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Myopathy in endogenous Cushing's Syndrome: Type II muscle fiber atrophy and its association with age and circulating IGF-1

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Abstract

Context:

Patients with endogenous Cushing syndrome (CS) exhibit reduced muscle strength compared with healthy individuals and frequently experience a further postoperative decline despite remission.

Objective:

To evaluate the histological and immunohistochemical patterns underlying myopathy in patients with active and remitted CS.

Patients and methods:

We included nine patients with active CS and eight patients with CS in remission, prospectively enrolled at XXX Hospital. Patients in remission had curative tumor surgery and biochemical remission for ≥24 months. Biopsies of the vastus lateralis muscle were obtained surgically and analyzed by histology, immunohistochemistry, and electron microscopy. IGF-1 concentrations were determined by IDS-iSYS analyzer.

Results:

In active CS, the mean cross-sectional area of type II muscle fibers was reduced. Nicotinamide-adenine-dinucleotide staining revealed mild moth-eaten fiber patterns in four patients, accompanied by ultrastructural pathologies on electron microscopy, including increased subsarcolemmal mitochondrial aggregates that were also present in remitted CS. In active CS circulating absolute IGF-1 concentrations positively (r_s=0.8, p=0.014) and age inversely (r_s= -0.77, p=0.021) correlated with mean cross-sectional area of type II muscle fibers. In remission from CS, type II fiber atrophy was not present in most patients. No substantial associations were found between the severity of glucocorticoid-excess and histological alterations during active or remitted disease.

Conclusion:

Active CS is characterized by type II fiber atrophy and is associated with circulating IGF-1 and age. In remission type II fiber atrophy was not present, whereas mitochondrial ultrastructural alterations could

be observed. These findings indicate sustained muscle alterations and highlight the need for mechanistic studies to guide targeted therapies.

Introduction

Endogenous Cushing syndrome (CS) is a rare endocrine disorder, compared to exogenous hypercortisolism caused by systemic glucocorticoid therapy. It is most commonly caused by a adrenocorticotropic hormone (ACTH)-secreting pituitary adenoma, followed by ACTH-independent adrenal causes, or rarely by ectopic sources of ACTH-excess [1]. The resulting cortisol excess leads to profound metabolic dysfunction and is associated with increased morbidity and mortality, which remains elevated even after successful surgical treatment [2,3]. These adverse outcomes are largely driven by persistent comorbidities induced by chronic hypercortisolism and the subsequent glucocorticoid (GC) withdrawal [1,2,4-7].

Myopathy is amongst the most common and most burdensome comorbidities in patients with CS. It can be observed in approximately 40% of patients with pituitary as well as adrenal CS and in up to 60% of patients with ectopic CS [8]. Patients with CS exhibit reduced handgrip strength, as well as impaired performance in the chair rising test, during the active disease stage [9]. CS-associated myopathy mainly affects the proximal muscles of the arms and especially the lower limbs. This weakness limits mobility and participation in daily activities, thereby further decreasing quality of life [10,11]. In a longitudinal study, we assessed improvement of muscle function through long-term follow-up of up to four years after surgical remission. Although hand grip strength was already impaired at diagnosis, compared to controls, it further declined after surgery and did not recover fully during follow-up of four years despite biochemical remission. This poor muscular outcome was associated with increased age, waist-to-hip ratio, and HbA1c, and persisted for up to four years or more and was associated with a worse quality of life [12]. More recently, we demonstrated that postoperative IGF-1 concentrations predicted long-term muscle outcomes, with lower levels associated with persistent myopathy [13].

However, studies of muscle histology including contemporary immunohistochemistry and electron microscopy in active CS patients and those in long-term remission are lacking. The aim of this study was to characterize myopathological changes during both active disease and CS in remission, and to

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evaluate potential associations between clinical and laboratory parameters, including the severity of glucocorticoid excess, and muscle histology.

Methods

Study setting and patient selection

This cross-sectional study was part of XXXXXXXXXX study. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee (study number: 18-0430). All participants provided written informed consent before participation. CS was diagnosed and subtyped according to current guideline recommendations [1,14]. None of the participants had a diagnosed neuromuscular disease. Remission from CS following successful tumor resection was defined as either the presence of prolonged adrenal insufficiency (confirmed by an ACTH-stimulation test) or normal test results of the 1 mg dexamethasone suppression test (LDDST) (<1.8 µg/dl), late-night salivary cortisol (LNSC) (<1.5 ng/ml) and urinary free cortisol (UFC) (≤ 83.0 µg/24h). Patients with adrenal insufficiency received physiological GC-replacement therapy with 20-25 mg of hydrocortisone divided into 2-3 doses per day.

Laboratory analyses

All blood samples were collected in the morning after an overnight fast. Cortisol, ACTH and insulin (Diasorin, Saluggia, Italy) and IGF-1 (IDS iSYS, Immunodiagnostic Systems, Boldon, UK) were measured using chemiluminescence immunoassay (CLIA) in the Endocrine Laboratory of the XXX hospital. All other laboratory analyses (creatine kinase, CK-MB-activity, myoglobin, fasting plasma glucose, HbA1c) were performed in the central laboratory of the XXX Hospital using standard methods.

Muscle biopsy and histological and immunohistochemical analyses

To obtain muscle tissue, an open muscle biopsy of the distal superficial part of the vastus lateralis muscle of the dominant leg was performed with local anaesthesia. As muscle fiber type distribution varies from proximal to distal regions and from superficial to deep layers, biopsies were taken from the

same anatomical area of each individual to improve comparability [15-17]. Immediately after collection, the samples were divided, embedded in tragacanth, and snap-frozen in liquid nitrogen before being sectioned at 10 µm, using a Leica CM1950 cryostat at the XXX Institute. Afterwards the samples were stained according to the protocol, in hematoxylin eosin (HE), Congo red, myoadenylate deaminase (MAD), nicotinamide adenine dinucleotide reduced (NADH), Oil Red O, periodic acid Schiff stain (PAS), phosphofructokinase (PFK), phosphorylase, succinate dehydrogenase/cytochrome c oxidase SDH/COX, acid phosphatase, Van Gieson`s stain and Masson`s trichrome stain. Additional immunohistochemistry was performed using monoclonal antibodies against CD4, CD8, CD68, MHC-I, MHC-II, C5B9, KLG, ISG15, P62 and neonatal myosin. Electron microscopy was performed using a Jeol JEM1400Flash. Analyses of cross-sectional area (CSA), fiber length, and fiber type composition were performed using QuPath version 0.6.0, with a minimum of 200 muscle fibers evaluated per sample; all muscle biopsy samples were anonymized and labelled only with numerical identifiers to ensure data protection and to blind the analyst to participant characteristics and group allocation.

Statistical analyses

Statistical analysis was performed using GraphPad Prism Version 10.4. Due to non-normally distributed data, continuous clinical and laboratory variables were expressed as median with interquartile ranges [IQR]. Fiber length, cross-sectional area and the fiber type distribution were expressed as mean ± SD due to normally distributed values. Spearman correlation (r_s) was used to assess associations between quantitative outcomes. Differences between patients with active CS and remitted CS were analyzed using the Mann-Whitney-U-Test. P values <0.05 were considered statistically significant. Due to the exploratory nature of the study and the small sample size no adjustments were made for multiple testing or potential confounding. For comparison, we used the female subgroup from the study by van de Casteele et al., which provides contemporary vastus lateralis muscle data from 40 healthy young women and men [18].

Results

Patient characteristics

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Nine patients with active CS and eight patients with CS in remission were included. **Table 1** summarizes clinical and biochemical characteristics of patients with active CS and patients in remission. All nine patients with active CS were female, with a median BMI of 25.7 kg/m² (23.4; 29.7). Four had Cushing's disease, four had adrenal CS, and one had ectopic CS. In the remission group, seven of eight participants were female and had a median BMI of 25.2 kg/m² (24.1; 30.2). In the remission group, six patients had Cushing's disease (five treated with transsphenoidal pituitary surgery, and one with bilateral adrenalectomy), and two had adrenal CS (both treated with unilateral adrenalectomy). Following surgery, CS patients experienced adrenal insufficiency. All patients received hydrocortisone replacement therapy with a maintenance dose of 20-25 mg/d; the dosage was subsequently adjusted according to clinical assessment and biochemical monitoring of adrenal insufficiency. The minimum remission period was 24 months, and the median duration of remission was four years (2; 6), with the longest remission lasting 11 years.

One patient with active CS and one with CS in remission had mildly elevated creatine kinase levels, whereas all other patients had values within the reference range. Serum myoglobin concentrations were not elevated in any participant. In patients with active CS, myoglobin concentrations were low and, in some cases, even below the lower reference limit.

Type II fiber atrophy during active glucocorticoid excess and its association with age and circulating IGF-1

Patients with active CS showed no relevant fast-to-slow switch in the fiber type composition (**Figure 1**), but a reduced mean cross-sectional area of type II fibers with no relevant type I fiber atrophy and occasional mild fiber caliber variability (**Figure 2).** In active CS, a strong positive correlation between serum IGF-1 concentrations and the cross-sectional area of type II fibers was found (r_s=0.80, p=0.014, **Figure 3**), whereas no such association was observed for type I fibers. After adjustment for age using IGF-1 SDS [19], there was no significant correlation with the cross-sectional area of type II fibers. On the other hand we also observed a strong negative correlation between age and type II fiber cross-sectional area (r_s= -0.77, p=0.021, **Figure 4**).

Patients with CS in remission showed normal type II cross-sectional area

A fast-to-slow fiber type switch could not be observed in remitted CS, with normal mean fiber type distribution (**Figure 1**). Reduced Type II fiber cross-sectional area was only present in few cases (**Figure 2; Table 1**). Compared to the active CS group, patients in remission, including one male participant, showed a clinically meaningful but not statistical significant trend (p=0.0745), towards higher mean cross-sectional area and mean fiber length of both type II (CSA: active CS 2887 ± 686 vs. remitted CS 3719 ± 1075 µm²; p=0.0745) and, in particular type I fibers (CSA: active CS 4065 ± 1197 vs. remitted CS 5676 ± 2078 µm² p=0.1388) (**Table 1**).

Histological, immunohistochemical and electron microscopy findings in patients with active CS

In all patients with active CS, fascicular architecture was preserved, and no fatty tissue infiltration was observed. Myonuclei were predominantly located subsarcolemmally, and no vacuoles were detected. There were no signs of myophagocytosis or inflammatory infiltrates (**Table 1**). In one patient disseminated angular atrophic fibers were noted.

Trichrome staining did not reveal ragged red fibers, rimmed vacuoles, or other myofibrillar structural pathologies in any patient. PAS, phosphorylase, phosphofructokinase, and MAD staining were unremarkable, and acid phosphatase activity was not increased (data not shown). Immunohistochemistry for slow and fast myosin showed a checkerboard-like fiber distribution, with type II fiber atrophy present in six of nine patients (**Figure 2 & 5A**).

NADH revealed mild moth-eaten fiber patterns in four patients (**Figure 5B**). However, no cores, defined as regions within muscle fibers with reduced or absent mitochondrial and oxidative enzyme activity, and no target fibers were observed. In one patient, a few trabecular fibers were detected. Congo red staining showed no evidence of amyloid deposition. Oil Red O staining demonstrated slight lipid inclusions in one patient (data not shown).

Electron microscopy revealed increased subsarcolemmal mitochondrial aggregates in one patient, while another patient also demonstrated subsarcolemmal mitochondrial aggregates with minor fat accumulation and lipofuscin deposits. However, there was no evidence of structural disruption of the

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myofibrillar architecture, no Z-line streaming and no fiber necrosis. Tubular aggregates or hyaline bodies were not detected (**Figure 6A-C**).

Histological, immunohistochemical and electron microscopy findings in patients with CS in remission

In one patient, a few trabecular fibers and occasional angular atrophic fibers with mildly increased fiber caliber variability were noted. Two patients showed COX-negative/SDH-positive fibers. Four patients revealed a moth-eaten pattern in NADH staining. In electron microscopy, one patient showed isolated tertiary lysosomes and minimal fat (**Table 1**).

Another patient revealed autophagic vacuoles and mitophagy in electron microscopy. Four patients demonstrated fat and glycogen accumulation, one lipofuscin deposits. Two patients showed increased subsarcolemmal mitochondrial aggregates, and three patients mitophagy (**Figure 6 D-E**).

Immunohistochemistry showed no evidence of immune cell infiltration in the muscle tissue (**Table 1**).

No correlation between the severity of initial GC excess or duration of remission and fiber cross-sectional area

Neither the active CS group, nor the remission group displayed significant correlations between the severity of the initial GC excess, as measured by UFC, LNSC, and LDDST during the active disease and the severity of type II or type I fiber atrophy. A longer remission duration was not substantially associated with higher cross-sectional area for type I or type II fibers (data not shown).

Discussion

We present a comprehensive contemporary series of open muscle biopsies from the vastus lateralis muscle, reporting structural and histopathological alterations in CS-associated myopathy both during active disease and in patients in long-term remission for at least two years. Our data show that most patients with active CS exhibit a pattern of type II fiber atrophy with occasional increased fiber caliber variability. However, no increase in connective tissue or fatty infiltration was observed. In active CS higher absolute IGF-1 levels correlated positively with greater type II fiber cross-sectional area, whereas age showed an inverse correlation with type II fiber cross sectional area. Most patients with CS in long-

term remission had no longer evidence of type II fiber atrophy. Nevertheless, ultrastructural alterations were evident.

In patients with endogenous CS or chronic exogenous GC exposure, fast-twitch type II muscle fibers are known to undergo atrophy, resulting in a reduction of cross-sectional area [20]. Investigations on GC-induced myopathy have predominantly been conducted in mouse or rat models using synthetic GC administration. Chronic GC excess induces muscle atrophy primarily through inhibition of protein synthesis, activation of proteolytic pathways, mitochondrial dysfunction, and reduced sarcolemmal excitability [21]. The anti-anabolic effects results from impaired amino acid transport, altered transcriptional regulation, suppression of IGF-1 and androgen receptor signaling, inhibition of myogenesis, and upregulation of myostatin [22]. Concurrently, GCs enhance proteolysis via activation of the ubiquitin-proteasome and lysosomal systems, notably through FOXO-mediated induction of atrogenes such as atrogin-1 and MuRF1 [22,23].

The low serum myoglobin concentrations observed in the active CS group may reflect the anti-anabolic effects of long-term glucocorticoid excess on skeletal muscle, consistent with previous observations by Minetto et al [24].

The vastus lateralis muscle, we used for this study, is frequently used for biopsies because of its accessibility and trainability [25]. It is particularly suitable for studying cortisol-induced myopathy, as this condition predominantly affects the proximal muscles of the lower limbs [10]. In active CS, a significant negative correlation was observed between age and type II fiber cross-sectional area. This partly reflects the known age-related decline in type II fiber cross-sectional area, especially in patients over 70 years, whereas type I fibers are less affected [26,27]. This fits well with our previous observation, that age is a strong predictor for severe CS-associated myopathy and a poorer long-term muscle strength outcome [12]. The inverse correlation of age and type II fiber cross-sectional area supports the higher susceptibility of older patients to cortisol-induced myopathy by demonstrating that histological type II fiber atrophy correlates with age. Muscle biopsies in active and remitted CS were assessed in two independent cross-sectional cohorts. Nevertheless, the cohort comparison provided a biologically informative

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finding: the remission group demonstrated a clinically meaningful trend toward a larger type II fiber cross-sectional area, despite the absence of statistical significance. This observation is particularly relevant, given that the median age in our active CS group was 31 years (27; 59) compared to 45 years (35; 51) in the CS in remission group, indicating that the observed changes especially the type II fiber atrophy cannot be attributed solely to age-related sarcopenia.

Physical exercise can increase type II CSA, so training after successful surgery might help increase type II fiber CSA [28,29].

We found no correlation between the severity of hypercortisolism and the observed histological muscle pattern. This parallels our previous observation that there was no association between the severity of cortisol excess and muscle strength, as assessed by handgrip strength [9]. UFC, LNSC, and LDDST provide only a snapshot of the current hormonal status. In addition, it often remains unclear how long the hypercortisolism had been present prior to diagnosis. The lack of correlation between the severity of GC excess may also be explained by the high individual sensitivity to cortisol [30,31].

Notably, despite higher circulating IGF-1 concentrations, patients with active CS were characterized by a smaller type II fiber cross sectional area compared with patients in remission. This finding suggests that hypercortisolism strongly suppresses type II fiber size despite age-related anabolic advantages, while relatively higher IGF-1 levels may confer partial histological protection within the disease state.

IGF-1 in the muscle can act as an anabolic factor, stimulating protein synthesis and myogenesis while reducing proteolysis, but glucocorticoids can inhibit the local production of IGF-1 [22]. We observed a correlation between serum IGF-1 levels and cross-sectional area of type II fibers in patients with active CS, which was no longer significant after correction for age [19]. However IGF-1 serum concentrations vary within the population depending on age, sex and further clinical characteristics and need to be interpreted carefully [32]. It is known that the administration of growth hormone (GH) can have a beneficial impact on the catabolic side effects induced by exogenous GC administration in humans [33,34]. In rats and hamsters, systemic administration of IGF-1 has been shown to exert a preventive effect against corticosteroid-induced muscle atrophy [35,36].

Our research group previously demonstrated that higher IGF-1 concentrations measured six months after surgery were associated with better long-term outcomes in handgrip strength [13]. Postoperative

circulating IGF-1 may therefore better represent the true anabolic recovery capacity. Muscle fibers with a larger type II fiber cross-sectional area are generally associated with greater muscle strength. This relationship is complex and influenced by additional factors [37]. Higher type II fiber cross-sectional area in active CS might be protective for a further decrease in muscle strength after surgery.

Moth-eaten lesions on NADH staining were observed in both active and remitted CS. Given their non-specific nature and their occurrence in various myopathies as well as in minimally abnormal muscle, these findings should be interpreted cautiously as generic indicators of focal oxidative or structural disturbance rather than as Cushing myopathy-specific lesions [38,39]. Ultrastructural analysis revealed subsarcolemmal mitochondrial aggregates both in active and remitted CS and evidence of mitophagy in remitted CS, indicating mild mitochondrial dysfunction. Mitochondrial function, however, is crucial for muscle strength and integrity [40]. Interestingly, mitophagy was frequently observed in patients with CS in remission. This may reflect persistent postoperative low grade inflammation following curative surgery, which has also been associated with poorer muscle strength outcomes [41]. Reduced mitochondrial membrane potential and accumulation of reactive oxygen species may activate multiple signaling pathways and non coding RNAs, thereby promoting an imbalance in mitochondrial dynamics and impaired mitophagy in muscle cells [42]. These findings suggest that mitochondrial function remains impaired despite long-term remission in some CS patients. Diabetes is also known to influence muscle function through impaired mitochondrial function and reduced cellular glucose uptake [43]. However, the ultrastructural changes we observed in the CS remission group in patients without a diabetes diagnosis indicate that a diabetic metabolic state cannot account for these alterations. IGF-1 regulates protein degradation through the PI3K/Akt/mTOR signaling pathway and also influences the balance between mitochondrial fission and fusion [40], which might be one of the reasons for the better muscle function outcome for patients with higher IGF-1 levels following curative surgery [13,40]. However, these structural findings need to be further evaluated by sequencing or transcriptomic analyses, as myopathy in CS is common and affects patients' quality of life in the long term [12].

Strengths and limiations:

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Strengths of this study include the comprehensive and standardized histological, immunohistochemical, and ultrastructural assessment of open muscle biopsies obtained from patients with active disease and long term remission. Nevertheless, the limited number of available muscle biopsies represents a limitation, which is largely attributable to the rarity of the condition. However the use of open muscle biopsies enabled a more comprehensive and reliable assessment of muscle structure. The cohort was heterogeneous and notably, the active CS group did not include male patients. Furthermore, different age groups were represented within the study population, which provides a broader perspective but also increases variability. Due to the cross-sectional design, direct comparisons between active CS and CS in remission are limited. Due to ethical and practical considerations, open muscle biopsies from healthy age- and sex-matched controls were not available for comparison with either study group. Future studies incorporating such reference data would further strengthen comparative analyses. Another limitation is the potential for selection bias, as this was a single-center study.

Conclusion

Type II fiber cross-sectional area was reduced in patients with active CS, accompanied by ultrastructural mitochondrial impairments. In active CS, IGF-1 levels were positively correlated, and age was inversely correlated, with type II fiber cross sectional area. In contrast, no correlation was observed between the severity of initial glucocorticoid excess and the histological pattern, either in active or remitted CS. In patients in remission for at least two years, type II fiber cross-sectional area normalized in most patients, but ultrastructural mitochondrial abnormalities were frequently observed. Further studies, including RNA sequencing and spatial transcriptomics of muscle biopsies from patients with CS, are needed to better elucidate the molecular pathophysiology and to guide the development of effective treatment strategies.

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Abbreviations: ACTH= Adrenocorticotropic hormone; BMI= body mass index; CK= Creatine kinase; CS= Cushing syndrome; CSA= cross-sectional area; HbA1c=Hemoglobin A1c; LDDST=low-dose dexamethasone suppression test; IGF-1= Insulin-like growth factor 1; LNSC=late night salivary cortisol; NADH = Nicotinamide adenine dinucleotide; UFC=24 hour urinary free cortisol

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Figures

Figure 1: Fiber type composition in patients with active Cushing syndrome and in remission

The panel shows the fiber type composition of the vastus lateralis muscle of the dominant leg in patients with active CS (Cushing syndrome) and patients with CS in remission. Each dot represents an individual, error bars represent the mean and SD. The square indicates the male patient, whereas circles indicate female patients. The dotted horizontal lines indicates the reference mean fiber type composition for type I muscle fibers (mean ± SD: 54.7 % ± 9.1%) in healthy young females as reported by Van de Casteel et al. [18].

Figure 2: Mean fiber cross-sectional area of vastus lateralis muscle in active Cushing syndrome and in remission

Panel 2 shows the mean fiber cross-sectional area of vastus lateralis muscle of the dominant leg in active CS (Cushing syndrome) and CS in remission for type I and type II fibers. Each dot represents an individual, error bars represent the mean and SD. The square indicates the male patient, whereas circles indicate female patients**.** The dotted horizontal lines indicate the reference mean cross-sectional area (CSA) for type I muscle fibers (mean ± SD: 4915 ± 1084 µm²) and the mean cross-sectional area for type II muscle fibers (mean ± SD: 4533 ± 1255 µm²) in healthy young women as reported by Van de Casteel et al. [18].

Figure 3: Correlation of IGF-1 with cross-sectional area of type II fibers of vastus lateralis muscle in active Cushing syndrome

The panel displays the relationship between IGF-1 serum concentrations (ng/ml) and cross-sectional area (µm²) during active CS (Cushingsyndrome). The line indicates the estimated linear regression line and the 95% fit bands. n=9, Spearman´s coefficient 0.80, R^2=0.529, p=0.014. Each dot represents one individual.

Figure 4: Correlation of age with cross-sectional area of type II fibers of vastus lateralis muscle in active Cushing syndrome

The panel displays the relationship between age (years) and cross-sectional area (µm²) during active Cushing syndrome (CS). Line indicates the estimated linear regression line and the 95% fit bands. n=9, Spearman´s coefficient - 0.77, R^2=0.515, p=0.021. Each dot represents one individual.

Figure 5: Type II muscle fiber atrophy and moth-eaten lesions in active Cushing syndrome

Type II fibers (pink color in myosin fast staining, see arrow) exhibit a markedly smaller cross-sectional area, consistent with fiber atrophy. Type I fibers (unstained, grey) display no reduced cross-sectional area (A) (x 20). The microscopic image displays moth-eaten lesions (arrow) on NADH staining of one biopsy (B) (x 20).

Figure 6: Electron microscopic analysis of Cushing syndrome muscle biopsy specimens

A-C: Active Cushing syndrome: All biopsies were taken from the vastus lateralis muscle. Overview shows a moderate increase in mitochondria and lipid content within a fully preserved skeletal muscle (A). Ballooned swollen mitochondria with still preserved cristae structure (B). Subsarcolemmal mitochondria aggregate with tertiary lysosomes and lipid droplets (C).

D-E: Cushing syndrome in remission: Panels (D) (X 3300) and (E) (higher magnification of panel D, X 8600) demonstrate a myelin-like structure adjacent to myonuclei, consistent with an end-stage of mitophagy.

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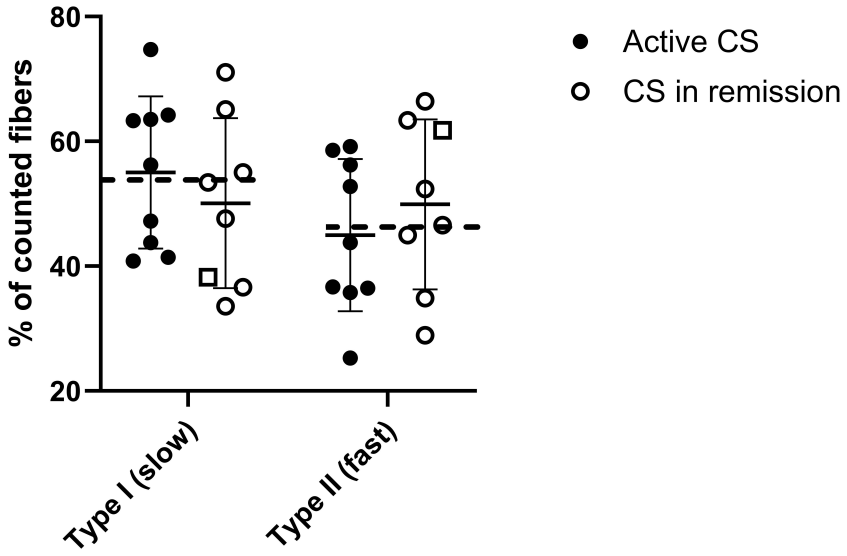

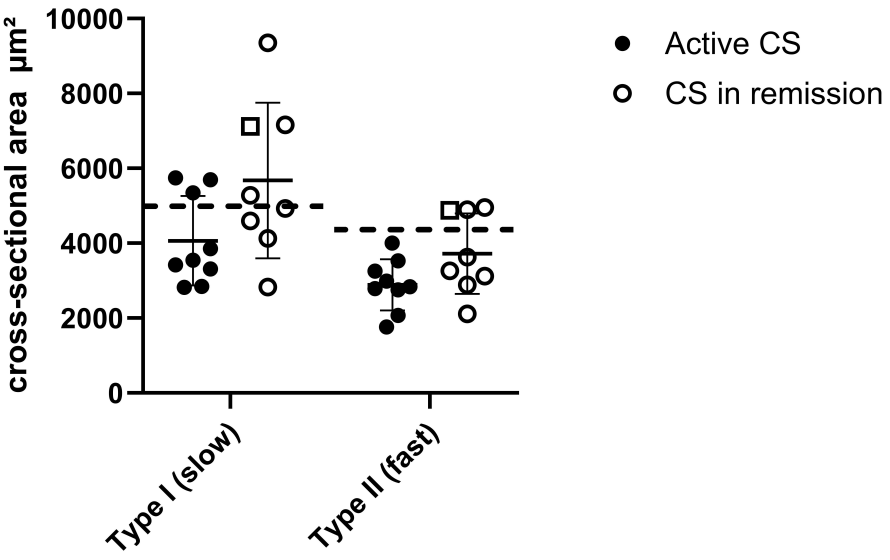

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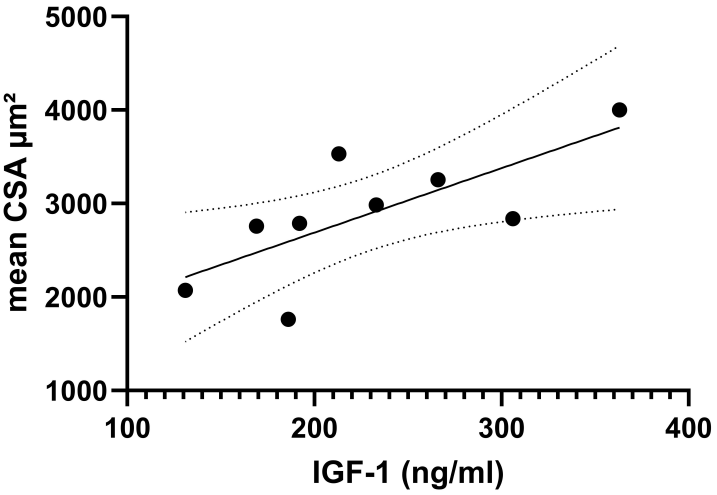

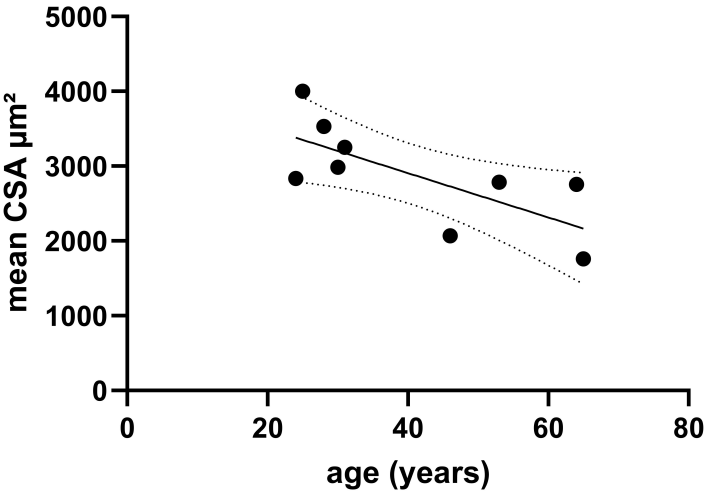

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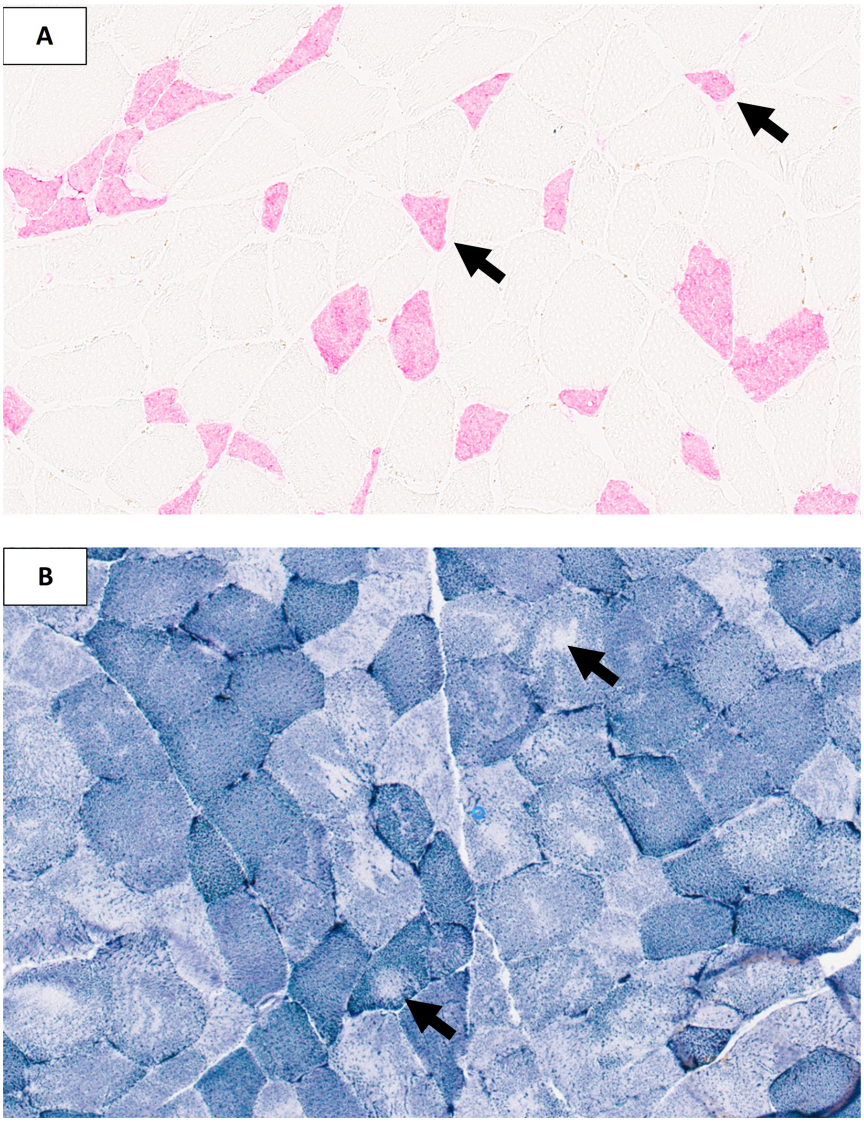

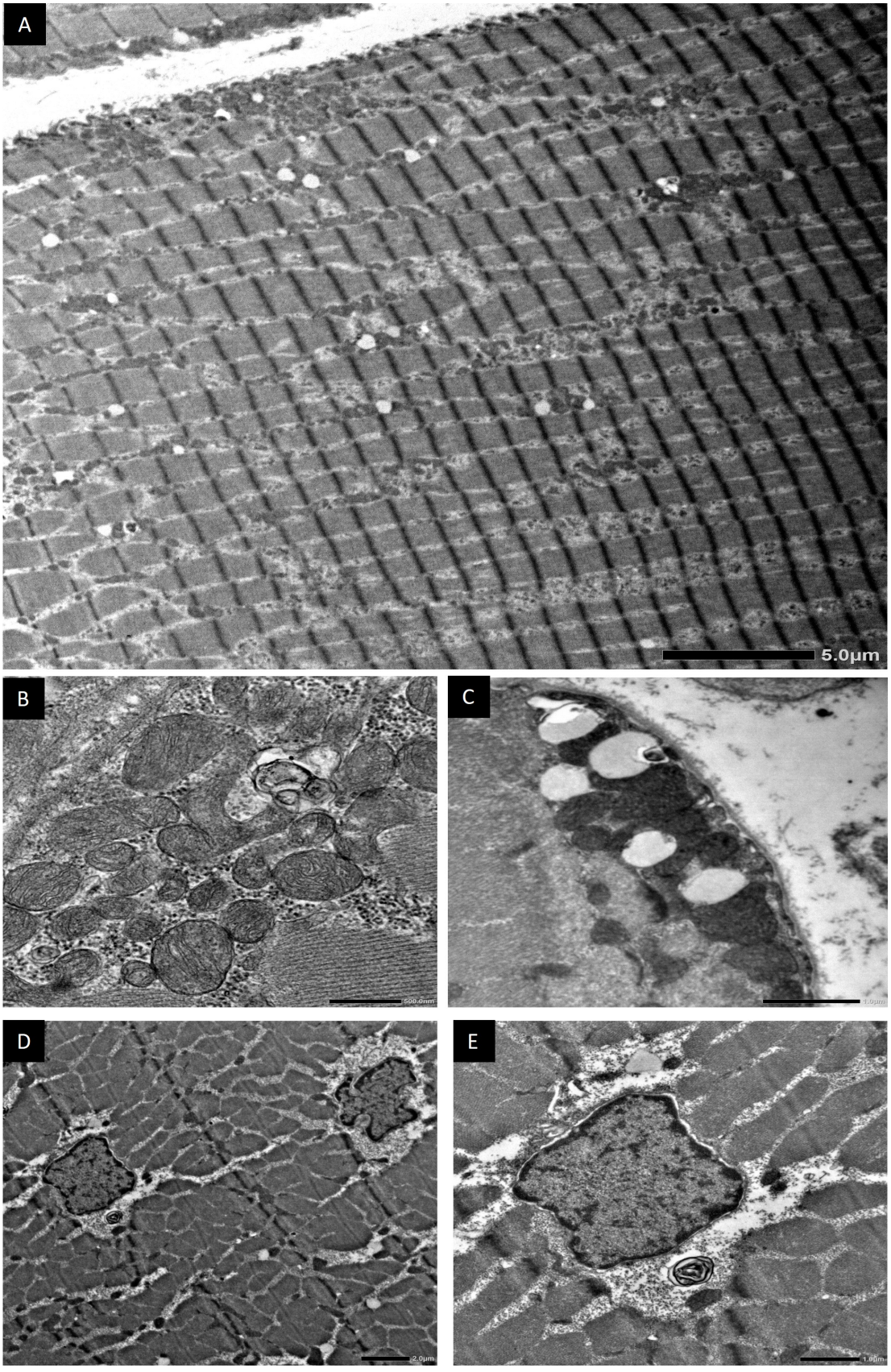

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Patient characteristics	**Active CS (n=9)**	**CS in remission (n=8)**	**Reference intervals and units**	**P value ***
Sex n (%) **Female** **Male**	 9 (100%) -	 7 (87.5) 1 (12.5)	-	
Age, years	31 (27; 59)	45 (35; 51)	-	0.4370
Tumor origin **Pituitary** **Adrenal** **Ectopic**	 4 4 1	 6 2 -	-	
BMI	25.7 (23.4; 29.7)	25.2 (24.1; 30.2)	kg/m²	0.8884
UFC	527 (355.5; 1016.0)	54 (47.6; 111.0)	≤ 83.0 µg/24h	**0.0002**
LNSC	10.3 (9.7; 21.1)	1.1 (0.9; 1.5)	< 1.5 ng/mL	**0.0003**
LDDST	16.1 (13.1; 20.45)	0.8 (0.5; 1.0)	< 1.8 µg/dL	**0.0007**
ACTH **In pituitary CS** **In adrenal CS** **In ectopic CS**	 57 (33.0; 58.5) 2.5 (2.0; 3.0) 88	 45.5 (15.0; 234.0) 11 (10.0; 12.0) -	4-61 pg/mL	
HbA1c	5.8 (5.5; 6.1)	5.4 (5.3; 5.7)	< 5.7%	0.1570
Fasting plasma glucose	93.0 (84.5; 96.5)	86.5 (85.3; 95.0)	60-90 mg/dL	0.3336
Insulin	14.0 (12.2; 16.0)	6.5 (4.7; 9.6)	3.2 - 16.3 µIU/mL	**0.0359**
Creatine kinase (CK)	69.0 (47.0; 109.0)	88.5 (77.5; 128.8)	≤ 169 U/L	0.5414
CK-MB activity	14.0 (13.0; 18.0)	13.0 (12.5; 14.0)	≤ 24 U/L	0.6777

Myoglobin	21.5 (21.0; 25.0)	24.0 (21.0; 32.8)	25-58 ng/mL	0.7846
IGF-1	213 (178-286)	156 (110-176)	ng/mL	**0.0079**
Mean cross-sectional area type I	4065 ± 1197	5676 ± 2078	µm²	0.1388
Mean cross-sectional area type II	2887 ± 686	3719 ± 1075	µm²	0.0745
Mean length muscle fiber type I	88.2 ± 14.5	99.8 ± 19.8	µm	0.1672
Mean length muscle fiber type II	75.1 ± 7.6	80.8 ± 12.5	µm	0.2359
Fiber type I	55 ± 12	50.1 ± 13.6	% of counted fibers	0.4807
Fiber type II	45 ± 12	49.9 ± 13.6	% of counted fibers	0.4807
NADH	In 4 of 9 biopsies moth eaten lesions	In 4 of 8 biopsies moth eaten lesions		
Subsarcolemmal mitochondrial aggregates	In 2 of 9 biopsies	In 2 of 8 biopsies		
Mitophagy	In 0 of 9 biopsies	In 3 of 8 biopsies		
Fat and glycogen accumulation	In 1 of 9 biopsies	In 4 of 8 biopsies		

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Immune cell infiltration	In 0 of 9 biopsies	In 0 of 9 biopsies		

Data are given as median and 25th and 75th percentile (except cross-sectional area, muscle fiber length and fiber type are displayed as mean and SD**)** in brackets. Bold p-values indicate statistical significance. * Active CS vs CS in remission

Abbreviations: ACTH= Adrenocorticotropic hormone; BMI= body mass index; CK= Creatine kinase; CS= Cushing syndrome; CSA= cross-sectional area; HbA1c=Hemoglobin A1c; LDDST=low-dosedexamethasone suppression test; IGF-1= Insulin-like growth factor 1; LNSC=late night salivary cortisol; NADH = Nicotinamide adenine dinucleotide; UFC=24 hour urinary free cortisol