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Abbreviations

ACTH	adrenocorticotropin hormone
ACRO	acromegaly
AM	adductor magnus
ASM	appendicular skeletal mass
ATP	adenosine triphosphate
BHL	biceps long head
BMD	bone mineral density
BMI	body mass index
CD	Cushing's disease
CK	creatine kinase
CRH	corticotropin releasing hormone
CS	Cushing's syndrome
CT	Computed tomography
DXA	dual-energy X-ray absorptiometry
EWGSOP2	European Working Group on Sarcopenia in Older People 2
FF	fat fraction
FFA	free fatty acids
G	gracilis
GC	glucocorticoids
GH	growth hormone
GHD	growth hormone deficiency
GHRH	growth hormone releasing hormone
GS	gait speed
HC	hydrocortisone
HRQoL	health-related quality of life
IGF-I	Insulin-like growth factor type I

IL-6	Interleukin-6
IMAT	intermuscular fat
IMCL	intramyocelular lipid
IPAQ	International Physical Activity Questionnaire
IQR	interquartile range
LDDST	48-h 2-mg/day low-dose dexamethasone suppression test
M	meters
MET	metabolic equivalents
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
ODST	1-mg overnight dexamethasone suppression test
RF	rectus femoris
rGH	recombinant GH
RIA	radioimmunoassay
ROS	reactive oxygen species
s	seconds
Sart	sartorious
SD	standard deviation
SN	semimembranosus
SSa	somatostatin analogs
TNF alpha	tumor necrosis factor-alpha
TSH	thyroid stimulating hormone
TSS	transsphenoidal surgery
TUG	timed up and go
VI	vastus intermedius
VL	vastus lteralis
VM	vastus medialis
WOMAC	Western Ontario McMaster Universities Osteoarthritis Index

Summary

Abstract

Cushing's syndrome (CS) and acromegaly (ACRO) present with myopathy and muscle weakness that persist after remission of excess hormones. Intramuscular fatty infiltration has been associated with the deterioration of muscle performance in several conditions. Muscle MRI with 2-point Dixon technique studies the degree of myosteatosis.

Objectives: To evaluate myosteatosis using MRI 2-point Dixon sequence, and correlate them with muscle functional tests in SC and acromegaly, where the body composition is altered.

Patients with Cushing's syndrome:

Methods: This was a cross-sectional study. Thirty-six women with CS in remission, and 36 controls matched for age, BMI, menopausal status, and level of physical activity. We analyzed the percentage fat fraction (FF) of the thigh muscles in the anterior, posterior, and combined anterior and posterior compartments using MRI and 2-point Dixon sequence. We assessed muscle function and strength using the following tests: gait speed (GS), timed up and go (TUG), 30-second chair stand, and hand grip strength.

Results: Fat fraction in all the compartments analyzed was increased in patients as compared with controls. The performance on TUG, 30-second chair stand, and GS was more impaired in CS patients versus controls. In patients, greater FF was negatively associated with performance on functional tests. Fat fraction in the combined anterior and posterior compartments predicted performance on TUG (ß 0.626, $p < 0.000$) and GS (ß -0.461, $p = 0.007$), after adjusting for age, BMI, menopausal status, and muscle mass.

Patients with acromegaly

Methods: In a cross-sectional study, we included 36 patients with controlled acromegaly and 36 matched controls. We assessed the percentage of fat fraction in each thigh muscle, using MRI 2-point Dixon sequence, and muscle performance and strength using the gait speed, timed up and go, 30-s chair stand, and hand grip strength tests. We evaluated joint symptoms using the Western Ontario McMaster Universities Osteoarthritis Index (WOMAC).

Results: Intramuscular fat fraction was greater in patients than controls ($p < 0.05$ for muscle compartments, rectus femoris (RF), vastus intermedius (VI), adductor magnus (AM) and semimembranosus). Patients had slower gait speed and poorer performance on the 30-s chair stand and timed up and go tests than controls ($p < 0.05$). The greater fat fraction in the combined anterior–posterior compartment and in each muscle was associated with worse performance on timed up and go ($p < 0.05$). The fat fraction in the anterior–posterior compartment predicted performance on timed up and go after adjusting for muscle area, IGF-I and WOMAC functional and pain scores ($\beta = 0.737$ $p < 0.001$).

Conclusions: Thigh muscle fatty infiltration is increased in CS patients with long term remission and in controlled ACRO patients and it is associated with poorer muscle performance and muscle weakness. Future studies are needed to establish therapeutic strategies to improve muscle weakness in these patients.

Resumen

El síndrome de Cushing (SC) y la acromegalia (ACRO) se presentan con miopatía y debilidad muscular que persisten después de la remisión del exceso de hormonas. La infiltración de grasa intramuscular se ha asociado con el deterioro del rendimiento muscular en varias condiciones. La resonancia magnética muscular con técnica de Dixon de 2 puntos estudia el grado de mioesteatosis.

Objetivos: Evaluar la mioesteatosis mediante resonancia magnética secuencia Dixon de 2 puntos, y correlacionarlas con pruebas funcionales musculares en SC y acromegalia, donde la composición corporal se encuentra alterada.

Pacientes con síndrome de Cushing:

Métodos: Este fue un estudio transversal. Treinta y seis mujeres con SC en remisión y 36 controles emparejados por edad, IMC, estado menopáusico y nivel de actividad física. Analizamos la fracción de grasa porcentual (FF) de los músculos del muslo en los compartimentos anterior, posterior y anterior y posterior combinados mediante resonancia magnética y secuencia Dixon de 2 puntos. Evaluamos la función y la fuerza muscular mediante las siguientes pruebas: velocidad de la marcha (GS), cronometrado hacia arriba y hacia adelante (TUG), parada en silla de 30 segundos y fuerza de agarre manual.

Resultados: La fracción de grasa en todos los compartimentos analizados aumentó en los pacientes en comparación con los controles. El rendimiento en TUG, soporte de silla de 30 segundos y GS se deterioró más en los pacientes con CS que en los controles. En los pacientes, una mayor FF se asoció negativamente con el rendimiento en las pruebas funcionales. La fracción de

grasa en los compartimentos anterior y posterior combinados predijo el rendimiento en TUG (ß 0,626, p <0,000) y GS (ß -0,461, p = 0,007), después de ajustar por edad, IMC, estado menopáusico y masa muscular.

Pacientes con acromegalia

Métodos: En un estudio transversal, incluimos a 36 pacientes con acromegalia controlada y 36 controles emparejados. Evaluamos el porcentaje de fracción de grasa en cada músculo del muslo, usando la secuencia de Dixon de 2 puntos de resonancia magnética, y el rendimiento y la fuerza muscular usando la velocidad de la marcha, cronometrado y listo, soporte de silla de 30 s y pruebas de fuerza de agarre. Evaluamos los síntomas articulares utilizando el Índice de Osteoartritis de las Universidades Western Ontario McMaster (WOMAC).

Resultados: La fracción de grasa intramuscular fue mayor en los pacientes que en los controles (p <0.05 para los compartimentos musculares, recto femoral (RF), vasto intermedio (VI), aductor mayor (AM) y semimembranoso). Los pacientes tenían una velocidad de marcha más lenta y un rendimiento más deficiente en el soporte de la silla de 30 segundos y las pruebas cronometradas para arriba y para adelante que los controles (p <0.05). La mayor fracción de grasa en el compartimento anterior-posterior combinado y en cada músculo se asoció con un peor rendimiento en el cronometrado up and go (p <0,05). La fracción de grasa en el compartimento anteroposterior predijo el rendimiento en cronometrado y listo después de ajustar el área muscular, las puntuaciones funcionales y de dolor de IGF-I y WOMAC (β = 0,737 p <0,001).

Conclusiones: La infiltración grasa en el muslo del muslo está aumentada en pacientes con SC con remisión a largo plazo y en pacientes controlados con ACRO y se asocia con un peor rendimiento muscular y debilidad muscular. Se necesitan estudios futuros para establecer estrategias terapéuticas para mejorar la debilidad muscular en estos pacientes

1. Introduction and background

1.1 Cushing's syndrome (definition, prevalence, etiology, clinical manifestations)

Endogenous Cushing's syndrome (CS) is a serious condition due to chronic exposure to high circulating levels of glucocorticoids (GC) which, if untreated, leads to impaired health-related quality of life (HRQoL) and significant shortening of life expectancy due to multisystemic morbidity, including neurocognitive dysfunction, cardiovascular diseases, metabolic syndrome, osteoporosis, increased fracture risk, and proximal myopathy (1–3). Cortisol is the main steroid hormone produced by the adrenal cortex in response to adrenocorticotropic hormone (ACTH), which is, in turn, regulated by hypothalamic secretion of corticotropin-releasing hormone (CRH) and vasopressin. Cortisol secretion is modulated by a mechanism of feedback at the hypothalamic and pituitary level, and exhibits circadian rhythm; in healthy individuals, cortisol production peaks early in the morning and decreases until a nadir around midnight (4). Endogenous CS is a rare disease, with an incidence between 0.7 to 2.5 cases / million inhabitants per year. In 70%-80% of cases CS is caused by excessive ACTH release from a pituitary corticotrope adenoma (Cushing's disease, CD) and less frequently by ectopic ACTH or (very rarely) CRH production. CS can also be ACTH-independent when caused by increased cortisol secretion by adrenocortical tumors or hyperplasia. Endogenous CS occurs most frequently in women of reproductive age, but it can affect males and females of any age (1).

The primary treatment is transsphenoidal pituitary or adrenal surgery, depending on the etiology. Pituitary surgery in CD is associated with remission of cortisol excess in approximately 60-70% of patients, although

success rate of transsphenoidal approach mainly depends upon surgeon's experience. If surgery is contraindicated or has not been successful, pituitary irradiation can be a therapeutic option; medical therapy can also be used to decrease circulating cortisol or block its action on peripherals tissues.

CS is associated with several metabolic features, including hypertension, atherosclerosis, cardiomyopathy, dyslipidemia, diabetes/impaired glucose tolerance and obesity (5). Specific symptoms related with protein wasting may also be present, including proximal weakness, thin skin, easy bruising, and decreased linear growth in children (4). Table 1 describes metabolic and catabolic features in CS.

Table 1. Metabolic and catabolic features in CS (1,5–8)

Morbidity	Prevalence (%)
Cardiometabolic symptoms	
Weight gain	81%
Hypertension	55-85%
Atherosclerosis	30%
Cardiomyopathy	70%
Dyslipidemia	37-71%
Diabetes/ Impaired glucose tolerance	35% / 21-64%
Catabolic symptoms	
Skin abnormalities	73%
Proximal weakness	67%
Decreased linear growth in children	80%
Fractures	21%

Clinical diagnosis of CS can be challenging due to the overlap between hypercortisolism-related features and signs/symptoms commonly found in the general population, such as obesity and hypertension. Moreover, the typical proteocatabolic manifestations of CS may remain unrecognized during a long period of time, due to interindividual differences in their severity degree. As a result, CS patients are exposed to cortisol excess during several months or even years prior to receive the correct diagnosis of their condition, which may lead to irreversible damage of several tissues such as bone and brain (9,10).

Indeed, recent evidence suggests that CS patients who achieve cortisol normalization after surgical removal of the source of their hormone excess, do not experience a parallel resolution of the systemic dysfunctions observed during the active phase of the disease (11).

Glucocorticoid are widely used in clinical practice as a therapy for many rheumatic, inflammatory or immune diseases and in transplanted patients (12). A study using a detailed pharmacological recording system that covers 7 million individuals in England and Wales, identified 1.6 million oral glucocorticoids prescriptions over a 10-year period (12). To disentangle the relative role of glucocorticoids in these conditions is challenging (13). Thus, CS is a unique model which allows elucidating the deleterious effects of chronic GC excess, without the confounding effect of another concomitant pathological condition.

1.2 Acromegaly (definition, prevalence, etiology, clinical manifestations)

Acromegaly (ACRO) is a clinical syndrome resulting from the excessive secretion of growth hormone (GH) and increased serum concentrations of insulin-like growth factor I (IGF-I). Acromegaly is associated with excess mortality, mainly due to cardiovascular diseases (14,15). GH is produced by somatotroph cells in the anterior pituitary and is released in a characteristic pulsatile manner. GH remains almost undetectable most part of the day and peaks during exercise and slow waves sleep. GH is mainly regulated by a dual-hypothalamic control: it is stimulated by growth hormone releasing hormone (GHRH) and inhibited by somatostatin. Finally, a third regulator of growth hormone secretion is ghrelin, a gastrointestinal peptide secreted by the oxyntic cells as a response to physical stimuli from the lumen and chemical stimuli from the basolateral site (16,17). Ghrelin, which has a

marked growth hormone-stimulating activity, is the first hormone linking gastrointestinal system and pituitary axis.

GH exerts its actions directly or indirectly through IGF-I, which is mainly synthesized in the liver as a consequence of GH stimulation. GH secretion is also modulated by IGF-I through a mechanism of negative feedback at the hypothalamic and pituitary level (18).

ACRO is a rare disease, with an estimated prevalence in Europe of 30 to 70 individuals per million and an annual incidence of 3-5 cases per million (19,20). The mean age at diagnosis is 40 to 45 years. The commonest cause of acromegaly is a somatotroph (GH-secreting) adenoma of the anterior pituitary, mainly macroadenomas (82%) (21). These adenomas account for approximately one-third of all hormone-secreting pituitary adenomas. Growth hormone excess that occurs before fusion of the epiphyseal growth plates in a child or adolescent is called pituitary gigantism. The incidence rate of the disease is similar between men and women (14).

ACRO is characterized by an acquired progressive somatic disfigurement, mainly involving the face and extremities. ACRO is also associated with several systemic morbidities, listed in Table 2. Cardiovascular comorbidities are considered the main causes of mortality in these patients, its main contributors being arterial hypertension, dyslipidemia and diabetes mellitus, which are associated with chronic GH excess effects (22). GH has been found to modulate the expression of various growth factors (besides IGF-I) and receptors in several tissues. Both GH and IGF-I receptors are found in myocardial tissue and blood vessels where regulate growth and remodeling. As a matter of fact, left ventricular hypertrophy, myocardial fibrosis, myocardial infarction and arterial hypertension are frequent complications of ACRO(23). Also, GH acts as an anti-natriuretic hormone possibly mediated

by the activation of the renin-angiotensin-aldosterone system which predisposes to sodium and water retention in the kidney, thus increasing plasma volume and arterial resistance (24). Increased sleep apnea syndrome in ACRO due to craniofacial deformities has been associated to increased cardiovascular events (25). GH excess also induces several metabolic derangements, including insulin resistance, increased endogenous glucose production, increased gluconeogenesis and decreased peripheral glucose disposal in muscle. These effects appear to be secondary to the stimulation of lipolysis and subsequent glucose-fatty acid substrate competition (26,27). Also, GH exerts an overall lipolytic action, promoting lipid mobilization and oxidation, and increasing circulating FFA (28).

Table 2 shows morbidities associated with ACRO and its prevalence (22).

Table 2. Morbidity in ACRO patients (22)

Morbidity	Prevalence (%)
Diabetes	37.4
Hypertension	39.1
Dyslipidemia	25.8
Arthropathy	19.6
Carpal tunnel syndrome	18.7
Goiter	22.4
Sleeping apnea	13.2
Cerebrovascular disease	7.1
Cardiovascular disease	14.1
Chronic pulmonary disease	4.9
Hypopituitarism,	25.7
Biliary stones	9.5

The clinical diagnosis is often delayed due to the slow progression of the signs of ACRO over a period of many years. At diagnosis, several comorbidities may be present already, secondary to chronic exposure to excessive GH and tumoral mass effect. Hypertension (40%), neoplasms mainly of thyroid, breast and colon (30%), hypopituitarism (22%), diabetes mellitus (17%), arthropathies (15%) and sleep disorders (14%) are the most frequent comorbidities (29).

The therapeutic goal in patients with ACRO is the total adenoma removal which is not possible in most of the cases due to the tumor extension and

invasion of peripheral tissues. Secondary goals are to lower serum IGF-I concentrations to within the normal range for the patient's age and gender, control adenoma size and reduce mass effects, improve symptoms, and reverse metabolic abnormalities such as diabetes mellitus (30). Surgery is the first choice of treatment in most of the cases, with a success rate of immediate disease control of roughly 85% and 45% in microadenomas and macroadenomas, respectively (21).

When serum GH and IGF-I concentrations decline to normal, the characteristic soft tissue overgrowth and related symptoms gradually recede and the metabolic abnormalities, such as diabetes mellitus, improve. In addition, life expectancy seems to return to that of the general population (31,32). Nonetheless, it is known that bone abnormalities, as well as joint symptoms and low HRQoL persist or even worsen long-term despite biochemical control of the disease (33–35).

1.3 Muscle in Cushing's syndrome & acromegaly

GC and GH exert important effects on muscle. Typically, circulating GCs excess causes an imbalance in protein formation due to its stimulatory effect on protein catabolism and the inhibition of anabolic signals, with ultimate loss of muscle tissue (36). Indeed, myopathy and muscle weakness are the most frequent and detrimental effects of chronic exposure to GC. Indeed, the European CS database, involving more than 1500 patients, reported that the presence of muscle weakness at diagnosis was associated with increased mortality rate at short term (1,37).

On the contrary, GH and IGF-I have a net anabolic effect in muscle as they stimulate protein synthesis and nitrogen balance, and inhibit proteolysis (38–40). However, while substitution treatment with physiological doses of GH in patients with GH deficiency (GHD) increased muscle mass and normalized

strength and performance (41), the effect of supraphysiological concentrations of this hormone, such as those observed in ACRO, has not been clearly established. Interestingly, patients with ACRO complain of persistent muscle weakness even after normalization of GH excess.

1.3.1 Muscle in Cushing's syndrome

1.3.1.1 Effects of glucocorticoids on muscle

Proximal and symmetric myopathy associated with muscle weakness is a frequent complication of long term exposure to exogenous or endogenous GC (36). Histological studies have shown atrophy of type 2a fibers (involved in rapid power contraction), loss of myosin filaments in sarcomeres, and necrosis in the muscles of patients treated with GC (42,43). Accordingly, patients with CS present with slow muscle fiber conduction and profound muscle weakness (42,44).

While around 70% of patients with chronic endogenous glucocorticoid excess complain of muscle weakness mainly affecting the appendicular muscles of the pelvic girdle and lower limbs, a decrease in lean mass by 15%, especially at the level of the legs, as measured using dual-energy X-ray absorptiometry (DXA), and a reduction of skeletal muscle mass, as assessed using whole-body MRI, have been found in active CS patients as compared with BMI-matched controls (43,45–47). Moreover, the presence of muscle weakness at diagnosis has been associated with a high mortality rate in a large cohort of patients who underwent surgical treatment (37).

1.3.1.2 Muscle dysfunction in active and "cured" Cushing's syndrome (CS)

A decrease in both hand grip strength and proximal muscle functionality has been described in CS patients during the active phase of the disease when compared with obese controls (1,48). Recent data analyzed from the German Cushing's Registry demonstrated that patients who had previously suffered

from CS not only maintained muscle weakness after 13 years of remission of hypercortisolism, but that muscle performance, corrected for age, was even lower than that measured during the active phase of disease (48). Furthermore, in a study with T1-weighted whole-body magnetic resonance imaging (MRI), a reduction in absolute muscle mass was found after 20 months of remission (47,49). These data indicate that previous chronic exposure to GC induces irreversible structural and functional alterations in the muscle, which seriously affects the physical capacity of patients and their quality of life in the long term.

Persistence of residual morbidity and HRQoL deterioration, as assessed using the disease-specific CushingQoL questionnaire, have been documented even long-term after biochemical "cure" is reached, and mortality appears to remain more elevated in CS patients in remission as compared with that in the general population (50). These findings suggest that prior exposure to hypercortisolism in CS patients may be associated with pathophysiology changes in several tissues, which are maintained over time despite hormone normalization (51,52). Data from the ERCUSYN registry indicate that patients with CS who have been in remission for 4 years reported a lower quality of life (measured using the specific CushingQoL questionnaire, than in the active phase also due to the presence of muscle fatigue (52). In addition, the impact of myopathy on bone health in CS is yet to be determined. Of interest, lower bone mineral density (BMD) was observed in premenopausal women who had suffered from CS as compared with healthy controls, after an average of 11 years of biochemical healing (9).

1.3.2 Muscle in acromegaly

1.3.2.1 Effects of growth hormone (GH) and insulin like grow factor type I (IGF-I) in muscle

GH and IGF-I exert important effects on skeletal muscle mass and metabolism. GH, both directly and indirectly via IGF-I, has a net anabolic effect on whole body protein metabolism, essential to maintain skeletal muscle protein mass (26–28). Muscle biopsies in patients with GH excess due to ACRO have shown hypertrophy of type 1 fibers and atrophy of fibers 2, but the muscle mass does not appear to significantly change in shape, contrary to what would be expected due to the anabolic effect of GH on muscle (53). In particular, a body composition study with DXA demonstrated an increase in lean mass in active ACRO patients, mainly due to increased extracellular collagen deposition and fluid retention in soft tissues rather than to muscle cell hyperplasia (54). Likewise, a study using whole-body T1 MRI images did not document any difference between the muscle mass of active ACRO patients and healthy controls (55). Despite this, ACRO patients had lower muscle mass 2 years after control of GH excess, as compared with that measured at diagnosis, suggesting that the loss of muscle tissue may progress over time regardless of hormone normalization (56).

1.3.2.2 Muscle dysfunction in acromegaly

Musculoskeletal symptoms are frequent at diagnosis of ACRO and they may persist or even worsen long-term after control of GH/IGF-I excess (53,57). There are few data on muscle functionality in ACRO patients, most of which only evaluating strength or balance in uncontrolled disease. Some studies found a balance impairment as well as increased risk of falls and poor muscle function in lower limbs, as compared with healthy controls (58–60). A longitudinal study evaluating muscle strength found low hand grip strength at

diagnosis, which normalized after biochemical control was reached. Interestingly, fast concentric extension and endurance were decreased in controlled patients after 11 years of disease control as compared with baseline, suggesting that previous exposure to overt GH concentrations may have determined irreversible changes of muscle structure and/or quality potentially leading to altered functionality (61). However, impaired muscle performance in ACRO patients cannot be explained by changes in muscle mass, since the latter did not differ from that of healthy controls, at diagnosis or after successful treatment (55,61).

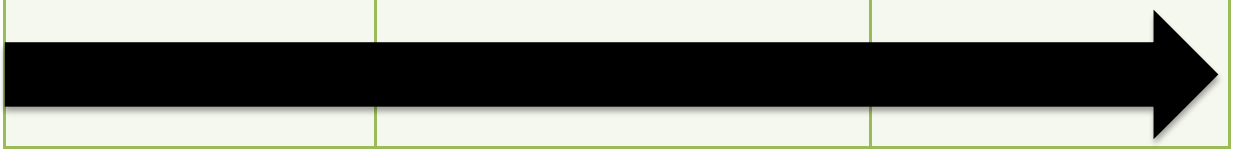
It should be noted that myopathy significantly contributes to the development and severity of ACRO arthropathy, one of the most frequent and disabling complications of this disease, which leads to irreversible degeneration of the osteoarticular structures (62). Musculoskeletal alterations have a serious impact on the HRQoL of patients and are associated with a significant deterioration in their physical function and psychological state, which persists beyond 10 years after remission of the disease (33). Also, the mechanisms whereby the decrease in muscle strength affects bone mineral density and the high risk of fractures observed in ACRO remains to be determined (35,63).

1.4 Potential role of myosteatosis on the development of muscle dysfunction

The decline in muscle strength that occurs with aging or obesity is associated with an accumulation of intramuscular fat infiltration (myosteatosis). The factors leading to myosteatosis are poorly understood, but recent evidence indicates that disuse, altered leptin signaling, sex steroid deficiency, and glucocorticoid treatment contribute to it (64–68). Conditions leading to intramuscular fat accumulation are summarized in Table 3.

Table 3. Conditions leading to intramuscular fat accumulation, and its consequences.

Aging GC Unloading/disuse Sex steroid deficiency Leptin deficiency Muscle injury Increase circulating lipids	Increased lipid stores and lipid droplet size in skeletal muscle Differentiation of fibro/adipogenic progenitors (FAPS's) towards to adipocyites	Insulin resistance Inflammation Functional deficit Fragility



Intramuscular fat is not an inert fat depot that simply fills the space left by lean mass loss, but an important factor and a possible predictor of muscle function that seems to act synergistically with sarcopenia (69). Both in vitro and in vivo studies suggest that it may be an important contributor to structural and metabolic changes leading to atrophy of muscle fibers, poor muscle performance, weakness, musculoskeletal fragility, and insulin resistance (70–75). Furthermore, it is associated with high all-cause mortality in the elderly, regardless of cardiovascular comorbidities (76). In particular, recent data indicates that the volume of intramuscular fat is associated with poor muscle functionality and mobility limitations in human aging, muscular dystrophies, and in models of muscle injury, such as massive rotator cuff tear (70,77–81).

Even though the specific mechanisms underlying the harmful effects of myosteatosis on local and systemic muscle metabolism are yet unknown, some theories involving metabolic, endocrine and mechanical factors have been proposed. The "metabolic theory" proposes that the close proximity of fat to the muscle fiber may damage the local muscle environment through the paracrine secretion of pro-inflammatory cytokines which have a direct impact on skeletal myocyte metabolism (82,83). Recent findings indicate that an increased number of inter-muscular adipocytes may enhance myotube mRNA expression of genes involved in oxidative metabolism (84,85). In animal models, high synthesis of ceramide, a product of muscle oxidative damage, was independently related to myocytes insulin resistance induced by adipocyte-derived cytokines such as interleukine-6 (IL6) and tumor necrosis factor-α (TNF- α) (86,87). Some studies have also suggested that proinflammatory cytokines may also favor proteolysis and muscle catabolism (82,83,88,89). One study suggested that myosteatosis may also lead to the transition of type II to type I muscle fibers (90), which ultimately impairs contractile function and muscle power (product of force and speed). Myosteatosis may also impair nutritive blood flow to muscle (91).

The "endocrine theory" proposes that myosteatosis may disrupt the secretion of the so-called myokines, cytokines synthesized and released by myocytes during muscular contraction (92). The muscle expression of myostatin, a myokine that inhibits the conversion of the muscle mesenchymal cell towards to the myogenic line, increases proportionally to the degree of myosteatosis in older people (93). Finally, myosteatosis may also impair the contractile capacity due to changes in muscle fiber orientation ("mechanical theory"). Future studies are needed to determine which are the mechanisms linking intramuscular fatty infiltration and muscle weakness.

1.4.1 What is known of the relationship between muscle and fat in Cushing's syndrome?

Cortisol excess in CS is associated with fat partitioning, consistent with glucocorticoids being prominent regulators of body fat metabolism and regional distribution (47,94,95). As a matter of fact, total fat, visceral fat, and trunk subcutaneous fat, as measured by whole-body MRI, were greater in active CS as compared with controls (47). Interestingly enough, Geer et al showed that whole-body intermuscular fat (IMAT), defined as the adipose tissue located between muscle groups and beneath the muscle fascia, did not change in patients with CD after a mean of 20 months of remission, despite a concomitant reduction of the other fat depots, including visceral, trunk subcutaneous, and pelvic bone marrow adipose tissue, as compared with the pretreatment measurements (96). In light of this evidence suggesting that abnormal fat distribution may persist in the muscles of CS patients despite remission, we aimed at assessing whether there is intramuscular fatty infiltration in CS patients after long-term remission, and whether this is associated with poor muscle function.

1.4.2 What is known of the relationship between muscle and fat in acromegaly?

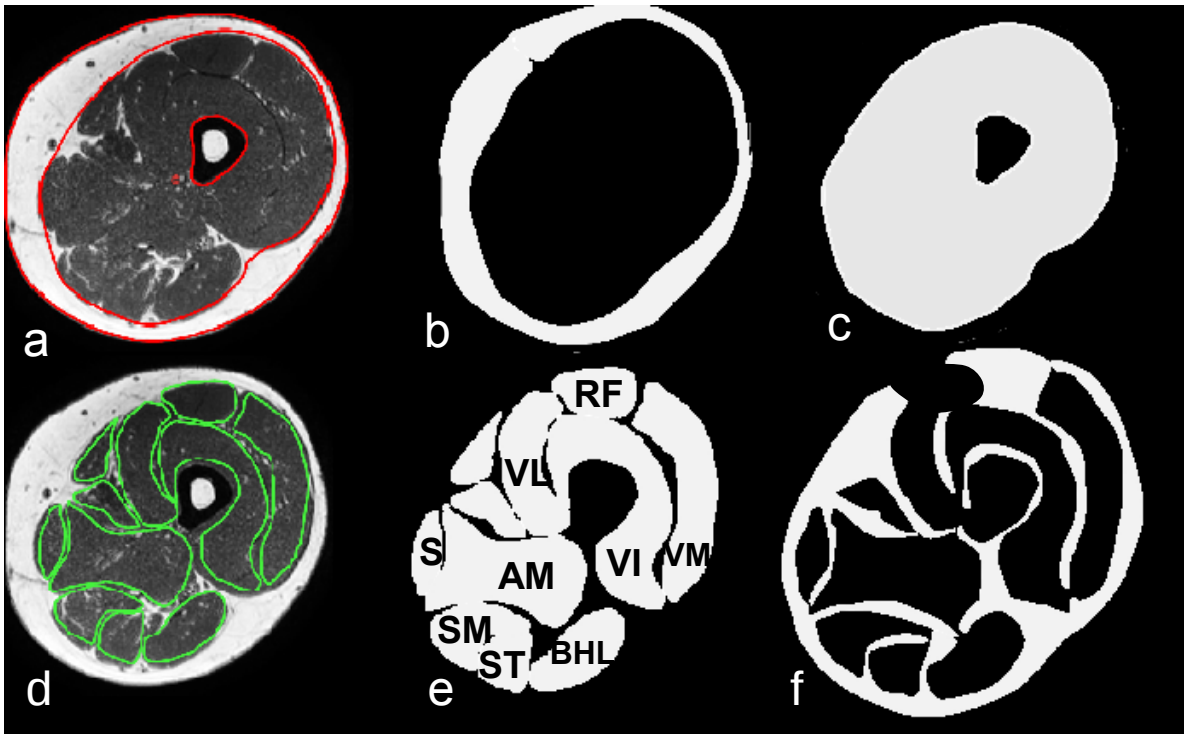
GH has important effects on muscle energy metabolism and its actions on fat metabolism are tissue-specific. Whereas GH is globally lipolytic and induces whole-body lipid oxidation, in muscle, it down-regulates genes involved in lipid β-oxidation, promoting lipid storage and carbohydrate utilization as primary fuel energy (49). It is well known that GH stimulates whole-body lipid oxidation in healthy humans (97–99). Administration of GH to adults with growth hormone deficiency (GHD) induces protein retention primarily by increasing protein synthesis, decrease in overall glucose turnover, and

stimulates of lipid oxidation, leading to an increase of plasma fatty acids levels (100–102). In skeletal muscle, GH action is rather the opposite. GH treatment down-regulated genes involved in lipid β-oxidation in skeletal muscle of aged rats (103–105). Moreover, GH replacement in adult GHD patients was found to be lipogenic in muscle, in that inhibited the expression of genes related to lipid metabolism, as well as tricarboxylic acid cycle activity and mitochondrial respiration in skeletal muscle, promoting lipid storage and anaerobic substrate metabolism for ATP synthesis (106).

Chronic excess of GH, as occurs in ACRO is associated with significant adipose tissue redistribution (107). In patients with uncontrolled ACRO, abdominal subcutaneous and visceral fat were lower than predicted, consistent with the well-known lipolytic effect of GH (107,108) . On the contrary, both the IMAT and intramyocelular lipids deposits were unexpectedly high in active ACRO patients compared to healthy subjects, despite the lipolytic effect of GH (109,110). Indeed, two years after hormonal excess control, the former persisted elevated, while intramuscular fat did not vary compared to the volume measured before biochemical control (109). These findings suggest that the metabolic derangements associated with GH/IGF-I excess may lead to fat accumulation in muscles of ACRO patients, which may persist despite hormone control (97,109,111), therefore we aimed at investigating whether there is increased fat accumulation in muscles in ACRO and this is associated with muscle function impairment.

In Figure 1 fat deposits compartments in the thigh are depicted in a 2point Dixon sequence MRI image.

Figure 1. Differentiation of fat deposits in the muscle: MRI Segmentation of muscle and fat compartments in thigh: (a) superimposed ROIs for subcutaneous fat and bone and bone marrow regions; (b) subcutaneous adipose tissue mask; (c) mask including all the muscle regions and excluding the bone and bone marrow regions; (d) muscle ROIs used for the evaluation of fat distribution; (e) masks of ten muscle ROIs; and (f) mask of soft tissue excluding subcutaneous fat and six muscular ROIs. Fat within the mask of (e) corresponds to intramuscular fat within the mask of (f) corresponds to intermuscular fat.

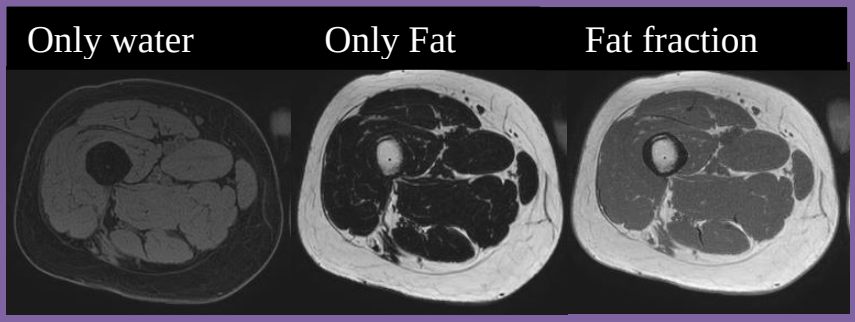
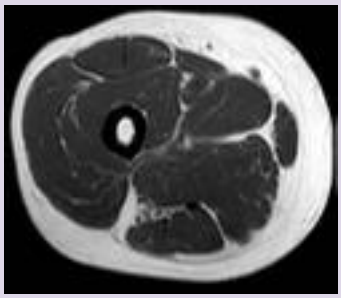
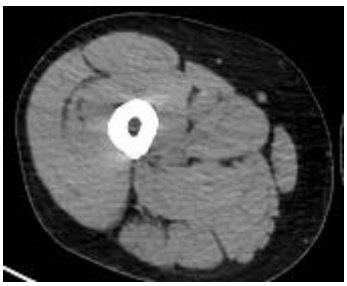
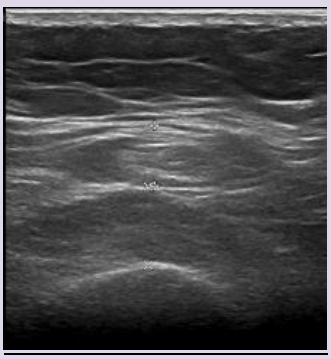


1.5 Techniques to assess intramuscular fatty infiltration in Cushing's syndrome (CS) and acromegaly (ACRO)

Muscle "quality" is a novel term, referring to both micro and macroscopic changes in muscle architecture and composition (112). Highly-sensitive imaging tools such as CT and MRI have been used to assess it in research settings, by using the attenuation of the muscle or determining infiltration of fat into muscle, respectively (69,113) (Table 4). An ideal specific tool that could be used as a biomarker of muscle quality should be quantitative and reflect specific pathological events with accuracy. These characteristics would allow determining to what extent muscle quality is altered (severity), monitoring pathological changes over time and, most importantly, evaluating skeletal muscle responses to therapeutic intervention. In this line, in the last years, quantitative muscle MRI has been used to quantify fatty infiltration in the skeletal muscle of patients with several muscle disorders (114). The ideal tool is the Dixon technique, a specific sequence applied to MRI images, which is a robust chemical-shift imaging application producing water- and fat-only images from different echo acquisitions and has excellent accuracy for intramuscular fat quantification, expressed as percentages of fat fraction (FF), in comparison to spectroscopy, and histology examination (115–117).

Importantly, the fat fraction maps acquired with Dixon sequences have proven their capability to detect subtle changes in muscle composition, as might occur in CS and ACRO, and have repeatedly shown superior sensitivity over standard functional evaluation and the semiquantitatives methods T1-weighted MRI and echo intensity of ultrasonography (118,119) (Table 4). Moreover, Dixon-assessed FF has proven to be a reliable outcome measure that is related to muscle function parameters in several neuromuscular disorders (80,114,116,119–121). Figure 3 summarizes the existing techniques to assess intramuscular fatty infiltration

Table 4. Techniques to assess intramuscular fatty infiltration

Quantitative MRI: Dixon Ectopic Fat: Exact Fat %	
Semiquantitative MRI: T1 weighted MRI: Estimated Fat %	
Computed Tomography: Muscle Attenuation	
Ultrasonography Muscle Echo Intensity	

1.6 New definition of sarcopenia applied to Cushing's syndrome

Although sarcopenia has been traditionally defined as the loss of muscle mass occurring in aging, recent evidence has recognized it as a muscle disease, mainly characterized by reduced muscle strength and function, which are not necessarily associated with low muscle mass (122,123). Traditionally, loss of muscle mass has been considered the *primum movens* for the development of sarcopenia and therefore, quantification of muscle mass has been used as the most reliable tool to diagnose sarcopenia in the elderly (124). This assumption has been recently challenged by the observation that reduction of muscle strength and/or performance is a key feature of aging-related sarcopenia, which appears to be more linked to muscle quality impairment rather than to a decrease in muscle mass (125). Indeed, sarcopenia is now considered as an independent clinical entity which may be observed in several groups of individuals, across the lifetime (125). In agreement with this, the European Working Group on Sarcopenia in Older People 2 (EWGSOP2) recently suggested a comprehensive clinical algorithm for case-finding, diagnosis, confirmation and severity classification of sarcopenia, taking into account muscle quality and quantity, muscle strength and physical performance (125). Patient's perception has also been recognized as an important parameter to suspect sarcopenia and measure its impact on patient's daily life (125).

Because diagnosis of sarcopenia is based on imaging that is technically difficult and expensive or not immediately available in primary care, several biomarkers have been advocated in recent years as a cost-effective tool to easily diagnose muscle dysfunction in clinical practice (126).

Creatinine (Cr) and cystatin C (CysC) are serum markers commonly used to measure glomerular filtration rate. Cr freely filters in the glomeruli, and is excreted unchanged in the urine except for a small but variable degree of proximal tubular secretion. Because Cr production varies with body composition, it could underestimate actual GFR in case of high muscle mass and *vice versa* (127). CysC is a low molecular weight protein which also freely filters in glomeruli but is reabsorbed completely by the proximal tubular cells. Since CysC is excreted by all nucleated cells, the impact of muscle mass on its production is negligible. The Sarcopenia Index (SI) [Cr (mg/dL) / CysC (mg/L) × 100] has recently been proposed as a potential marker of muscle mass, muscle quality and muscle function in patients with normal renal function hospitalized in intensive care units, as well as in patients with type 2 diabetes, hepatocellular carcinoma, and in lung transplant candidates (128–130).

Because of the lack of studies determining the prevalence of sarcopenia in patients previously exposed to endogenous cortisol excess and its potential impact on HRQoL we aimed at investigating which is the prevalence of sarcopenia in CS patients in long-term remission using the EWGSOP2 criteria and the impact of sarcopenia on HRQoL in these patients. We also aimed at determine if the Sarcopenia Index (SI) is a reliable surrogate marker of sarcopenia in CS.

2. Hypothesis

This evidence led us to hypothesize that:

- Muscle architecture remains impaired long-term after normalization of cortisol levels in Cushing's syndrome (CS) patients, and control of GH excess in acromegaly patients (ACRO) due to increased fatty infiltration.

- Intramuscular fatty infiltration in "cured" CS and controlled ACRO patients may be associated with muscle weakness and poor functionality

3. Objectives

Main Objective:

- To study the degree of intramuscular fat fraction (%), (by MRI with 2-point Dixon) in patients with Cushing syndrome with long term remission and in patients with controlled acromegaly, to clarify mechanisms responsible for persistent sarcopenia and muscle weakness in these patients.

Secondary objective:

- To correlate myosteatosis and structural alterations of the muscle with muscle strength and performance in patients with Cushing syndrome with long term remission and in patients with controlled acromegaly.

4. Compendium of publications

4.1 Manuscript I

Martel-Duguech L, Alonso-Jiménez A, Bascuñana H, Díaz-Manera J, Llauger J, Nuñez-Peralta C, Biagetti B, Montesinos P, Webb SM, Valassi E. Thigh Muscle Fat Infiltration Is Associated With Impaired Physical Performance Despite Remission in Cushing's Syndrome. J Clin Endocrinol Metab. 2020 May 1;105(5): :2039–2049 dgz329. doi: 10.1210/clinem/dgz329. PMID: 31912154.

Impact factor 5.958

Martel-Duguech L, Alonso-Jiménez A, Bascuñana H, Díaz-Manera J, Llauger J, Nuñez-Peralta C, Biagetti B, Montesinos P, Webb SM, Valassi E. Thigh Muscle Fat Infiltration Is Associated With Impaired Physical Performance Despite Remission in Cushing's Syndrome. J Clin Endocrinol Metab. 2020 May 1;105(5): :2039–2049 dgz329. doi: 10.1210/clinem/dgz329. PMID: 31912154.

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4.2 Manuscript II

Martel-Duguech L, Alonso-Perez J, Bascuñana H, Díaz-Manera J, Llauger J, Nuñez-Peralta C, Montesinos P, Webb SM, Valassi E. Intramuscular fatty infiltration and physical function in controlled acromegaly. Eur J Endocrinol. 2021 May 1:EJE-21-0209.R1. doi: 10.1530/EJE-21-0209. Epub ahead of print. PMID: 33950861.

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Martel-Duguech L, Alonso-Perez J, Bascuñana H, Díaz-Manera J, Llauger J, Nuñez-Peralta C, Montesinos P, Webb SM, Valassi E. Intramuscular fatty infiltration and physical function in controlled acromegaly. Eur J Endocrinol. 2021 May 1:EJE-21-0209.R1. doi: 10.1530/EJE-21-0209. Epub ahead of print. PMID: 33950861.

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5. Global summary of the results

5.1 Patients with Cushing's syndrome

Primary outcome

Patients with Cushing's syndrome (CS) showed greater muscle fat fraction (FF) in both the anterior and posterior compartment as well as in the combined anterior-posterior compartments of the thigh as compared with controls controls (18.9±4.8% vs. 16.2±4.8%, p = 0.027 for anterior compartment; 22.5±5.9%vs.19.2 ± 4.8%, p = 0.015 for posterior compartment; 20.6±4.8% vs. 17.9±4.7%, p = 0.027 for anterior-posterior compartment). The lean muscle volume was not different in CS as compared with controls (13198±1879 cm^3 vs. 13589±1901cm^3; p = 0.24). When only premenopausal and postmenopausal women were analyzed, separately, FF in the anterior compartment and in the posterior compartment, respectively, was still greater in CS patients as compared with controls (16±4 vs. 13±2.3; p = 0.027 for anterior compartment in premenopausal women, 24.9±5.4 vs. 21.2±4.4; p = 0.022 for posterior compartment in postmenopausal women).

Performance on all functional tests was worse in CS patients as compared with controls as demonstrated by longer time needed to complete the timed up and go (TUG) test, lower average number of times the former stood from a chair during 30-seconds in the 30-seconds chair stand test (30-s) and lower velocity in their usual space, in the gait speed test (GS) (6.7±1.6 vs. 5.9±1.5s; p = 0.023 for TUG, 14.6±4.1 vs. 17.6±5; p = 0.011 for 30-s, 1.1±0.2s/m vs. 1.2±0.2s/m; p = 0.034 for GS). Also, performance on 30-s remained significantly more impaired in either premenopausal or postmenopausal patients as compared with their counterpart in the control group, as well as performance in TUG test was still significantly poorer in postmenopausal

patients as compared with controls (17.2±4 vs. 21.3±4.3; p = 0.021 in premenopausal women and 13.2±3.4 vs. 15.4±3.9; p = 0.033 in postmenopausal women for 30-s, 7.2±1.8 vs. 6.3±1.6; p = 0.033 in postmenopausal women for TUG). These results suggest impaired static and dynamic balance, lower strength in lower limbs and lower functional capacity in patients.

Secondary outcome

Considering the association between FF and muscle function, only in CS patients and not in controls, greater mean FF in all thigh muscle compartments was negatively associated with performance on TUG, 30-s and GS ($p < 0.05$ for all associations).

Regard predictors of muscle function, only in CS patients FF in the combined anterior-posterior compartments were predictors of performance on TUG and GS, after adjusting for age, BMI, menopause and muscle volume in a multiple linear regression model (ß .626, $p < 0.0001$ for TUG; ß -.461, $p = 0.007$ for GS)

5.2 Patients with acromegaly

Primary outcome

Patients with acromegaly (ACRO) showed greater muscle FF in the anterior, posterior and combined anterior-posterior compartment of the thigh, as compared with controls (25.6 ± 7.2% vs. 18.4 ± 5.7%, $p < 0.001$ for anterior compartment; 33.4 ± 5.9% vs. 21.6 ± 7.5%, $p < 0.001$ for posterior compartment; 29.5 ± 7.7% vs. 19.9± 6.4%, $p < 0.001$ for combined anterior-posterior compartment). When each muscle was evaluated separately, FF in muscle rectus femoris (RF), vastus intermedius (VI), adductor magnus (AM) and semimembranosus (SM) was greater in ACRO patients as compared with controls (24.1 ± 8.9% vs. 18.2 ± 5.5%, $p = 0.03$, for RF; 18.7 ± 5.4% vs. 15.6

± 5.1%, p = 0.012, for VI; 21.3 ± 4.7% vs. 18.4 ± 3.7%, p = 0.019, for AM; 25.1 ± 5.5% vs. 21.4 ± 4.7%, p = 0.038 for SM). When women were analyzed separately, FF in anterior, posterior and combined anterior-posterior compartment, as well as in RF, VI, AM and SM was still greater in ACRO patients as compared with controls ($p < 0.05$ for all comparisons). Also, FF in sartorius (Sart) was greater in ACRO women as compared with the controls (36.2 ± 31.3, p = 0.029). When males were analyzed separately, FF of the anterior, posterior and combined anterior-posterior compartment was still greater in ACRO patients as compared with controls ($p < 0.05$ for all comparisons).

ACRO patients had worse performance in all muscle functional test as reflected by longer time needed to complete TUG, less sit to stand times in the 30-s and slower speed at their usual pace on the GS as compared with controls (6.8 ± 1.3 vs. 5.5 ± 0.2, P <0.001 for TUG; 16.4 ± 5.5 vs. 19.9 ± 6.1, p = 0.021 for 30-s; 1.15 ± 0.21 m/s vs. 1.34 ± 0.21m/s, p = 0.001 for GS). When ACRO women and men were analyzed separately, they still had poorer performance on TUG as compared with their controls (7.07 ±.1.19 vs. 5.54 ± 0.98, $p < 0.001$ for women; 6.4 ± 1.3 vs. 5.4 ±0.6, p = 0.041 for men). Performance on GS was still significantly poorer in ACRO males when analyzed separately (1.15 ± 0.24 vs. 1.5 ± 0.19, p = 0.001). These results suggest that ACRO patients have worse mobility, balance, walking ability and greater fall risk as well as decreased strength in lower limbs and lower functional mobility.

ACRO patients showed greater median values (interquartile range, IQR) for Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) score on pain, stiffness and functionality subscales as compared with controls (3.5 (6) vs. 1 (1.7), $p < 0.001$ for pain; 3 (3) vs. 0.94 (1.4), $p < 0.001$, for stiffness; 18.5 (25.5) vs. 1.6 (2.19), $p < 0.001$; for functionality).

Secondary outcome

In ACRO patients only, worse performance on TUG was correlated with greater mean FF in the anterior compartment, combined anterior-posterior compartment, RF, VI, VL (vastus lateralis), AM, BHL, ST, Sart, SM, gracilis (G) and vastus medialis (VM) ($p < 0.05$ for all correlations)

In ACRO patients, slower GS was associated with higher WOMAC functional and stiffness score, indicating that higher degree of functional impairment and joint stiffness have slower pace ($r = -0.525$, $p = 0.025$; $r = -0.469$, $P= 0.043$). Also, in ACRO patients, worse performance on TUG was associated with higher WOMAC pain score, and WOMAC functional score, indicating that patients with greater lower limb joint pain and greater functional impairment have worse mobility, balance, walking ability, and greater fall risk ($r = -0.483$, $p = 0.036$; $r = -0.500$ $p = 0.029$). In ACRO patients, worse performance in 30-second chair stand test was associated with higher WOMAC functional score, indicating that patients with higher degree of functional impairment have greater muscle weakness in lower limbs ($r = -0.464$, $p = 0.045$).

For the multiple logistic regression model, we examined all variables that may be involved in muscle function. Only in patients the FF in the combined anterior-posterior compartment was associated with the performance on TUG, regardless of muscle mass, IGF-I SDS and WOMAC functional and pain score indicating that higher FF in thigh muscles altogether predict longer time to complete TUG test, meaning worse functional mobility in ACRO patients ($\beta = 737$, $p < 0.001$)

6. Global summary of the discussion of the results obtained

6.1 Intramuscular fatty infiltration in patients with Cushing's syndrome and patients with acromegaly and its relationship with muscle performance

We have shown that patients with Cushing's syndrome (CS) in long-term remission as well as patients with controlled acromegaly (ACRO) have increased intramuscular fatty infiltration in thigh muscles, as compared with controls carefully matched for age, gender, BMI and degree of physical activity. Also, we demonstrated that this is associated with impaired performance on functional tests which assess individual functional capacity, balance, and strength in both groups of patients. Moreover, this relationship was independent of factors which are known to affect physical performance, such as low muscle mass and joint symptoms.

"Cured" CS patients frequently complain of fatigability at follow-up visits. Indeed, Berr et al. showed that performance on chair rising test, which was more impaired in active CS as compared with controls, did not improve 6 months after successful treatment of hypercortisolism (48). Moreover, the chair rising test performance was not different in CS patients after a mean of 13 years of remission in comparison to that described in active CS patients (48)

Muscle weakness, mostly involving the lower limbs, is one of the commonest complications of chronic exposure to cortisol excess in CS (1). Steroid-induced myopathy, which is characterized by the preferential atrophy of type IIa, fast-twitch muscle fibers, is partly related to the well-known anti-anabolic, proteolytic, and pro-apoptotic effects of glucocorticoids, resulting in the breakdown of the contractile proteins and suppression of myofibrillar protein synthesis (1,36). The imbalance of anabolic and catabolic processes

would ultimately cause muscle loss in patients exposed to hypercortisolism, which is reflected by the slowing of muscle fiber conduction and decrease in circulating muscle protein levels (44). As a matter of fact, studies of body composition using both dual-energy X-ray absorptiometry (DXA) and whole-body MRI showed a decrease in lean mass of the limbs and reduced skeletal muscle mass respectively, in active CS patients as compared with BMI-matched controls (47,95). Thus, one could speculate that, after successful treatment of CS, persistent impairment of muscle function is due to the irreversible loss of muscle mass that occurred during the active phase of the disease (48). Yet, we have shown that after 13 years of remission, muscle volume in the thigh is not different in CS patients as compared with controls, and is unrelated to physical performance. Our findings are in line with previous studies using DXA or bioelectrical impedance analysis to assess body composition in CS after more than one year of eucortisolism, which did not find any differences in lean body mass or skeletal muscle mass in patients as compared with BMI-matched controls (122–124). Burt el al. documented the restoration of protein synthesis 13 months after successful treatment of CS along with a significant increase in percentage of lean body mass, suggesting that changes in muscle mass may, at least in part, be reversible following hormone normalization (125).

We have demonstrated that fat infiltration was elevated in thigh muscles of "cured" CS patients, despite long lasting biochemical remission. Glucocorticoid excess in CS is associated with changes in fat distribution, such as elevated total fat, visceral fat and trunk subcutaneous fat (124,126). Persistent elevation of both DXA-measured total fat and trunk fat was documented in CS patients after long-term remission as compared with controls (124). Noteworthy, Geer et al. found that the intermuscular adipose tissue (IMAT), as measured by whole-body semi-quantitative MRI, was the

only fat depot that did not decrease after 20 months of remission in 14 CS patients, as compared with their preoperative measurements, suggesting that the metabolic derangements leading to fat deposition between muscles may be maintained over time (47).

Pathways whereby glucocorticoids regulate fat metabolism and mediate specific fat accumulation in muscle are complex and not completely understood. Glucocorticoids are known to enhance both the conversion of pre-adipocytes to adipocytes and the differentiation of the bone marrow mesenchymal stem cells towards the adipocyte lineage (71). While dexamethasone administration to mice enhanced adiposity and insulin-resistance in muscle, an in vitro study showed that dexamethasone promoted the differentiation of murine muscle adipogenic progenitors to adipocytes through the inhibition of inteleukin-4 signaling (127,128). Role of glucocorticoids in the modulation and maintenance of fat infiltration in muscles of CS patients should be clarified in future studies. Moreover, to elucidate the impact of hypercortisolism-related comorbidities, such as type 2 diabetes and osteoporosis, on fatty accumulation in muscle of CS patients should be an important goal of future research.

Estrogen deficiency is also known to increase both lipid content and the expression of adipogenic genes in muscle and, consistently, intramuscular fatty infiltration has been documented in postmenopausal women (129). However, the relationship between menopause and muscle performance is not well-established, with most of the studies suggesting that muscle dysfunction in postmenopausal women is due to many factors, especially aging, physical activity and adiposity, rather than to estrogen deprivation *per se* (130). In agreement with this, when we analyzed separately patients and controls according to the menopausal status, fat fraction (FF) remained greater while

muscle performance on some tests was still poorer in either premenopausal or postmenopausal women with CS as compared with controls. These findings suggest that the negative impact of prior exposure to cortisol excess on muscle quality prevails on any potential protective or detrimental effect of estrogen-sufficiency or deficiency, respectively. Accordingly, previous studies showed altered body composition and low bone mineral density in estrogen-sufficient women with CS as compared with estrogen-sufficient controls, indicating that the protective effects of estrogens is lost in the presence of cortisol excess (9,131).

The small sample size is a limitation of the study as well as the inclusion of patients with both pituitary-dependent and adrenal-dependent CS. However, a large population-based study did not show any differences in self-reported muscle weakness between patients with pituitary-dependent and those with adrenal-dependent CS (1). Also, we have only included patients in long-term remission. Future studies are needed to prospectively evaluate structural and functional changes in muscle of CS patients before and after surgical correction of cortisol excess. Although we have only included female patients with CS in order to analyze a more homogeneous sample, inter-gender differences in muscle structure and function may exist and therefore, future studies should also evaluate these parameters in men (142).

We have shown that patients with long-term controlled ACRO have increased fatty infiltration in thigh muscles, as compared with controls. GH has important effects on muscle energy metabolism and composition. GH stimulates whole-body lipid oxidation in humans (97–99). Administration of GH to healthy men increased lipid oxidation, circulating free fatty acid levels and intra-myocellular triglyceride content (99). GH excess in ACRO was associated with significant increase in the flux of free fatty acids into the

muscle due to enhanced whole-body lipolysis in extra-muscle fat depots (98,111). It may be speculated that the increased intramuscular fatty infiltration documented in our study may be the result of the elevated lipid uptake which occurred in muscle during the active phase of the disease.

In vivo studies also showed that GH treatment down-regulated genes involved in lipid β-oxidation in skeletal muscle of aged rats (103–105). Moreover, GH replacement in adult GH-deficient (GHD) patients inhibited the expression of genes related to lipid metabolism, as well as tricarboxylic acid cycle activity and mitochondrial respiration in muscle, suggesting that GH may enhance lipid storage and anaerobic substrate metabolism for ATP synthesis (106). Future studies are needed to clarify if GH may also play muscle-specific metabolic effects which could promote, under certain conditions, a lipogenic pattern, opposite to its extra-muscle lipolytic action (132).

Reyes-Vidal et al. prospectively examined the potential changes occurring in both whole-body adipose tissue depots, as assessed using semi-quantitative MRI, and intramyocellular lipid content (IMCL), measured using proton MR spectroscopy, in the tibialis anterior of 14 ACRO patients before and 2 years after successful surgical treatment (107). They did not find any differences in the IMCL of controlled ACRO as compared with baseline, despite postsurgical increase in trunk adipose tissue, abdominal subcutaneous and visceral fat, and intrahepatic lipid content. Moreover, intermuscular fat (IMAT) was greater than predicted in active ACRO and decreased after surgery only in women (107,109). These findings suggest that fat accumulation may occur in muscle of uncontrolled ACRO and persist despite normalization of GH and IGF-I, likely due to tissue-specific differences in the responsiveness of the adipose tissue depots to the metabolic effects of GH (107).

We have shown that patients with long-term controlled ACRO have slower gait speed and poorer performance on muscle function tests. In particular, worse performance on the timed up and go test (TUG), which reflects impaired mobility, balance, walking ability, and higher fall risk, was associated with greater degree of fatty infiltration independent of muscle area and joint symptoms (133). TUG is an easy test which reflects daily functional ability and was recently recognized by the European Working Group on Sarcopenia in Older People (EWGSOP) as a reliable tool to assess physical performance and identify those patients with severe sarcopenia, a muscle disease characterized by muscle dysfunction and low muscle mass and/or altered muscle quality (133–135).

One previous study found less peripheral muscle strength and greater fatigability, as assessed using isometric dynamometry, surface electromyography and a six-minute walk test in 12 young controlled ACRO patients as compared with controls (58). A longitudinal study evaluating muscle strength found decreased fast concentric extension and endurance in controlled patients after 11 years of follow-up since the start of treatment, as compared with baseline (61).

Studies in humans showed that GHD is associated with changes in both muscle contractile properties and neural activation independent of muscle mass (136–138). Growth-hormone administration to both healthy subjects and GHD patients increased body cell mass without a concomitant improvement of muscle strength and endurance (137–139). These findings suggest that the mechanisms whereby GH influences muscle function are multiple and not merely limited to its pro-anabolic effects.

An independent relationship between the deterioration of muscle quality, mainly due to increased fatty infiltration, and impaired muscle function has

also been described in several neuromuscular disorders, in Cushing's syndrome as well as in human aging (78,140–142).

We found that CS patients after long term remission as well as controlled ACRO patients have increased intramuscular fatty infiltration and this is associated with impaired performance on functional tests which assess individual functional capacity, balance and strength (143–146). Moreover, the relationship we have described between fatty infiltration of skeletal muscle and poor functionality was independent of factors which are known to affect physical performance, such as low muscle mass, older age, menopausal status as well as joint symptoms (133).

These results support the hypothesis that the sustained alteration of physical performance in these patients may be associated with the deterioration of muscle quality due to intramuscular fat accumulation rather than to decreased muscle mass (147). This is consistent with previous observations in human aging (77,78,141). In a large cohort of elderly people followed-up over three years, a progressive decline of muscle strength was reported, which was only minimally paralleled by concomitant loss of muscle mass (141). Moreover, aged subjects who were able to increase their muscle mass through exercise, did not attain a relevant gain of strength, suggesting that qualitative changes of muscle may be one of the main determinants of functional impairment (142). Indeed, fat infiltration in the thigh muscles, as assessed using CT, progressively increases with aging and has been found to be an independent predictor of both loss of strength and mobility limitation in weight-stable aged men and elderly people, respectively (140). An inverse association between the degree of skeletal muscle fatty infiltration and physical performance has also been described in patients with neuromuscular disorders (79,80,148–152).

We used the Dixon technique to quantify, for the first time in CS and in ACRO, the exact percentage of fat in thigh muscles, which is the adipose tissue located inside the muscle and surrounding the muscle fibers.

A strength of our study is the low prevalence of hypopituitarism in our patients in with CS. While pituitary hormone deficiency is a frequent complication of surgery in many patients with pituitary-dependent CS, only 5% of our patients presented with hypopituitarism. They were on long-term hormone replacement and had normal hormone levels at study entry. Moreover, after excluding both patients with GH deficiency, and the two who were on hydrocortisone replacement, our results did not change. Also, we have performed an extensive evaluation of muscle function in patients and carefully matched control group, and evaluated the potential impact of joints symptoms, a common feature in ACRO, on muscle dysfunction. Interestingly, the relationship we have found between impaired performance on TUG and FF remained significant after adjusting for the coexistence of WOMAC functional limitation and pain scores. Furthermore, we included 10 patients on somatostatin analogs and 2 on pegvisomant. Potential, muscle-specific effects of medical treatment in ACRO patients have not been extensively studied thus far. Madsen et al. showed that co-treatment with somatostatin analog and GH receptor antagonist decreased intramyocellular content in the tibialis anterior muscle in 12 ACRO after 24 weeks, as compared with patients treated with somatostatin analogs only, supporting the hypothesis that the latter may exert independent metabolic actions on peripheral tissues, including skeletal muscle (167,168). However, when we excluded patients on medical treatment from the analysis, the difference in both fatty infiltration and muscle functionality between ACRO and controls remained significant. Larger studies are needed to confirm our results and evaluate potential effects of comorbidities and

concomitant medical treatment on muscle quality and function in ACRO patients.

Limitations of this study include the relatively small size of the total and sex-specific sample, and the cross-sectional design which prevents from inferring causality based on our data. Another limitation is that we included 22% of patients with hypopituitarism, which may affect muscle status. However, all our patients had been on stable hormone replacement for more than one year and their hormone levels were not different as compared with those in controls. In addition, the outcomes analyzed were similar between patients with hypopituitarism as compared with those without.

Interpretation of our results needs to consider that we included only 4 estrogen sufficient women (20%). Because estrogens contribute to the regulation of adipogenic pathways in muscle, future studies should specifically address the question of whether fatty infiltration and muscle function differ in ACRO women depending on the menopausal status (129).

6.2 Mechanisms linking intramuscular fat accumulation and impaired muscle contractility in patients with Cushing's syndrome and acromegaly

We postulated that this fat depot may be persistently elevated patients previously exposed to overt hypercortisolism as well as overt GH concentrations determining irreversible changes of muscle structure and/or quality possibly perpetuating functional impairment after biochemical control (80,116,121,153–155)

Although the mechanisms linking intramuscular fat accumulation and muscle contractility are still to be elucidated, it has been suggested that the close proximity of adipocytes and myofibers in muscle may promote muscle dysfunction, through the production of fatty acids, adipokines and

chemokines from adipose tissue that induce inflammation and oxidative stress (73,156–159). Increased flux of free fatty acid into the muscle and intramuscular lipid deposition have been associated with altered insulin signaling and mitochondrial dysfunction, leading to ATP pool depletion and impairment of myofibril contractile function (84). Indeed, decreased ATP synthesis and oxidative capacity, as measured using magnetic resonance spectroscopy (MRS), and respiratory gas exchange, have been described in the skeletal muscle of 7 ACRO patients long-term after successful treatment, as compared with healthy controls (160). In vitro studies showed that intramuscular secretion of pro-inflammatory adipokines blunts the expression of contractile proteins in myotubes, impairs myoblast differentiation and promotes myofibers atrophy (73,74,156). Moreover, fatty infiltration of muscle is associated with insulin-resistance which may determine glycolytic disturbances and reduced cellular uptake of glucose, ultimately leading to reduced ATP pool and impaired myofibril contractile function (161,162). Fatty infiltration may also induce direct, mechanical alteration in the muscle. Yoshida et al. demonstrated that the presence of non-contractile tissue, such as intramuscular fat, blocked muscle activation and force production in older adults, inducing both the compression of the motor unit and changes in muscle fiber orientation and fiber pennation angle (163).

6.3 Addressing fatty infiltration to prevent and treat sarcopenia in Cushing's syndrome patients and acromegaly.

While the mechanisms underlying this progressive deterioration of muscle functionality are still to be determined, efforts should be made to prevent and, possibly, revert it through the adoption of a healthy lifestyle. The beneficial effects of exercise on physical and mental health are well known. A sedentary lifestyle has been associated with poor muscle performance and significant

alterations of muscle composition and quality (i.e. excessive accumulation of fat within the muscle) in elderly people (164). A structured physical training program positively changed muscle composition in them, which was associated with significant amelioration of physical performance and, if continued, even prevented age-related decline of muscle function (165,166). Thus, physical activity should be considered as an important therapeutic intervention integrating and supporting other treatments, such as surgery and drugs, aimed at pursuing changes in patient's health. Exercise trainers should be part of the multidisciplinary health care team accompanying patients during all the phases of their disease.

According to the evaluation of Postgraduat subcomission, the Manuscript "Prevalence of sarcopenia after remission of hypercortisolism and its impact on HRQoL" cannot be part of the main body of the thesis, since it is published in a Q3 Journal. However, it is interesting information added about the new definition of sarcopenia applied to Cushing's syndrome and its impact on health related quality of life in these patients.

6.4 Prevalence of sarcopenia and its impact on quality of life in patients with Cushing's syndrome (*Annex*)

We have demonstrated that 19% (7 patients) of CS patients in long-term remission have sarcopenia, being greater than in controls. Also, from these 7 patients, 1 had severe sarcopenia. Such prevalence is similar to that described in both people older than 70 years (1-29%) and patients with type 2 diabetes mellitus (7.5–19.5%), and suggest that prior exposure to glucocorticoid excess plays a negative effect on muscle performance, strength and muscle quality, which persists even many years after its resolution (166,167). This important clinical information deserves to be taken into account to optimize treatment in CS as well as in patients on chronic glucocorticoid treatment. Indeed,

prevalence of secondary sarcopenia in inflammatory diseases range from 14% to 37%, depending on the disease, sample characteristics, and criteria used to define it (13). Traditionally, loss of muscle mass has been considered the *primum movens* for the development of sarcopenia and therefore, quantification of muscle mass has been used as the most reliable tool to diagnose sarcopenia in the elderly (124). This assumption has been recently challenged by the observation that reduction of muscle strength and/or performance is a key feature of aging-related sarcopenia, which appears to be more linked to muscle quality impairment rather than to a decrease in muscle mass (125). Indeed, deterioration of muscle function in older people was two- to five-fold faster than muscle mass wasting and was not reverted/prevented by specific training programs aimed at increasing muscle mass (168). Thus, sarcopenia has recently been recognized as a complex disease characterized by unfavourable changes in both muscle structure and function potentially occurring across a lifetime (125).

Based on our findings, the EWGSOP2 criteria should be used to diagnose sarcopenia in CS patients, since it is the only consensus including muscle quality and 30-second chair stand test as reliable parameters as an alternative to muscle quantity and hand grip strength, which are not deteriorated in our patients. However, it includes tools, such as the MRI 2-point Dixon, which are not always available in the clinical setting, due to elevated costs and specific technical requirements (169).

CS patients scored worse on the Sar-QoL questionnaire (auto-administered patient-reported outcome measure specific for sarcopenia, to evaluate how muscle weakness affects QoL), as well as on five of the eight dimensions of the generic Short-Form 36-Item (SF-36) HRQoL questionnaire. CS patients with sarcopenia had a lower SarQoL score as compared with those without

sarcopenia, indicating that the former perceived a poorer well-being. CS patients with sarcopenia scored worse on the functionality domain as compared with those without. Patients with sarcopenia also scored worse on CushingQoL pain, easy bruising, and worries on physical appearance as compared with patients without sarcopenia.

6.5 Usefulness of the Sarcopenia Index (SI) (*Annex*)

We have shown that a cut-off value of 72 for the Sarcopenia Index (SI) [Creatinine-Cr- (mg/dl)/cystatin C-CysC- (mg/L) × 100] identified those CS patients at risk of having muscle weakness and poor physical function. The SI was associated with low muscle mass, impaired muscle quality, poor functionality, and decreased HRQoL. Interestingly, while CysC levels were proportional to the amount of adipose tissue, the SI was associated with body muscle mass and total body fat in obese patients (170). Thus, it could be speculated that CysC levels may reflect changes in muscle quality, and potentially predict muscular functional decline in CS patients with sarcopenia. Future prospective studies are needed to confirm the usefulness of the SI in the clinical setting, to evaluate physical function in CS patients at every stage of the disease, and monitor the responsiveness to specific treatments aimed at improving muscle performance.

Interestingly, our middle-aged CS patients with sarcopenia had a similar SarQoL to that described in older sarcopenic patients(138). Moreover, CS patients with sarcopenia had impaired physical functioning and a greater score for easy bruising than patients without sarcopenia, indicating that the former had a more severe proteocatabolic state associated with significant physical limitations. In light of such an important impact of muscle dysfunction on patient's well-being, SarQoL could complement the CushingQoL questionnaire to evaluate health perception in patients with CS in remission.

7. Final conclusions

- Patients previously exposed to cortisol excess present with greater fatty infiltration in the thigh skeletal muscle along with poorer muscle function as compared with healthy controls.

- There is an independent association between fatty infiltration and impairment of performance on several function tests in “cured” CS patients. This suggests that clinicians should be aware of this sustained deterioration of muscle health and specifically address this problem during follow-up.

- Patients previously exposed to long-standing high circulating levels of GH and IGF-I have greater fatty infiltration in thigh muscles along with poorer muscle functionality, as compared with healthy controls.

- There is an independent association between fatty infiltration and worse performance on the timed up and go test (TUG), reflecting impaired mobility, balance, walking ability, and greater fall risk in ACRO patients. This indicates that muscle performance should be evaluated in ACRO patients even long-term after normalization of GH and IGF-I levels.

8. Future lines

- Larger studies are needed to confirm our results and evaluate the potential effects of comorbidities and concomitant medical treatment on muscle quality and function in patients with Cushing's syndrome (CS) and in patients with controlled acromegaly (ACRO).

- Pathways whereby glucocorticoids and GH/IGF-I regulate fat metabolism and mediate specific fat accumulation in muscles are complex and not completely understood.

- Mechanisms linking intramuscular fat accumulation and impaired muscle function in patients with CS with long term remission and patients with controlled ACRO are unknown.

- Further studies are needed to increase its understanding and to establish therapeutic strategies aimed at reducing intramuscular fat accumulation, with the purpose of reversing muscle dysfunction in these patients.

9. Bibliographic references

1. Valassi E, Santos A, Yaneva M, et al. The European Registry on Cushing's syndrome: 2-year experience. Baseline demographic and clinical characteristics. *Eur J Endocrinol*. 2011;165(3):383-392. doi:10.1530/EJE-11-0272

2. Valassi E, Feelders R, Maiter D, et al. Worse Health-Related Quality of Life at long-term follow-up in patients with Cushing's disease than patients with cortisol producing adenoma. Data from the ERCUSYN. *Clin Endocrinol (Oxf)*. 2018;88(6):787-798. doi:10.1111/cen.13600

3. Valassi E, Crespo I, Santos A, Webb SM. Clinical consequences of Cushing's syndrome. *Pituitary*. 2012;15(3):319-329. doi:10.1007/s11102-012-0394-8

4. Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. *Lancet*. 2006;367(9522):1605-1617. doi:10.1016/S0140-6736(06)68699-6

5. Ferraù F, Korbonits M. Metabolic comorbidities in Cushing's syndrome. *Eur J Endocrinol*. 2015;173(4):M133-M157. doi:10.1530/EJE-15-0354

6. Neary NM, Booker OJ, Abel BS, et al. Hypercortisolism is associated with increased coronary arterial atherosclerosis: Analysis of noninvasive coronary angiography using multidetector computerized tomography. *J Clin Endocrinol Metab*. 2013;98(5):2045-2052. doi:10.1210/jc.2012-3754

7. Lupoli R, Ambrosino P, Tortora A, Barba L, Lupoli GA, Di Minno MND. Markers of atherosclerosis in patients with Cushing's syndrome: a meta-analysis of literature studies. *Ann Med*. 2017;49(3):206-216. doi:10.1080/07853890.2016.1252055

8. Minnetti M, Caiulo S, Ferrigno R, et al. Abnormal linear growth in paediatric adrenal diseases: Pathogenesis, prevalence and management. *Clin Endocrinol (Oxf)*. 2020;92(2):98-108. doi:10.1111/cen.14131

9. Barahona M-J, Sucunza N, Resmini E, et al. Deleterious effects of glucocorticoid replacement on bone in women after long-term remission of Cushing's syndrome. *J Bone Miner Res*. 2009;24(11):1841-1846. doi:10.1359/jbmr.090505

10. Andela CD, van Haalen FM, Ragnarsson O, et al. MECHANISMS IN

ENDOCRINOLOGY: Cushing's syndrome causes irreversible effects on the human brain: a systematic review of structural and functional magnetic resonance imaging studies. *Eur J Endocrinol*. 2015;173(1):R1-14. doi:10.1530/EJE-14-1101

11. Webb SM, Valassi E. Morbidity of Cushing's Syndrome and Impact of Treatment. *Endocrinol Metab Clin North Am*. 2018;47(2):299-311. doi:10.1016/j.ecl.2018.01.001

12. Van Staa TP, Leufkens HGM, Abenhaim L, Begaud B, Zhang B, Cooper C. Use of oral corticosteroids in the United Kingdom. *QJM*. 2000;93(2). doi:10.1093/QJMED/93.2.105

13. Barone M, Viggiani M, Anelli M, et al. Sarcopenia in Patients with Rheumatic Diseases: Prevalence and Associated Risk Factors. *J Clin Med*. 2018;7(12):504. doi:10.3390/jcm7120504

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

15. Holdaway IM, Bolland MJ, Gamble GD. A meta-analysis of the effect of lowering serum levels of GH and IGF-I on mortality in acromegaly. *Eur J Endocrinol*. 2008;159(2):89-95. doi:10.1530/EJE-08-0267

16. Steyn FJ, Tolle V, Chen C, Epelbaum J. Neuroendocrine regulation of growth hormone secretion. *Compr Physiol*. 2016;6(2):687-735. doi:10.1002/cphy.c150002

17. Xu L, Gong Y, Wang H, et al. The stimulating effect of ghrelin on gastric motility and firing activity of gastric-distension-sensitive hippocampal neurons and its underlying regulation by the hypothalamus. *Exp Physiol*. 2014;99(1):123-135. doi:10.1113/expphysiol.2013.074716

18. Murray PG, Higham CE, Clayton PE. The hypothalamo-GH axis: The past 60 years. *J Endocrinol*. 2015;226(2):T123-T140. doi:10.1530/JOE-15-0120

19. Hoskuldsdottir GT, Fjalldal SB, Sigurjonsdottir HA. The incidence and prevalence of acromegaly, a nationwide study from 1955 through 2013. *Pituitary*. 2015;18(6):803-807. doi:10.1007/s11102-015-0655-4

20. Holdaway IM, Rajasoorya C. Epidemiology of Acromegaly. *Pituitary*. 1999;2(1):29-41. doi:10.1023/A:1009965803750

21. Sun H, Brzana J, Yedinak CG, Gultekin SH, Delashaw JB, Fleseriu M.

Factors associated with biochemical remission after microscopic transsphenoidal surgery for acromegaly. *J Neurol Surgery, Part B Skull Base*. 2014;75(1):47-52. doi:10.1055/s-0033-1354578

22. Mestrón A, Webb SM, Astorga R, et al. Epidemiology, clinical characteristics, outcome, morbidity and mortality in acromegaly based on the Spanish Acromegaly Registry (Registro Español de Acromegalia, REA). *Eur J Endocrinol*. 2004;151(4):439-446. doi:10.1530/eje.0.1510439

23. Fleseriu M. Insight into cardiovascular risk factors in patients with acromegaly. *Endocrine*. 2014;47(1):1-2. doi:10.1007/s12020-013-0157-y

24. Kamenický P, Mazziotti G, Lombès M, Giustina A, Chanson P. Growth hormone, insulin-like growth factor-1, and the kidney: Pathophysiological and clinical implications. *Endocr Rev*. 2014;35(2):234-281. doi:10.1210/er.2013-1071

25. Javaheri S, Barbe F, Campos-Rodriguez F, et al. Sleep Apnea: Types, Mechanisms, and Clinical Cardiovascular Consequences. *J Am Coll Cardiol*. 2017;69(7):841-858. doi:10.1016/j.jacc.2016.11.069

26. Møller N, Schmitz O, Jørgensen JOL, et al. Basal- and insulin-stimulated substrate metabolism in patients with active acromegaly before and after adenomectomy. *J Clin Endocrinol Metab*. 1992;74(5):1012-1019. doi:10.1210/jcem.74.5.1569148

27. Møller N, Butler PC, Antsiferov MA, Alberti KGMM. Effects of growth hormone on insulin sensitivity and forearm metabolism in normal man. *Diabetologia*. 1989;32(2):105-110. doi:10.1007/BF00505182

28. Rabinowitz D, Zierler KL. A metabolic regulating device based on the actions of human growth hormone and of insulin, singly and together, on the human forearm [23]. *Nature*. 1963;199(4896):913-915. doi:10.1038/199913a0

29. Colao A, Ferone D, Marzullo P, Lombardi G. Systemic Complications of Acromegaly: Epidemiology, Pathogenesis, and Management. *Endocr Rev*. 2004;25(1):102-152. doi:10.1210/er.2002-0022

30. Melmed S. Acromegaly pathogenesis and treatment. *J Clin Invest*. 2009;119(11):3189-3202. doi:10.1172/JCI39375

31. Swearingen B, Barker FG, Katznelson L, et al. Long-Term Mortality

after Transsphenoidal Surgery and Adjunctive Therapy for Acromegaly 1 . *J Clin Endocrinol Metab*. 1998;83(10):3419-3426. doi:10.1210/jcem.83.10.5222

32. Abosch A, Tyrrell JB, Lamborn KR, Hannegan LT, Applebury CB, Wilson CB. Transsphenoidal microsurgery for growth hormone-secreting pituitary adenomas: Initial outcome and long-term results. *J Clin Endocrinol Metab*. 1998;83(10):3411-3418. doi:10.1210/jcem.83.10.5111

33. Biermasz NR, Pereira AM, Smit JWA, Romijn JA, Roelfsema F. Morbidity after Long-Term Remission for Acromegaly: Persisting Joint-Related Complaints Cause Reduced Quality of Life. *J Clin Endocrinol Metab*. 2005;90(5):2731-2739. doi:10.1210/jc.2004-2297

34. Webb SM, Badia X. Quality of Life in Acromegaly. *Neuroendocrinology*. 2016;103(1):106-111. doi:10.1159/000375451

35. Valassi E, Crespo I, Malouf J, et al. Reduction of trabecular and cortical volumetric bone mineral density at the proximal femur in patients with acromegaly. *Eur J Endocrinol*. 2016;174(2):107-114. doi:10.1530/EJE-15-0931

36. Dirks-Naylor AJ, Griffiths CL. Glucocorticoid-induced apoptosis and cellular mechanisms of myopathy. *J Steroid Biochem Mol Biol*. 2009;117(1-3):1-7. doi:10.1016/j.jsbmb.2009.05.014

37. Valassi E, Tabarin A, Brue T, et al. High mortality within 90 days of diagnosis in patients with Cushing's syndrome: Results from the ERCUSYN Registry. *Eur J Endocrinol*. 2019;181(5):461-472. doi:10.1530/EJE-19-0464

38. D L Russell-Jones 1, A J Weissberger, S B Bowes, J M Kelly, M Thomason, A M Umpleby, R H Jones PHS. Protein metabolism in growth hormone deficiency, and effects of growth hormone replacement therapy - PubMed. *Acta Endocrinol*. 1993;128(Suppl 2):44-47.

39. Russell-Jones DL, Bowes SB, Rees SE, et al. Effect of growth hormone treatment on postprandial protein metabolism in growth hormone-deficient adults. *Am J Physiol - Endocrinol Metab*. 1998;274(6):1050-1056. doi:10.1152/ajpendo.1998.274.6.e1050

40. Fukagawa NK, Minaker KL, Rowe JW, et al. Insulin-mediated reduction of whole body protein breakdown. Dose-response effects on leucine metabolism in postabsorptive men. *J Clin Invest*. 1985;76(6):2306-2311. doi:10.1172/JCI112240

41. Janssen YJH, Doornbos J, Roelfsema F. Changes in Muscle Volume, Strength, and Bioenergetics during Recombinant Human Growth Hormone (GH) Therapy in Adults with GH Deficiency1. *J Clin Endocrinol Metab*. 1999;84(1):279-284. doi:10.1210/jcem.84.1.5411

42. Khaleeli AA, Edwards RH, Gohil K, et al. Corticosteroid myopathy: a clinical and pathological study. *Clin Endocrinol (Oxf)*. 1983;18(2):155-166. Accessed June 20, 2019. http://www.ncbi.nlm.nih.gov/pubmed/6851197

43. Schakman O, Gilson H, Thissen JP. Mechanisms of glucocorticoid-induced myopathy. *J Endocrinol*. 2008;197(1):1-10. doi:10.1677/JOE-07-0606

44. Minetto MA, Lanfranco F, Botter A, et al. Do muscle fiber conduction slowing and decreased levels of circulating muscle proteins represent sensitive markers of steroid myopathy? A pilot study in Cushing's disease. *Eur J Endocrinol*. 2011;164(6):985-993. doi:10.1530/EJE-10-1169

45. Valassi E, Santos A, Yaneva M, et al. The European Registry on Cushing's syndrome: 2-Year experience. Baseline demographic and clinical characteristics. *Eur J Endocrinol*. 2011;165(3):383-392. doi:10.1530/EJE-11-0272

46. Wajchenberg BL, Bosco A, Marone MM, et al. Estimation of body fat and lean tissue distribution by dual energy X-ray absorptiometry and abdominal body fat evaluation by computed tomography in Cushing's disease. *J Clin Endocrinol Metab*. 1995;80(9):2791-2794. doi:10.1210/jcem.80.9.7673425

47. Geer EB, Shen W, Gallagher D, et al. MRI assessment of lean and adipose tissue distribution in female patients with Cushing's disease. *Clin Endocrinol (Oxf)*. 2010;73(4):469-475. doi:10.1111/j.1365-2265.2010.03829.x

48. Berr CM, Stieg MR, Deutschbein T, et al. Persistence of myopathy in Cushing's syndrome: evaluation of the German Cushing's Registry. *Eur J Endocrinol*. 2017;176(6):737-746. doi:10.1530/EJE-16-0689

49. Geer EB, Shen W, Strohmayer E, Post KD, Freda PU. Body composition and cardiovascular risk markers after remission of Cushing's disease: a prospective study using whole-body MRI. *J Clin Endocrinol Metab*. 2012;97(5):1702-1711. doi:10.1210/jc.2011-3123

50. Dekkers OM, Horváth-Puḥo E, Jørgensen JOL, et al. Multisystem

morbidity and mortality in cushing's syndrome: A cohort study. *J Clin Endocrinol Metab*. 2013;98(6):2277-2284. doi:10.1210/jc.2012-3582

51. Santos A, Resmini E, Martínez Momblán MA, Valassi E, Martel L, Webb SM. Quality of Life in Patients With Cushing's Disease. *Front Endocrinol (Lausanne)*. 2019;10. doi:10.3389/fendo.2019.00862

52. Webb SM, Santos A, Resmini E, Martínez-Momblán MA, Martel L, Valassi E. Quality of Life in Cushing's disease: A long term issue? *Ann Endocrinol (Paris)*. 2018;79(3):132-137. doi:10.1016/j.ando.2018.03.007

53. Khaleeli AA, Levy RD, Edwards RHT, et al. The neuromuscular features of acromegaly: A clinical and pathological study. *J Neurol Neurosurg Psychiatry*. 1984;47(9):1009-1015. doi:10.1136/jnnp.47.9.1009

54. O'sullivan AJ, Kelly JJ, Hoffman DM, Freund J, Ho KKY. Body composition and energy expenditure in acromegaly. *J Clin Endocrinol Metab*. 1994;78(2):381-386. doi:10.1210/jcem.78.2.8106626

55. Freda PU, Shen W, Reyes-Vidal CM, et al. Skeletal muscle mass in acromegaly assessed by magnetic resonance imaging and dual-photon X-ray absorptiometry. *J Clin Endocrinol Metab*. 2009;94(8):2880-2886. doi:10.1210/jc.2009-0026

56. Reyes-Vidal C, Fernandez JC, Bruce JN, et al. Prospective study of surgical treatment of acromegaly: Effects on ghrelin, weight, adiposity, and markers of CV risk. *J Clin Endocrinol Metab*. 2014;99(11):4124-4132. doi:10.1210/jc.2014-2259

57. Miller A, Doll H, David J, Wass J. Impact of musculoskeletal disease on quality of life in long-standing acromegaly. *Eur J Endocrinol*. 2008;158(5):587-593. doi:10.1530/EJE-07-0838

58. Guedes da Silva DP, Guimarães FS, Dias CM, et al. On the functional capacity and quality of life of patients with acromegaly: are they candidates for rehabilitation programs? *J Phys Ther Sci*. 2013;25(11):1497-1501. doi:10.1589/jpts.25.1497

59. Homem TS, Guimarães FS, Soares MS, Kasuki L, Gadelha MR, Lopes AJ. Balance control and peripheral muscle function in aging: A comparison between individuals with acromegaly and healthy subjects. *J Aging Phys Act*. 2017;25(2):218-227. doi:10.1123/japa.2016-0100

60. Haliloglu O, Topsakal N, Camliguney F, et al. Static and dynamic

balances of patients with acromegaly and impact of exercise on balance. *Pituitary*. 2019;22(5):497-506. doi:10.1007/s11102-019-00979-3

61. Füchtbauer L, Olsson DS, Bengtsson BA, Norrman LL, Sunnerhagen KS, Johannsson G. Muscle strength in patients with acromegaly at diagnosis and during long-term follow-up. *Eur J Endocrinol*. 2017;177(2):217-226. doi:10.1530/EJE-17-0120

62. Claessen KMJA, Ramautar SR, Pereira AM, et al. Progression of acromegalic arthropathy despite long-term biochemical control: A prospective, radiological study. *Eur J Endocrinol*. 2012;167(2):235-244. doi:10.1530/EJE-12-0147

63. Gadelha MR, Kasuki L, Lim DST, Fleseriu M. Systemic complications of acromegaly and the impact of the current treatment landscape: An update. *Endocr Rev*. 2018;40(1):268-332. doi:10.1210/er.2018-00115

64. Cree MG, Paddon-Jones D, Newcomer BR, et al. Twenty-eight-day bed rest with hypercortisolemia induces peripheral insulin resistance and increases intramuscular triglycerides. *Metabolism*. 2010;59(5):703-710. doi:10.1016/j.metabol.2009.09.014

65. Leite RD, Prestes J, Bernardes CF, et al. Effects of ovariectomy and resistance training on lipid content in skeletal muscle, liver, and heart; fat depots; and lipid profile. *Appl Physiol Nutr Metab*. 2009;34(6):1079-1086. doi:10.1139/H09-116

66. Frechette DM, Krishnamoorthy D, Adler BJ, Chan ME, Rubin CT. Diminished satellite cells and elevated adipogenic gene expression in muscle as caused by ovariectomy are averted by low-magnitude mechanical signals. *J Appl Physiol*. 2015;119(1):27-36. doi:10.1152/japplphysiol.01020.2014

67. Chang D, Joseph DJ, Ebert MA, et al. Effect of androgen deprivation therapy on muscle attenuation in men with prostate cancer. *J Med Imaging Radiat Oncol*. 2014;58(2):223-228. doi:10.1111/1754-9485.12124

68. Matson AP, Kim C, Bajpai S, Green CL, Hash TW, Garrigues GE. The effect of obesity on fatty infiltration of the rotator cuff musculature in patients without rotator cuff tears. *Shoulder Elb*. 2019;11(1_suppl):30-38. doi:10.1177/1758573217736008

69. Reinders I, Murphy RA, Brouwer IA, et al. Muscle Quality and Myosteatosis: Novel Associations with Mortality Risk. *Am J Epidemiol*. 2016;183(1):53-60. doi:10.1093/aje/kwv153

70. Valencia AP, Lai JK, Iyer SR, et al. Fatty Infiltration Is a Prognostic Marker of Muscle Function After Rotator Cuff Tear. *Am J Sports Med*. 2018;46(9):2161-2169. doi:10.1177/0363546518769267

71. Hamrick MW. The skeletal muscle secretome: an emerging player in muscle-bone crosstalk. *Bonekey Rep*. 2012;1(4):60. doi:10.1038/bonekey.2012.60

72. Choi SH, Chung KY, Johnson BJ, et al. Co-culture of bovine muscle satellite cells with preadipocytes increases PPARγ and C/EBPβ gene expression in differentiated myoblasts and increases GPR43 gene expression in adipocytes. *J Nutr Biochem*. 2013;24(3):539-543. doi:10.1016/j.jnutbio.2012.01.015

73. Pellegrinelli V, Rouault C, Rodriguez-Cuenca S, et al. Human Adipocytes Induce Inflammation and Atrophy in Muscle Cells During Obesity. *Diabetes*. 2015;64(9):3121-3134. doi:10.2337/db14-0796

74. Takegahara Y, Yamanouchi K, Nakamura K, Nakano S, Nishihara M. Myotube formation is affected by adipogenic lineage cells in a cell-to-cell contact-independent manner. *Exp Cell Res*. 2014;324(1):105-114. doi:10.1016/j.yexcr.2014.03.021

75. Hamrick MW, McGee-Lawrence ME, Frechette DM. Fatty Infiltration of Skeletal Muscle: Mechanisms and Comparisons with Bone Marrow Adiposity. *Front Endocrinol (Lausanne)*. 2016;7:69. doi:10.3389/fendo.2016.00069

76. Miljkovic I, Kuipers AL, Cauley JA, et al. Greater Skeletal Muscle Fat Infiltration Is Associated With Higher All-Cause and Cardiovascular Mortality in Older Men. *Journals Gerontol Ser A Biol Sci Med Sci*. 2015;70(9):1133-1140. doi:10.1093/gerona/glv027

77. Goodpaster BH, Carlson CL, Visser M, et al. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *J Appl Physiol*. 2001;90(6):2157-2165. doi:10.1152/jappl.2001.90.6.2157

78. Visser M, Goodpaster BH, Kritchevsky SB, et al. Muscle Mass, Muscle Strength, and Muscle Fat Infiltration as Predictors of Incident Mobility Limitations in Well-Functioning Older Persons. *Journals Gerontol Ser A Biol Sci Med Sci*. 2005;60(3):324-333. doi:10.1093/gerona/60.3.324

79. Diaz-Manera J, Fernandez-Torron R, LLauger J, et al. Muscle MRI in patients with dysferlinopathy: pattern recognition and implications for clinical trials. *J Neurol Neurosurg Psychiatry*. 2018;89(10):1071-1081. doi:10.1136/jnnp-2017-317488

80. Figueroa-Bonaparte S, Llauger J, Segovia S, et al. Quantitative muscle MRI to follow up late onset Pompe patients: a prospective study. *Sci Rep*. 2018;8(1):10898. doi:10.1038/s41598-018-29170-7

81. Wokke BH, van den Bergen JC, Versluis MJ, et al. Quantitative MRI and strength measurements in the assessment of muscle quality in Duchenne muscular dystrophy. *Neuromuscul Disord*. 2014;24(5):409-416. doi:10.1016/j.nmd.2014.01.015

82. Vettor R, Milan G, Franzin C, et al. The origin of intermuscular adipose tissue and its pathophysiological implications. *Am J Physiol - Endocrinol Metab*. 2009;297(5). doi:10.1152/ajpendo.00229.2009

83. Zoico E, Rossi A, Di Francesco V, et al. Adipose tissue infiltration in skeletal muscle of healthy elderly men: Relationships with body composition, insulin resistance, and inflammation at the systemic and tissue level. *Journals Gerontol - Ser A Biol Sci Med Sci*. 2010;65 A(3):295-299. doi:10.1093/gerona/glp155

84. Roden M. Muscle triglycerides and mitochondrial function: Possible mechanisms for the development of type 2 diabetes. *Int J Obes*. 2005;29:S111-S115. doi:10.1038/sj.ijo.0803102

85. Kovalik JP, Slentz D, Stevens RD, et al. Metabolic remodeling of human skeletal myocytes by cocultured adipocytes depends on the lipolytic state of the system. *Diabetes*. 2011;60(7):1882-1893. doi:10.2337/db10-0427

86. Summers SA, Nelson DH. A role for sphingolipids in producing the common features of type 2 diabetes, metabolic syndrome X, and Cushing's syndrome. *Diabetes*. 2005;54(3):591-602. doi:10.2337/diabetes.54.3.591

87. Tardif N, Salles J, Guillet C, et al. Muscle ectopic fat deposition contributes to anabolic resistance in obese sarcopenic old rats through eIF2α activation. *Aging Cell*. 2014;13(6):1001-1011. doi:10.1111/acel.12263

88. Ebisui C, Tsujinaka T, Morimoto T, et al. Interleukin-6 induces proteolysis by activating intracellular proteases (cathepsins B and L, proteasome) in C2C12 myotubes. *Clin Sci*. 1995;89(4):431-439. doi:10.1042/cs0890431

89. Hegarty BD, Cooney GJ, Kraegen EW, Furler SM. Increased efficiency of fatty acid uptake contributes to lipid accumulation in skeletal muscle of high fat-fed insulin-resistant rats. *Diabetes*. 2002;51(5):1477-1484.

doi:10.2337/diabetes.51.5.1477

90. Mastrocola R, Collino M, Nigro D, et al. Accumulation of advanced glycation end-products and activation of the SCAP/SREBP Lipogenetic pathway occur in diet-induced obese mouse skeletal muscle. Guillou H, ed. *PLoS One*. 2015;10(3):e0119587. doi:10.1371/journal.pone.0119587

91. Lee D-E, Kehlenbrink S, Lee H, Hawkins M, Yudkin JS. Getting the message across: mechanisms of physiological cross talk by adipose tissue. *Am J Physiol Endocrinol Metab*. 2009;296(6):E1210-29. doi:10.1152/ajpendo.00015.2009

92. Brandt C, Pedersen BK. The role of exercise-induced myokines in muscle homeostasis and the defense against chronic diseases. *J Biomed Biotechnol*. 2010;2010:520258. doi:10.1155/2010/520258

93. Zoico E, Corzato F, Bambace C, et al. Myosteatosis and myofibrosis: relationship with aging, inflammation and insulin resistance. *Arch Gerontol Geriatr*. 2013;57(3):411-416. doi:10.1016/j.archger.2013.06.001

94. Rebuffé-Scrive M, Walsh UA, McEwen B, Rodin J. Effect of chronic stress and exogenous glucocorticoids on regional fat distribution and metabolism. *Physiol Behav*. 1992;52(3):583-590. doi:10.1016/0031-9384(92)90351-2

95. Wajchenberg BL, Bosco A, Marone MM, et al. Estimation of body fat and lean tissue distribution by dual energy X-ray absorptiometry and abdominal body fat evaluation by computed tomography in Cushing's disease. *J Clin Endocrinol Metab*. 1995;80(9):2791-2794. doi:10.1210/jcem.80.9.7673425

96. Geer EB, Shen W, Strohmayer E, Post KD, Freda PU. Body Composition and Cardiovascular Risk Markers after Remission of Cushing's Disease: A Prospective Study Using Whole-Body MRI. *J Clin Endocrinol Metab*. 2012;97(5):1702-1711. doi:10.1210/jc.2011-3123

97. Møller N, Jørgensen JOL, Alberti KG, Flyvbjerg A, Schmitz O. Short-term effects of growth hormone on fuel oxidation and regional substrate metabolism in normal man. *J Clin Endocrinol Metab*. 1990;70(4):1179-1186. doi:10.1210/jcem-70-4-1179

98. Møller N, Schmitz O, Pørksen N, Møller J, Jørgensen JOL. Dose-response studies on the metabolic effects of a growth hormone pulse in humans. *Metabolism*. 1992;41(2):172-175. doi:10.1016/0026-

0495(92)90147-3

99. Krag MB, Gormsen LC, Guo ZK, et al. Growth hormone-induced insulin resistance is associated with increased intramyocellular triglyceride content but unaltered VLDL-triglyceride kinetics. *Am J Physiol - Endocrinol Metab*. 2007;292(3):920-927. doi:10.1152/ajpendo.00374.2006

100. Jørgensen JOL, Møller J, Alberti, K. G. Schmitz O, Christiansen JS, Ørskov H, Moller N. Marked effects of sustained low growth hormone (GH) levels on day-to-day fuel metabolism: Studies in GH-deficient patients and healthy untreated subjects. *J Clin Endocrinol Metab*. 1993;77(6):1589-1596. doi:10.1210/jcem.77.6.8263146

101. Wolthers T, Hoffman DM, Nugent AG, Duncan MW, Umpleby M, Ho KKY. Oral estrogen antagonizes the metabolic actions of growth hormone in growth hormone-deficient women. *Am J Physiol - Endocrinol Metab*. 2001;281(6):1191-1196. doi:10.1152/ajpendo.2001.281.6.e1191

102. Gibney J, Wolthers T, Johannsson G, Umpleby AM, Ho KKY. Growth hormone and testosterone interact positively to enhance protein and energy metabolism in hypopituitary men. *Am J Physiol - Endocrinol Metab*. 2005;289(2):266-271. doi:10.1152/ajpendo.00483.2004

103. Flores-Morales A, Ståhlberg N, Tollet-Egnell P, et al. Microarray analysis of the in vivo effects of hypophysectomy and growth hormone treatment on gene expression in the rat. *Endocrinology*. 2001;142(7):3163-3176. doi:10.1210/endo.142.7.8235

104. Tollet-Egnell P, Flores-Morales A, Ståhlberg N, Malek RL, Lee N, Norstedt G. Gene expression profile of the aging process in rat liver: Normalizing effects of growth hormone replacement. *Mol Endocrinol*. 2001;15(2):308-318. doi:10.1210/mend.15.2.0594

105. Tollet-Egnell P, Parini P, Ståhlberg N, et al. Growth hormone-mediated alteration of fuel metabolism in the aged rat as determined from transcript profiles. *Physiol Genomics*. 2004;16(2):261-267. doi:10.1152/physiolgenomics.00093.2002

106. Sjögren K, Leung KC, Kaplan W, Gardiner-Garden M, Gibney J, Ho KKY. Growth hormone regulation of metabolic gene expression in muscle: A microarray study in hypopituitary men. *Am J Physiol - Endocrinol Metab*. 2007;293(1):364-371. doi:10.1152/ajpendo.00054.2007

107. Reyes-Vidal CM, Mojahed H, Shen W, et al. Adipose tissue redistribution and ectopic lipid deposition in active acromegaly and effects of surgical treatment. *J Clin Endocrinol Metab*. 2015;100(8):2946-2955. doi:10.1210/jc.2015-1917

108. Moøller N, Joørgensen JOL. Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. *Endocr Rev*. 2009;30(2):152-177. doi:10.1210/er.2008-0027

109. Freda PU, Shen W, Heymsfield SB, et al. Lower visceral and subcutaneous but higher intermuscular adipose tissue depots in patients with growth hormone and insulin-like growth factor I excess due to acromegaly. *J Clin Endocrinol Metab*. 2008;93(6):2334-2343. doi:10.1210/jc.2007-2780

110. Bredella MA, Schorr M, Dichtel LE, et al. Body composition and ectopic lipid changes with biochemical control of acromegaly. *J Clin Endocrinol Metab*. 2017;102(11):4218-4225. doi:10.1210/jc.2017-01210

111. Rabinowitz D, Klassen GA, Zierler KL. Effect of human growth hormone on muscle and adipose tissue metabolism. *J Clin Invest*. 1965;44(1):51-61. doi:10.1172/JCI105126

112. McGregor RA, Cameron-Smith D, Poppitt SD. It is not just muscle mass: A review of muscle quality, composition and metabolism during ageing as determinants of muscle function and mobility in later life. *Longev Heal*. 2014;3(1). doi:10.1186/2046-2395-3-9

113. Heymsfield SB, Gonzalez MC, Lu J, Jia G, Zheng J. Skeletal muscle mass and quality: Evolution of modern measurement concepts in the context of sarcopenia. In: *Proceedings of the Nutrition Society*. Vol 74. Cambridge University Press; 2015:355-366. doi:10.1017/S0029665115000129

114. Díaz-Manera J, Llauger J, Gallardo E, Illa I. Muscle MRI in muscular dystrophies. *Acta Myol myopathies cardiomyopathies Off J Mediterr Soc Myol*. 2015;34(2-3):95-108.

115. Elliott JM, Walton DM, Rademaker A, Parrish TB. Quantification of cervical spine muscle fat: a comparison between T1-weighted and multi-echo gradient echo imaging using a variable projection algorithm (VARPRO). *BMC Med Imaging*. 2013;13(1):30. doi:10.1186/1471-2342-13-30

116. Noble JJ, Keevil SF, Totman J, Charles-Edwards GD. In vitro and in

vivo comparison of two-, three- and four-point Dixon techniques for clinical intramuscular fat quantification at 3 T. *Br J Radiol*. 2014;87(1036):20130761. doi:10.1259/bjr.20130761

117. Smith AC, Parrish TB, Abbott R, et al. Muscle-fat MRI: 1.5 tesla and 3.0 tesla versus histology. *Muscle Nerve*. 2014;50(2):170-176. doi:10.1002/mus.24255

118. Young HJ, Jenkins NT, Zhao Q, Mccully KK. Measurement of intramuscular fat by muscle echo intensity. *Muscle and Nerve*. 2015;52(6):963-971. doi:10.1002/mus.24656

119. Carlier PG, Marty B, Scheidegger O, et al. Skeletal Muscle Quantitative Nuclear Magnetic Resonance Imaging and Spectroscopy as an Outcome Measure for Clinical Trials. *J Neuromuscul Dis*. 2016;3(1):1-28. doi:10.3233/JND-160145

120. Murphy AP, Morrow J, Dahlqvist JR, et al. Natural history of limb girdle muscular dystrophy R9 over 6 years: searching for trial endpoints. *Ann Clin Transl Neurol*. 2019;6(6):1033-1045. doi:10.1002/acn3.774

121. Burakiewicz J, Sinclair CDJ, Fischer D, Walter GA, Kan HE, Hollingsworth KG. Quantifying fat replacement of muscle by quantitative MRI in muscular dystrophy. *J Neurol*. 2017;264(10):2053-2067. doi:10.1007/s00415-017-8547-3

122. Ueland T, Kristo C, Godang K, Aukrust P, Bollerslev J. Interleukin-1 Receptor Antagonist Is Associated with Fat Distribution in Endogenous Cushing's Syndrome: A Longitudinal Study. *J Clin Endocrinol Metab*. 2003;88(4):1492-1496. doi:10.1210/jc.2002-021030

123. Ragnarsson O, Glad CAM, Bergthorsdottir R, et al. Body composition and bone mineral density in women with Cushing's syndrome in remission and the association with common genetic variants influencing glucocorticoid sensitivity. *Eur J Endocrinol*. 2015;172(1):1-10. doi:10.1530/EJE-14-0747

124. Barahona M-J, Sucunza N, Resmini E, et al. Persistent body fat mass and inflammatory marker increases after long-term cure of Cushing's syndrome. *J Clin Endocrinol Metab*. 2009;94(9):3365-3371. doi:10.1210/jc.2009-0766

125. Burt MG, Gibney J, Ho KKY. Protein metabolism in glucocorticoid excess: study in Cushing's syndrome and the effect of treatment. *Am J Physiol Metab*. 2007;292(5):E1426-E1432. doi:10.1152/ajpendo.00524.2006

126. Geer EB, Shen W, Gallagher D, et al. MRI assessment of lean and adipose tissue distribution in female patients with Cushing's disease. *Clin Endocrinol (Oxf)*. 2010;73(4):469-475. doi:10.1111/j.1365-2265.2010.03829.x

127. Dong Y, Silva KAS, Dong Y, Zhang L. Glucocorticoids increase adipocytes in muscle by affecting IL-4 regulated FAP activity. *FASEB J*. 2014;28(9):4123-4132. doi:10.1096/fj.14-254011

128. Gounarides JS, Korach-André M, Killary K, Argentieri G, Turner O, Laurent D. Effect of Dexamethasone on Glucose Tolerance and Fat Metabolism in a Diet-Induced Obesity Mouse Model. *Endocrinology*. 2008;149(2):758-766. doi:10.1210/en.2007-1214

129. Abildgaard J, Danielsen ER, Dorph E, et al. Ectopic Lipid Deposition Is Associated With Insulin Resistance in Postmenopausal Women. *J Clin Endocrinol Metab*. 2018;103(9):3394-3404. doi:10.1210/jc.2018-00554

130. Ward-Ritacco CL, Adrian AL, Johnson MA, Rogers LQ, Evans EM. Adiposity, physical activity, and muscle quality are independently related to physical function performance in middle-aged postmenopausal women. *Menopause*. 2014;21(10):1114-1121. doi:10.1097/GME.0000000000000225

131. Garrapa GGM, Pantanetti P, Arnaldi G, Mantero F, Faloia E. Body Composition and Metabolic Features in Women with Adrenal Incidentaloma or Cushing's Syndrome. *J Clin Endocrinol Metab*. 2001;86(11):5301-5306. doi:10.1210/jcem.86.11.8059

132. Chikani V, Ho KKY. Action of GH on skeletal muscle function: Molecular and metabolic mechanisms. *J Mol Endocrinol*. 2013;52(1). doi:10.1530/JME-13-0208

133. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing*. 2019;48(4):601. doi:10.1093/ageing/afz046

134. Bischoff HA, Stähelin HB, Monsch AU, et al. Identifying a cut-off point for normal mobility: A comparison of the timed "up and go" test in community-dwelling and institutionalised elderly women. *Age Ageing*. 2003;32(3):315-320. doi:10.1093/ageing/32.3.315

135. Christopher A, Kraft E, Olenick H, Kiesling R, Doty A. The reliability and validity of the Timed Up and Go as a clinical tool in individuals with and without disabilities across a lifespan: a systematic review: Psychometric properties of the Timed Up and Go. *Disabil Rehabil*.

Published online 2019:1-15. doi:10.1080/09638288.2019.1682066

136. Cuneo RC, Salomon F, Wiles CM, Sönksen PH. Skeletal muscle performance in adults with growth hormone deficiency. *Horm Res Paediatr*. 1990;33:55-60. doi:10.1159/000181585

137. Yarasheski KE, Campbell JA, Smith K, Rennie MJ, Holloszy JO, Bier DM. Effect of growth hormone and resistance exercise on muscle growth in young men. *Am J Physiol - Endocrinol Metab*. 1992;262(3 Pt 1):261-267. doi:10.1152/ajpendo.1992.262.3.e261

138. Johannsson G, Grimby G, Sunnerhagen KS, Bengtsson B-Å. Two Years of Growth Hormone (GH) Treatment Increase Isometric and Isokinetic Muscle Strength in GH-Deficient Adults 1. *J Clin Endocrinol Metab*. 1997;82(9):2877-2884. doi:10.1210/jcem.82.9.4204

139. Chikani V, Cuneo RC, Hickman I, Ho KKY. Growth hormone (GH) enhances anaerobic capacity: impact on physical function and quality of life in adults with GH deficiency. *Clin Endocrinol (Oxf)*. 2016;85(4):660-668. doi:10.1111/cen.13147

140. Delmonico MJ, Harris TB, Visser M, et al. Longitudinal study of muscle strength, quality, and adipose tissue infiltration. *Am J Clin Nutr*. 2009;90(6):1579-1585. doi:10.3945/ajcn.2009.28047

141. Goodpaster BH, Park SW, Harris TB, et al. The Loss of Skeletal Muscle Strength, Mass, and Quality in Older Adults: The Health, Aging and Body Composition Study. *Journals Gerontol Ser A Biol Sci Med Sci*. 2006;61(10):1059-1064. doi:10.1093/gerona/61.10.1059

142. Goodpaster BH, Chomentowski P, Ward BK, et al. Effects of physical activity on strength and skeletal muscle fat infiltration in older adults: a randomized controlled trial. *J Appl Physiol*. 2008;105(5):1498-1503. doi:10.1152/japplphysiol.90425.2008

143. Guralnik JM, Simonsick EM, Ferrucci L, et al. A Short Physical Performance Battery Assessing Lower Extremity Function: Association With Self-Reported Disability and Prediction of Mortality and Nursing Home Admission. *J Gerontol*. 1994;49(2):M85-M94. doi:10.1093/geronj/49.2.M85

144. Bernabeu Mora R, Medina-Mirapeix F, Llamazares Herrán E, García Guillamón G, Giménez Giménez LM, Sánchez Nieto JM. The Short Physical Performance Battery is a discriminative tool for identifying patients with COPD at risk of disability. *Int J Chron Obstruct Pulmon Dis*. Published online December 2015:2619. doi:10.2147/COPD.S94377

145. Motl RW, Learmonth YC, Wójcicki TR, et al. Preliminary validation of the short physical performance battery in older adults with multiple sclerosis: secondary data analysis. *BMC Geriatr*. 2015;15(1):157. doi:10.1186/s12877-015-0156-3

146. van den Berg M, Sherrington C, Killington M, et al. Video and computer-based interactive exercises are safe and improve task-specific balance in geriatric and neurological rehabilitation: a randomised trial. *J Physiother*. 2016;62(1):20-28. doi:10.1016/J.JPHYS.2015.11.005

147. Drey M, Berr CM, Reincke M, et al. Cushing's syndrome: a model for sarcopenic obesity. *Endocrine*. 2017;57(3):481-485. doi:10.1007/s12020-017-1370-x

148. Figueroa-Bonaparte S, Segovia S, Llauger J, et al. Muscle MRI Findings in Childhood/Adult Onset Pompe Disease Correlate with Muscle Function. *PLoS One*. 2016;11(10):e0163493. doi:10.1371/journal.pone.0163493

149. Sarkozy A, Deschauer M, Carlier R-Y, et al. Muscle MRI findings in limb girdle muscular dystrophy type 2L. *Neuromuscul Disord*. 2012;22:S122-S129. doi:10.1016/j.nmd.2012.05.012

150. Wary C, Azzabou N, Giraudeau C, et al. Quantitative NMRI and NMRS identify augmented disease progression after loss of ambulation in forearms of boys with Duchenne muscular dystrophy. *NMR Biomed*. 2015;28(9):1150-1162. doi:10.1002/nbm.3352

151. Morrow JM, Sinclair CDJ, Fischmann A, et al. MRI biomarker assessment of neuromuscular disease progression: a prospective observational cohort study. *Lancet Neurol*. 2016;15(1):65-77. doi:10.1016/S1474-4422(15)00242-2

152. Bugiardini E, Morrow JM, Shah S, et al. The Diagnostic Value of MRI Pattern Recognition in Distal Myopathies. *Front Neurol*. 2018;9:456. doi:10.3389/fneur.2018.00456

153. Smith AC, Parrish TB, Abbott R, et al. Muscle-fat MRI: 1.5 tesla and 3.0 tesla versus histology. *Muscle Nerve*. 2014;50(2):170-176. doi:10.1002/mus.24255

154. Murphy AP, Morrow J, Dahlqvist JR, et al. Natural history of limb girdle muscular dystrophy R9 over 6 years: searching for trial endpoints. *Ann Clin Transl Neurol*. 2019;6(6):1033-1045. doi:10.1002/acn3.774

155. Fischer MA, Pfirrmann CWA, Espinosa N, Raptis DA, Buck FM.

Dixon-based MRI for assessment of muscle-fat content in phantoms, healthy volunteers and patients with achillodynia: comparison to visual assessment of calf muscle quality. *Eur Radiol*. 2014;24(6):1366-1375. doi:10.1007/s00330-014-3121-1

156. Carter CS, Justice JN, Thompson L. Lipotoxicity, aging, and muscle contractility: does fiber type matter? *GeroScience*. 2019;41(3):297-308. doi:10.1007/s11357-019-00077-z

157. Buck M, Chojkier M. Muscle wasting and dedifferentiation induced by oxidative stress in a murine model of cachexia is prevented by inhibitors of nitric oxide synthesis and antioxidants. *EMBO J*. 1996;15(8):1753-1765.

158. Baumann CW, Kwak D, Liu HM, Thompson L V. Age-induced oxidative stress: how does it influence skeletal muscle quantity and quality? *J Appl Physiol*. 2016;121(5):1047-1052. doi:10.1152/japplphysiol.00321.2016

159. Lamounier-Zepter V, Ehrhart-Bornstein M, Bornstein SR. Metabolic syndrome and the endocrine stress system. *Horm Metab Res*. 2006;38(7):437-441. doi:10.1055/s-2006-947837

160. Szendroedi J, Zwettler E, Schmid AI, et al. Reduced basal ATP synthetic flux of skeletal muscle in patients with previous acromegaly. *PLoS One*. 2008;3(12):3958. doi:10.1371/journal.pone.0003958

161. Doehner W, Turhan G, Leyva F, et al. Skeletal muscle weakness is related to insulin resistance in patients with chronic heart failure. *ESC Hear Fail*. 2015;2(2):85-89. doi:10.1002/ehf2.12035

162. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. *Lancet*. 2010;375(9733):2267-2277. doi:10.1016/S0140-6736(10)60408-4

163. Yoshida Y, Marcus RL, Lastayo PC. Intramuscular adipose tissue and central activation in older adults. *Muscle Nerve*. 2012;46(5):813-816. doi:10.1002/mus.23506

164. Shaw CS, Clark J, Wagenmakers AJM. The effect of exercise and nutrition on intramuscular fat metabolism and insulin sensitivity. *Annu Rev Nutr*. 2010;30:13-34. doi:10.1146/annurev.nutr.012809.104817

165. Prior SJ, Joseph LJ, Brandauer J, Katzel LI, Hagberg JM, Ryan AS. Reduction in midthigh low-density muscle with aerobic exercise training and weight loss impacts glucose tolerance in older men. *J Clin*

Endocrinol Metab. 2007;92(3):880-886. doi:10.1210/jc.2006-2113

166. Goodpaster BH, Chomentowski P, Ward BK, et al. Effects of physical activity on strength and skeletal muscle fat infiltration in older adults: A randomized controlled trial. *J Appl Physiol*. 2008;105(5):1498-1503. doi:10.1152/japplphysiol.90425.2008

167. Madsen M, Krusenstjerna-Hafstrmø T, Mløler L, et al. Fat content in liver and skeletal muscle changes in a reciprocal manner in patients with acromegaly during combination therapy with a somatostatin analog and a GH receptor antagonist: A randomized clinical trial. *J Clin Endocrinol Metab*. 2012;97(4):1227-1235. doi:10.1210/jc.2011-2681

168. Neggers SJ, Kopchick JJ, Jørgensen JOL, Van Der Lely AJ. Hypothesis: Extra-hepatic acromegaly: A new paradigm? *Eur J Endocrinol*. 2011;164(1):11-16. doi:10.1530/EJE-10-0969

10. Annex

10.1. Publication 1

Martel-Duguech L, Alonso-Jimenez A, Bascuñana H, Díaz-Manera J, Llauger J, Nuñez-Peralta C, Montesinos P, Webb SM, Valassi E. Prevalence of sarcopenia after remission of endogenous hypercortisolism and its impact on Health-Related Quality of Life. Accepted for publication in Clinical Endocrinology July 2021.

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