²)	79.7±13.8	62.2±12.6	**0.003**
LV-ESV (mL)	71.2±21.2	42.8±11.7	**0.001**
LV-ESVi (ml/m²)	33.9±7.9	23.4±5.7	**0.001**
LV-SV (mL)	95.6±24.1	70.4±18.7	**0.010**
LV-SVi (ml/m²)	45.8±9.2	38.7±9.1	0.065
LV-EF (%)	57.5±6.1	61.6±6.4	0.108
LVM (g)	118.9±36.8	85.5±14.5	**0.002**
LVMi (g/m²)	57.1±14.2	46.9±8.4	0.056
LVH (yes/no)/(%)	1/20 (5%)	0/18 (0%)	0.495
Concentricity index (g/mL)	0.7±0.1	0.8±0.2	0.231
IVS-thick	11.5±3.0	9.4±1.9	0.073
RV-EDV (mL)	188.3±53.4	113.2±20.5	**0.000**
RV-EDVi (ml/m²)	89.7±19.7	62.4±10.9	**0.001**
RV-ESV (mL)	94.4±33.7	44.8±9.7	**0.000**
RV-ESVi (ml/m²)	44.8±13.0	25.1±6.5	**0.000**
RV-SV (mL)	93.9±24.3	68.4±18.6	**0.010**
RV-SVi (ml/m²)	44.9±9.3	38.0±9.5	0.454
RV-EF (%)	50.6±6.0	60.0±8.6	**0.002**
T1-preMean (ms)	995.5±31.1	997.1±18.7	0.869
T1-postMean (ms)	446.8±55.0	405.4±46.6	0.063
ECV (%)	26.0±2.8	26.0±2.7	0.996
ECV>30% (yes/no)/(%)	3/20 (15%)	2/18 (11.1%)	0.741

Values are presented as mean±SD

CS: Cushing's syndrome; n: number; LV: left ventricle; EDV: end-diastolic volume; EDVi: end-diastolic volume indexed; ESV: end-systolic volume; ESVi: end-systolic volume indexed; SV: stroke volume; SVi: stroke volume indexed; EF: ejection fraction; LVM: left vetricular mass; LVMi: left vetricular mass indexed; LVH: left ventricular hypertrophy; IVS: interventricular sept; RV: right ventricle; ECV: extra-cellular volume

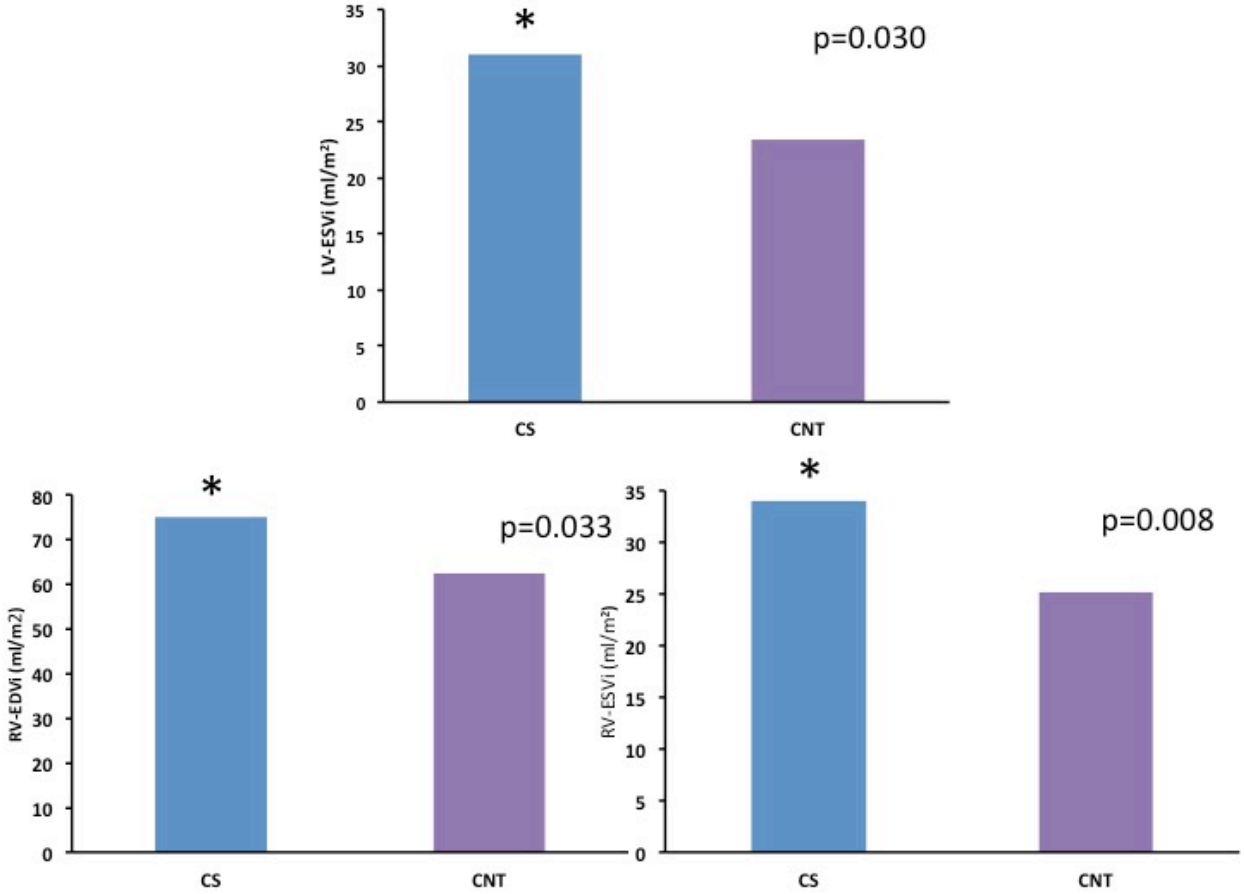

Figure 1: Mean LV-ESVi, RV-EDVi and RV-ESVi in Cusing's syndrome patients and controls.

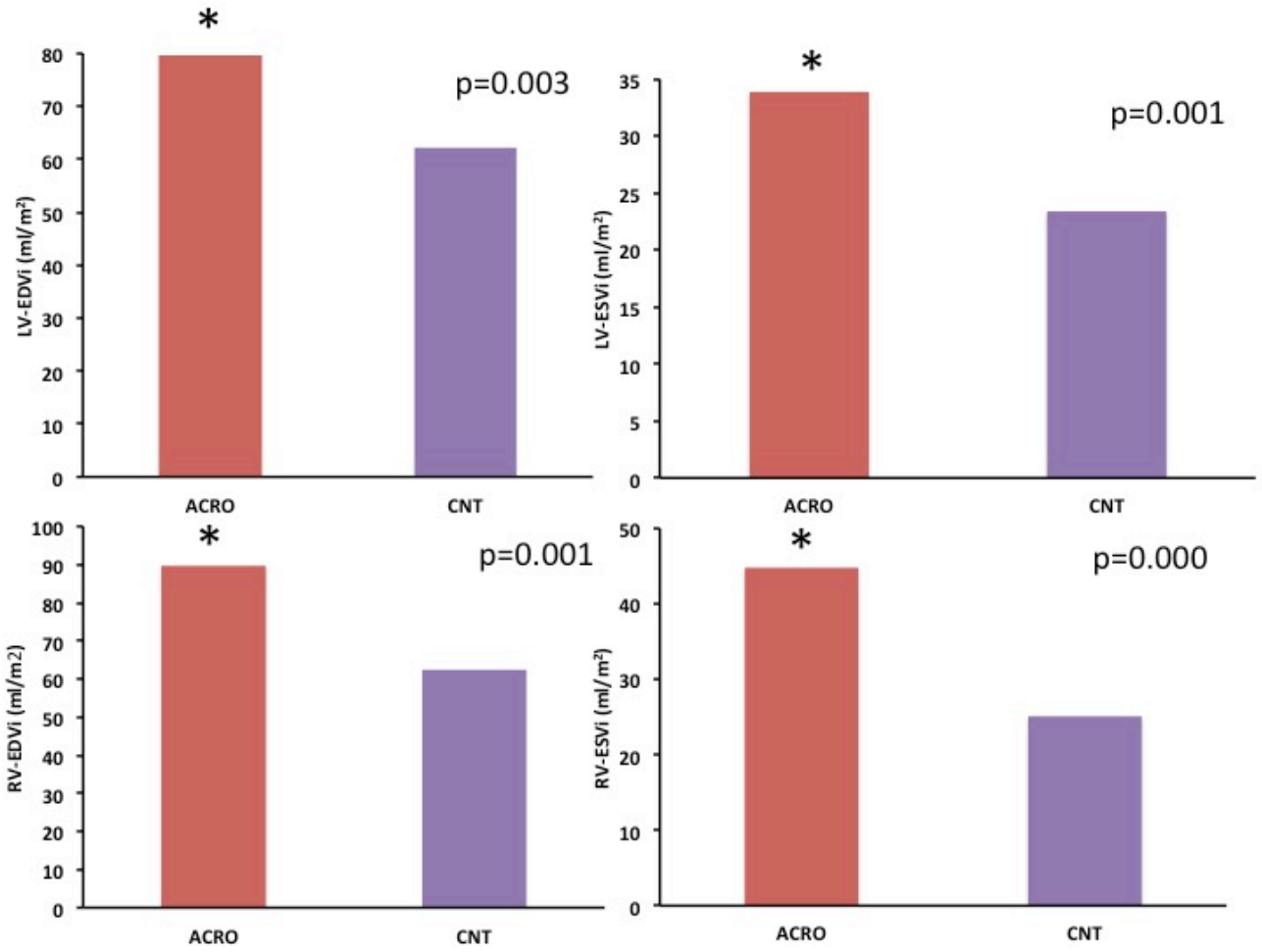

Figure 2: Mean LV-EDVi, LV-ESVi, RV-EDVi and RV-ESVi in acromegaly patients and controls.

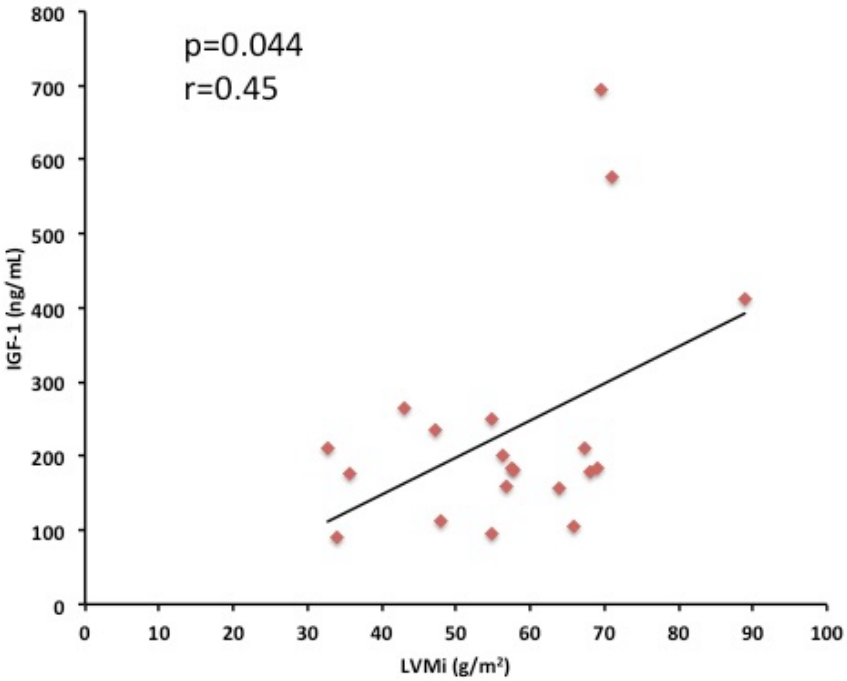

Figure 3: Correlation between IGF-1 levels and LVMi in acromegaly patients.