Persistence of myopathy in Cushing's syndrome: evaluation of the German Cushing's Registry

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Abstract

Background: Cushing's syndrome (CS) is characterized by an excessive secretion of glucocorticoids that results in a characteristic clinical phenotype. One feature of clinical hypercortisolism is breakdown of protein metabolism translating into clinical consequences including glucocorticoid-induced myopathy. While surgery is effective in control of cortisol excess, the effect of biochemical remission on muscular function is yet unclear.

Methods: In a cross-sectional study we analyzed 47 patients with CS during the florid phase (ActiveCS). 149 additional patients were studied 2–53 years (mean: 13 years) after surgery in biochemical long-term remission (RemissionCS). Also, 93 rule-out CS patients were used as controls (CON). All subjects were assessed for grip strength using a hand grip dynamometer and underwent the chair rising test (CRT).

Results: Hand grip strength (85% vs 97% of norm, $P=0.002$) and the CRT performance (9.5 s vs 7.1 s, $P=0.001$) were significantly lower in ActiveCS compared to the CON group. Six months after treatment grip strength further decreased in CS ($P=0.002$) and CRT performance remained impaired. The RemissionCS group (mean follow-up 13 years) had reduced hand grip strength (92% compared to normal reference values for dominant hand, $P<0.001$). The chair rising test performance was at 9.0 s and not significantly different from the ActiveCS group ($P=0.45$).

Conclusion: CS affects muscle strength in the acute phase, but functional impairment remains detectable also during long-term follow-up despite biochemical remission.

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Introduction

Endogenous Cushing's syndrome (CS) is a rare disease with devastating metabolic, cardiovascular, psychiatric and musculoskeletal consequences. Hypercortisolism associated myopathy resulting in muscle weakness is one of the characteristic clinical features of CS which has been studied so far in small series of patients using electromyography, histology, imaging and functional testing (1, 2, 3, 4, 5). Muscle weakness has been reported to be present in 40–70% of patients with florid CS (6, 7)

and it appears to be more pronounced in females (8). The most commonly impaired part of the body is the proximal musculature of the lower limbs. Accordingly, patients typically complain about the inability to get up from a squatting position or to climb stairs whereas running or walking is less frequently affected (9).

Despite these well-characterized clinical features, the long-term prognosis of Cushing associated myopathy is not known. Therefore, we analyzed the outcome of

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muscular function in a large series of patients with CS and controls.

Patients and methods

Patients

We performed a multicenter cross-sectional study (as part of the German Cushing's Registry) analyzing 196 adult CS patients (47 with active Cushing's syndrome, 149 patients 2–53 years (mean: 13 years) after successful surgery) from the centers in Munich (Ludwig-Maximilians-Universität München (LMU, $n=129$) and Max Planck Institute of Psychiatry (MPI, $n=26$)), University of Würzburg ($n=23$), and Endocrinology in Charlottenburg, Berlin ($n=18$). Additionally, ninety-three control subjects (CON) were recruited at the LMU. Details on the German Cushing's Registry have been described recently (10). Diagnosis of CS and subtype differentiation was established according to current guidelines (11, 12). Patients with subclinical hypercortisolism defined by the absence of typical signs/symptoms of CS in the presence of abnormal biochemical test results for hypercortisolism were excluded from the current analysis.

We studied 47 patients with active CS (ActiveCS) recruited between 2013 and 2015 mainly at LMU. The ActiveCS group consisted of 33 patients with Cushing's disease (CD), 10 patients with adrenal CS and 4 patients with ectopic CS.

Short-term follow-up data was available in nineteen of the 47 prospective patients with ActiveCS (13 patients with CD, 5 with adrenal CS and 1 with ectopic CS). They were re-assessed on average 6.5 ± 1.3 months after successful surgery inducing biochemical remission. Remission was defined as postoperative adrenal insufficiency requiring glucocorticoid replacement therapy as recently described (13). The hydrocortisone dose during the first months after surgery was individually adjusted according to the maximal tolerable level of glucocorticoid withdrawal symptoms without concomitant gastrointestinal complaints. If required, patients also received vitamin D supplementation after surgery.

We studied 149 patients (82% female) with CS 2–53 years after successful surgery for CS (RemissionCS). All subjects had to be at least 2 years in biochemical and also in clinical remission after 2 years. Remission of CS was defined by tertiary adrenal insufficiency requiring hydrocortisone replacement therapy or by normal biochemical screening tests (24h urinary free cortisol (UFC), 23:00h salivary cortisol and a 1 mg dexamethasone

suppression test). Patients receiving adrenostatic therapy or radiotherapy were excluded from analysis. Clinical remission was defined by the absence of Cushing stigmata e.g. buffalo hump, moon face, livid discoloration of striae. Mean remission time, defined by the day of successful treatment until the day of examination, was 13.0 ± 10 years and the mean age at clinical examination was 54.5 ± 12.7 years (25–80 years). The RemissionCS group consisted of 95 patients with CD, 38 patients with adrenal CS and 16 patients with ectopic CS.

The control group (CON) was recruited in parallel between 2013 and 2015 at LMU and consisted of 93 patients referred with suspicion of CS which was subsequently ruled out by repeated biochemical assessment and clinical follow-up after 3–12 months. All patients underwent determination of UFC, 23:00h salivary cortisol and a 1mg dexamethasone suppression test initially and after a follow-up of 3–6 months. The clinical characteristics and laboratory parameters of ActiveCS and CON are shown in Table 1.

All patients and controls gave written informed consent. The protocol was approved by the ethics committee of the participating centers.

Muscle strength measurements

Grip strength was measured three times on both hands in a standardized fashion with a JAMAR dynamometer (Patterson Medical, Nottinghamshire, UK) as specified by the manufacturer. The average of the three measurements was calculated. The hand with the higher performance in the initial evaluation was defined as the dominant hand for initial and follow-up examinations. Grip strength was then standardized to age and gender according to the manufacturer's information ('normalized grip strength'). Limbs with known muscle dysfunction (e.g. due to stroke or intracerebral hemorrhage) were excluded from analysis.

The chair rising test was performed as described in (14) by rising from a sitting position from a chair of 45 cm height as fast as possible. Five repetitions at maximum speed were performed starting while the patient was seated and ending while standing. Patients kept arms crossed over their chest while performing the chair rising test and raised until full knee and hip extension. The read-out was the time (s) needed to execute this task, with higher numbers indicating more severe muscle impairment.

Estimation of muscle mass

Body cell mass comparable to muscle mass and body fat mass was estimated by using a bio impedance measuring

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Table 1 Basic characteristics and laboratory parameters of ActiveCS and CON (control group). For normally distributed values the mean $\pm$ s.d. is given. For non-normally distributed values the median with range is given.

	Standard value	All patients with CS	Pituitary CS (n=33)	Ectopic CS (n=4)	Adrenal CS (n=10)	Control (n=93)	P-value control vs all CS
Mean age (years)		46.5 $\pm$ 13.7	45.9 $\pm$ 14.0	57.0 $\pm$ 11.3	44.5 $\pm$ 13.2	42.7 $\pm$ 15.7	0.16
Mean BMI (kg/m ²) before treatment		29.9 $\pm$ 5.8	30.5 $\pm$ 6.4	25.9 $\pm$ 3.2	29.6 $\pm$ 3.8	33.1 $\pm$ 8.1	0.009
% Female		64	67	50	60	72	0.32
% Hypertension		94	93	100	100	61	<0.001
% Diabetes mellitus		29	25	50	40	14	0.59
Median baseline ACTH (pg/dL)	4–50	52	55 (16–114)	194 (85–544)	2 (1–9)	13 $\pm$ 10	<0.001
Median urinary cortisol (μ g/24h), range	<150	714	727 (176–727)	2014 (257–24586)	610 (164–921)	207 $\pm$ 91	<0.001
Median DST* 1 mg, serum cortisol (μ g/dL) range	<2.0	14	14 (3–36)	48 (22–149)	15 (3–21)	1.0 $\pm$ 0.9	<0.001
Median midnight salivary cortisol (ng/mL), range	<1.5	10.0	10.0 (2–48)	19.0 (6–40)	8.0 (2–12)	1.5 $\pm$ 1.5	<0.001
Mean TSH (μ U/mL)	0.3–4.0	1.2 $\pm$ 1.1	1.1 $\pm$ 0.4	0.5 $\pm$ 0.4	1.8 $\pm$ 2.7	1.6 $\pm$ 1.3	0.35
Mean PTH** (pg/mL)	15–65	60 $\pm$ 28	63 $\pm$ 31	63 $\pm$ 12	44 $\pm$ 8	53 $\pm$ 28	0.91
Mean calcium (mmol/L)	2.10–2.55	2.48 $\pm$ 0.11	2.48 $\pm$ 0.12	2.47 $\pm$ 0.01	2.45 $\pm$ 0.09	2.44 $\pm$ 0.12	0.13
Mean 25-hydroxyvitamin D (ng/mL)	20–65	24 $\pm$ 10	24 $\pm$ 11	26 $\pm$ 3	26 $\pm$ 8	28 $\pm$ 13	0.2

A unpaired *t*-test was used for comparison between groups; *P* < 0.05 was considered statistical significant.

*DST, dexamethasone suppression test; **PTH, parathyroid hormone.

device at 50kHz with 400 μ A by Data Input (Poecking, Germany) according to the manufacturer's information. Absolute muscle mass is the estimated weight of muscle in kg according to bio impedance measurements. Relative muscle mass is the absolute muscle mass divided by whole body weight.

Laboratory testing

Measurement of serum cortisol and plasma ACTH was performed with Solid Phase Antigen linked Technique (Cortisol, SPALT, Liaison, DiaSorin, Saluggia, Italy) and chemiluminescence immuno-assay (ACTH, CLIA, Liaison, DiaSorin). Within- and between-assay coefficients of variation were below 5 and 7% (Cortisol) and below 13% (ACTH) respectively. Urinary cortisol measurement was performed with chemiluminescence immuno-assay (ADVIA Centaur, Siemens) with within- and between-assay coefficients variations below 7%. Salivary cortisol was measured by a luminescence immunoassay (Cortisol LIA, IBL, Hamburg, Germany) with within- and between-assay coefficients variations below 9% and 6%. The last laboratory values before surgery were used for statistical analysis.

Statistical analysis

Comparison of ActiveCS and CON was done using unpaired *t*-test. For multi-factorial testing ANOVA was used in normally distributed variables. In non-normally distributed variables the Mann–Whitney *U* test was performed. The Kolmogorov–Smirnov test and the Shapiro–Wilk test were applied to test for Gaussian distribution. For small sample sizes Fisher's exact test was applied for comparison of ActiveCS and RemissionCS regarding inability to perform the chair rising test. Multiple regression analysis was performed to investigate factors associated with the chair rising test performance. Data of muscle strength before successful treatment and 6 months afterward were analyzed by paired *t*-test in normally distributed values. For correlations the Pearson correlation coefficient was calculated. Case-control matching was performed by propensity score matching (15). All statistical analyses were performed using SPSS Statistics 20 (IBM), R Version 2.12.0 (R Foundation for Statistical Computing, Vienna, Austria (16)) and R essentials for SPSS (IBM). Graphics were created with SPSS Statistics 20 and GraphPad Software Inc. Version 5.03. For normally distributed values the mean $\pm$ s.d. is given. For non-normally distributed values the median with

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range is given. *P*-values less than 0.05 were considered statistically significant.

Results

Muscle function in patients with active Cushing's syndrome

Grip strength was tested in 47 ActiveCS patients and in 93 CON. Grip strength was analyzed with respect to hand side, handedness and corrected for gender and age. ActiveCS patients showed significantly lower grip strength than CON. They showed 84% vs 98% ($P=0.002$) of age and gender corrected grip strength on the non-dominant hand (Table 2). Patients with ectopic CS were hereby the most severely affected with a mean grip strength of 67% of norm ($P=0.007$) whereas patients with adrenal CS were not significantly affected ($P=0.79$). Patients with pituitary CS had a significant reduction to 82% ($P=0.002$) on the non-dominant hand (Fig. 1A and B). Normalized grip strength of the total cohort or of the subgroup of CD patients did not correlate with the biochemical parameters of disease activity listed in Table 1, e.g. urinary cortisol ($n=37$, $r=0.02$, $P=0.926$).

Patients with ActiveCS had a significantly prolonged chair rising test ($P=0.001$) compared to CON (9.5 vs 7.1 s; Fig. 2A, B and Table 2). Similar to grip strength the average chair rising test time was worst in patients with ectopic CS with a mean of 11.0 s ($P=0.011$) compared to 10.8 s and 9.0 s in adrenal CS and CD (Fig. 1C and Table 2). There was no significant gender difference in test performance. By applying multiple linear regression

analysis to the total cohort (ActiveCS and CON), diagnosis of CS ($P<0.001$) and age ($P<0.001$) contributed to the performance of the chair rising test (Fig. 3, Supplementary Table 1, see section on supplementary data given at the end of this article), but not gender ($P=0.11$) and BMI ($P=0.09$). Within the ActiveCS group performance in chair rising test was not associated with age ($P=0.08$), gender ($P=0.60$) or BMI ($P=0.25$) (Supplementary Table 2). Chair rising test performance remained significantly impaired compared to CON using a case-control approach matching for age, sex and BMI ($n=35$, $P=0.001$, Supplementary Table 3).

Short-term follow-up of muscle function after successful therapy of CS

Tests of muscle function were repeated in 19 patients with ActiveCS on average 6.5 months after successful surgery. Remission of CS was associated with a mean weight loss of 11.2 ± 7.6 kg ($P<0.001$) and a reduction of BMI from 31.1 to 26.7 kg/m² ($P<0.001$, Fig. 4A). The relative fat mass dropped from 35% to 29% ($P<0.001$). Muscle cell mass measured by bio impedance also declined from 25.6 kg to 24.0 kg without reaching statistical significance ($P=0.062$) while relative muscle mass increased from 31% to 33% ($P=0.011$, Fig. 4D, $n=17$). Normalized hand grip strength decreased by 22% on the dominant hand ($P=0.002$), whereas chair rising test showed a non-significant improvement from 10.3 to 8.4 s ($P=0.09$, Fig. 4B and C). Six months after treatment the test could not be performed in three patients due to back and knee pain. In

Table 2 Tests on muscle function in patients with ActiveCS and in CON (control group), mean values are given.

	Control (n=93)	All patients with CS	Pituitary CS (n=33)	Ectopic CS (n=4)	Adrenal CS (n=10)	P-value all CS vs control	P-value pituitary CS vs control	P-value ectopic CS vs control	P-value adrenal CS vs control
Grip strength left hand in kg	32	27	26	21	32	0.032	0.027	0.029	0.735
Grip strength right hand in kg	34	30	29	22	34	0.05	0.076	0.022	0.856
Grip strength non-dominant hand in kg	31	26	25	20	31	0.032	0.022	0.023	0.725
Grip strength dominant hand in kg	35	31	30	23	35	0.058	0.080	0.023	0.853
Grip strength non-dominant hand corrected for age and sex in percent	98	84	82	69	95	0.003	0.002	0.027	0.786
Grip strength dominant hand, corrected for age and sex in percent	97	85	85	67	92	0.002	0.006	0.007	0.501
Chair rising test in seconds	7.1	9.5	9.0	11.0	10.8	0.001	0.031	0.011	0.020

An unpaired *t*-test was used for comparison between normally distributed groups (all CS vs control). An unpaired *t*-test or a Mann–Whitney *U* test was used according to statistical distribution of subgroups; $P<0.05$ was considered statistical significant.

Figure 1

Age and gender corrected grip strength and chair rising test in subtypes of ActiveCS and CON (control group).

(A) dominant hand, (B) non-dominant hand; (C) chair rising test; CS, Cushing's syndrome; A unpaired *t*-test was used for comparison between groups; $P < 0.05$ was considered statistical significant.

a subgroup of 8 patients similar results partly with further decrease were still present 12 months after surgery (data not shown).

All of these 19 patients developed adrenal insufficiency following surgery and were replaced with hydrocortisone. The mean daily dose of hydrocortisone was $26.0 (\pm 6.2)$ mg/day (range 14.5–31.3 mg/day) calculated over 6 months. Patients with adrenal CS needed significant more hydrocortisone ($P = 0.03$), but the daily hydrocortisone dose per BMI ($P = 0.16$) and per weight in kg ($P = 0.40$) was not significantly different.

The relative reduction in normalized grip strength was not related to subtype ($P = 0.37$), gender ($P = 0.15$) or initial biochemical parameters. The function of thyroid gland and bone parameters as possible co-factors influencing muscle function showed no differences before and after treatment (Supplementary Table 4).

Long-term muscle function in CS patients in remission

Grip strength was tested in 149 RemissionCS patients at least two years after successful treatment. The age and gender corrected hand grip strength of RemissionCS overall was significantly lower in comparison to normal reference values (92% for dominant hand, $P < 0.001$, Supplementary Table 5). There were no significant differences between subtypes of CS, gender and time since successful treatment or the participating centers ($P = 0.71$, $P = 0.99$, $P = 0.23$ and $P = 0.86$ respectively, for the dominant hand, Supplementary Table 6). Age and gender corrected grip strength and chair rising test were negatively correlated ($n = 138$, $r = -0.262$, $P = 0.002$).

The total cohort of RemissionCS patients performed the chair rising test in a mean time of 9.0s. The percentage of patients unable to perform the test was lower in RemissionCS than in ActiveCS (7% vs 13% in ActiveCS, $P = 0.16$). There was no correlation between chair rising test performance and time elapsed since successful treatment ($P = 0.18$). To exclude a bias introduced by major differences in age, sex and BMI between patients with RemissionCS and the CON group we performed a propensity score matching between groups. This resulted in 46 matched pairs. Similar to the unmatched cohorts matched RemissionCS performed significantly poorer in the chair rising test than CON ($P = 0.016$, Supplementary Table 7). Chair rising performance was similar in the ActiveCS group, both in an unmatched ($P = 0.45$; Fig. 2B and C) and matched approach ($n = 29$, $P = 0.69$, Supplementary Table 8).

Figure 2

Distribution of times to complete the chair rising test in the control group (A; $n=93$), in patients with active Cushing's syndrome (B; $n=46$) and in patients with >2 years in remission of Cushing's syndrome (C; $n=148$); Patients in panel C are on average 8.0 years older than A and B.

50% of RemissionCS ($n=149$) were on hydrocortisone replacement therapy at examination. We did not find significant differences between patients with and without hydrocortisone replacement therapy ($P=0.53$ for grip strength of dominant hand; $P=0.53$ for chair rising). The mean hydrocortisone replacement dose was 22 ± 6 mg at last follow-up. There was no correlation between hydrocortisone dose and muscle strength (chair rising $P=0.55$, $n=43$; age and gender corrected grip strength of dominant hand $P=0.63$, $n=47$). All patients were in clinical and biochemical remission.

A complete status of pituitary hormonal insufficiency was known in 67 of 95 patients with CD. Eighteen of the patients (27%) had documented growth hormone deficiency accompanied by other pituitary insufficiencies, and 5 received GH replacement therapy (Supplementary Table 9). Patients receiving growth hormone replacement therapy had the lowest performance in grip strength (age and sex corrected grip strength of dominant hand, $P=0.039$ vs RemissionCS without hypopituitarism) and a similar chair rising test excluding a major beneficial effect of growth hormone replacement therapy in this small group (Supplementary Table 10). Similar analysis regarding thyrotropic and gonadotropic axis did not show significant differences

between patients with insufficiency with and without substitution in comparison to patients without insufficiency.

Discussion

This is the first study that systematically analyzes grip strength and proximal muscle function with the chair rising test in a large cohort of patients with endogenous CS. We here showed that patients with active CS have severe impairment of muscle strength compared to obese controls. Specifically, in particular 30% of ActiveCS presented with a grip strength performance at least one s.d. below that of the control group and 50% performed the chair rising test above the 75th percentile of the control group. More importantly, our data suggest that muscle function remains impaired short-term (6–8 months) and long-term (>2 years) after achieving biochemical remission of hypercortisolism. As a consequence muscle function can be regarded as another feature of CS that is impaired in the long-term as it is known for cognitive function (17). These results emphasize the need for further studies clarifying the mechanisms by which muscle function remains impaired and investigating the impact of exercise on muscle function.

Figure 3

Relationship between performance in chair rising test and age in ActiveCS (filled circles) and CON (open circles). Lines indicate estimated linear regression lines.

An Italian multicenter study evaluated gender differences in clinical presentation in 280 patients with CD (233 females, 47 males) (8). This study reported a higher prevalence of impaired muscle function in males compared to females (64% vs 45%, $P < 0.05$). Thereby, these findings are in contrast to our cohort of ActiveCS patients which showed similar rates of impaired muscle function between both sexes. One reason for the difference might be the retrospective collection of data from patient charts in the Italian investigation.

Another interesting feature of our study is the difference in muscle function according to subtypes of CS. As one would expect, patients with ectopic CS had the most severe cortisol excess compared to other subtypes. Accordingly, this group had the lowest grip strength and lowest chair rising test performance although we did not find a correlation between cortisol levels and grip strength in the whole Cushing cohort. Glucocorticoids are known to change muscle fiber composition and muscle strength with a loss of strong and fast contracting type II fibers already after a short time of glucocorticoid treatment (18, 19). Thereby, it can be hypothesized that the loss of grip strength and chair rising test performance might be due to this effect. Why the distribution pattern of muscle strength is different between subtypes of Cushing's syndrome remains unclear.

Figure 4

Grip strength and chair rising test before and six months following successful intervention in ActiveCS patients ($n=19$); muscle mass was estimated by bio impedance measurements ($n=17$). A paired t -test was used for comparison between groups; $P < 0.05$ was considered statistical significant.

Muscle wasting in endogenous or exogenous CS is a well-known clinical feature which has already been mentioned in early reports from the late 1950s (1). Functional studies were focused on electromyography (1, 2, 3), imaging (20, 21, 22, 23) and muscle biopsy (2, 24). Steroid induced myopathy is characterized by a decrease in muscle fiber conduction velocity on electromyography (3) and histology reveals an atrophy of type IIa muscle fibers (2). The latter implies that the impairment of muscle function is more on fast power than on slow continuous contraction making tests like grip strength and chair rising more reasonable.

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Our results highlight a deleterious effect on hand grip strength with continuous impairment of muscular function that is prevailing for at least one year. A similar observation was reported as early as 1959 by Müller & Kugelberg (1). Three of the 6 patients were investigated 5–60 months after surgical cure revealing electromyographic and histopathologic abnormalities. In contrast, Mills *et al.* reported an increase in lower limb muscle strength in four patients (4) and Khaleeli *et al.* observed an improvement of muscle strength in four patients within 18 months for maximum voluntary contraction of the lower limbs (25). The authors showed that this improvement was paralleled by an increase in relative muscle mass. We found a decrease in fat mass and a similar increase in relative muscle mass (as assessed by bio impedance), but not in absolute muscle mass. However, we could not confirm improved lower limb function using the chair rising test.

The reason why grip strength of the upper limb remains severely affected and even decreases after achieving remission of CS remains unclear. While this observation is of clinical significance, the underlying pathophysiology is likely to be multi-factorial. Anabolic factors, such as growth hormone and sex hormones which are known to influence myopathy (26) are often suppressed in florid CS (27, 28). These might only slowly recover after successful surgical correction of cortisol excess and might contribute to the deterioration of muscle function (27, 29, 30). Regarding growth hormone secretion, pre- and postoperative somatotrophic insufficiency might contribute to the observed effect and animal models showed that IGF-1 administration can prevent muscle fiber loss induced by glucocorticoid treatment (31). Additionally, a study in men by Johannsson *et al.* showed an increase in isometric knee extensor strength in long-term remission patients with somatotrophic insufficiency under growth hormone replacement therapy (32). However, we did not observe a trend toward normalization of muscle function in a very small sub-cohort of patients receiving growth hormone treatment or other replacement therapy. Therefore this data should be interpreted with caution.

Another important reason for decrease of muscle strength after successful treatment might be the glucocorticoid withdrawal syndrome. It is a prevalent condition after successful therapy that is associated with arthralgia and myalgia which might impair regular physical exercise. Although these symptoms rarely last longer than one year (33), the accompanying general lack of energy could further aggravate and sustain muscle wasting. Furthermore some individual improvements in this group could be due to muscle exercises on individual

initiative. It is therefore likely that the first year following treatment of CS is of particular importance for regaining long-term muscle strength.

The persisting muscle weakness observed in our cohort cured from CS for more than 2 years (mean: 13 years) is rather unexpected. Despite correction for age and gender, grip strength and chair rising test performance remained altered suggesting a long lasting effect of glucocorticoids on muscle function. In the light of rodent steroid-myopathy models and muscle biopsies, showing not hypotrophy, but also atrophy of type IIa muscle fibers following steroid treatment (34), one could assume an irretrievable loss of muscle that can be measured for many years after active CS. We did not observe lower muscle function in patients receiving glucocorticoid replacement, and impaired muscle performance tests were not associated with higher replacement doses, excluding a major effect of glucocorticoid replacement *per se*.

The results of this study have implication beyond muscle function. The performance in tests for muscle strength is a predictor of morbidity and mortality in the general population. Grip strength measured by a dynamometer has shown to be a predictor of all-cause death, cardiovascular death and cardiovascular disease in the PURE study with about 140000 participants of 17 countries (35). The authors mentioned that grip strength was a better predictor of all-cause death than systolic blood pressure (36). Mid-life muscle strength (assessment at 56–68 years) was also shown to be a predictor of longevity (37). As our cohort of RemissionCS was assessed at a mean age of 55 years, there is a striking parallelism and there are still contradictory statements on mortality in treated CS patients (38).

In summary, muscle weakness appears to be an important feature of CS not only during the active phase but also on long-term follow-up. We hypothesize potential associations with an adverse long-time outcome such as mortality (38) although as in grip strength the underlying mechanisms are yet unclear (35). The aim should be to prevent a further decline in muscular function after diagnosis of CS. Although there are promising medical treatments for sarcopenia evolving, drugs for augmentation of muscle function eligible for this purpose are not available yet and physical exercise is currently the only treatment (39). Animal experiments on steroid myopathy propose moderate exercise to be more suitable than high-intensity exercise to prevent further deterioration of muscle fiber composition during steroid treatment (40). Whether specific physical training before and in the aftermath of successful surgery could improve

long-term outcomes has to be addressed by randomized interventional trials.

Limitations and strength of our study

As we do not have full data on IGF-1-levels of patients we cannot rule out the effects of growth hormone deficiency on muscle strength in our patient. We could rule out other influencing factors as BMI, hydrocortisone replacement therapy, thyroid function, vitamin D levels, PTH and (with low level evidence) growth hormone replacement. Strength of the study includes the high number of patients and a long follow-up in the RemissionCS group of 13 years.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-16-0689>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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References

- Müller R & Kugelberg E. Myopathy in Cushing's syndrome. *Journal of Neurology, Neurosurgery, and Psychiatry* 1959 **22** 314–319. (doi:10.1136/jnnp.22.4.314)
- Khaleeli AA, Edwards RH, Gohil K, McPhail G, Rennie MJ, Round J & Ross EJ. Corticosteroid myopathy: a clinical and pathological study. *Clinical Endocrinology* 1983 **18** 155–166. (doi:10.1111/j.1365-2265.1983.tb03198.x)
- Minetto MA, Lanfranco F, Botter A, Motta G, Mengozzi G, Giordano R, Picu A, Ghigo E & Arvat E. 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. *European Journal of Endocrinology* 2011 **164** 985–993. (doi:10.1530/EJE-10-1169)
- Mills GH, Kyroussis D, Jenkins P, Hamnegard CH, Polkey MI, Wass J, Besser GM, Moxham J & Green M. Respiratory muscle strength in Cushing's syndrome. *American Journal of Respiratory and Critical Care Medicine* 1999 **160** 1762–1765. (doi:10.1164/ajrccm.160.5.9810028)
- Olafsson E, Jones HR Jr, Guay AT & Thomas CB. Myopathy of endogenous Cushing's syndrome: a review of the clinical and electromyographic features in 8 patients. *Muscle and Nerve* 1994 **17** 692–693. (doi:10.1002/mus.880170625)
- Bolland MJ, Holdaway IM, Berkeley JE, Lim S, Dransfield WJ, Conaglen JV, Croxson MS, Gamble GD, Hunt PJ & Toomath RJ. Mortality and morbidity in Cushing's syndrome in New Zealand. *Clinical Endocrinology* 2011 **75** 436–442. (doi:10.1111/j.1365-2265.2011.04124.x)
- Ammini AC, Tandon N, Gupta N, Bhalla AS, Devasenaspathy K, Kumar G, Sahoo JP, Chittawar S, Philip J, Baruah MP *et al*. Etiology and clinical profile of patients with Cushing's syndrome: a single center experience. *Indian Journal of Endocrinology and Metabolism* 2014 **18** 99–105. (doi:10.4103/2230-8210.126586)
- Pecori Giralaldi F, Moro M, Cavagnini F & Study Group on the Hypothalamo-Pituitary-Adrenal Axis of the Italian Society of Endocrinology. Gender-related differences in the presentation and course of Cushing's disease. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 1554–1558. (doi:10.1210/jc.2002-021518)
- Minetto MA, Lanfranco F, Motta G, Allasia S, Arvat E & D'Antona G. Steroid myopathy: some unresolved issues. *Journal of Endocrinological Investigation* 2011 **34** 370–375. (doi:10.1007/BF03347462)
- Osswald A, Plomer E, Dimopoulou C, Milian M, Blaser R, Ritzel K, Mickisch A, Knerr F, Stanojevic M, Hallfeldt K *et al*. Favorable long-term outcomes of bilateral adrenalectomy in Cushing's disease. *European Journal of Endocrinology* 2014 **171** 209–215. (doi:10.1530/EJE-14-0214)
- Arnaldi G, Angeli A, Atkinson AB, Bertagna X, Cavagnini F, Chrousos GP, Fava GA, Findling JW, Gaillard RC, Grossman AB *et al*. Diagnosis and complications of Cushing's syndrome: a consensus statement. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 5593–5602. (doi:10.1210/jc.2003-030871)
- Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM & Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 1526–1540. (doi:10.1210/jc.2008-0125)
- Berr CM, Di Dalmazi G, Osswald A, Ritzel K, Bidlingmaier M, Geyer LL, Treitl M, Hallfeldt K, Rachinger W, Reisch N *et al*. Time to recovery of adrenal function after curative surgery for Cushing's syndrome depends on etiology. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** 1300–1308. (doi:10.1210/jc.2014-3632)
- Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, Scherr PA & Wallace RB. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *Journals of Gerontology* 1994 **49** M85–M94. (doi:10.1093/geronj/49.2.M85)
- Thoemmes F & Liao W. Propensity score matching (with multilevel data) using SPSS and R. *Modern Modeling Methods Conference*, Storrs, CT, USA 2013.
- R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2014 (<http://www.R-project.org/>).
- Ragnarsson O, Berglund P, Eder DN & Johannsson G. Long-term cognitive impairments and attentional deficits in patients with Cushing's disease and cortisol-producing adrenal adenoma in remission. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** E1640–E1648. (doi:10.1210/jc.2012-1945)
- Fitts RH, Romatowski JG, Peters JR, Paddon-Jones D, Wolfe RR & Ferrando AA. The deleterious effects of bed rest on human skeletal muscle fibers are exacerbated by hypercortisolemia and ameliorated by dietary supplementation. *American Journal of Physiology: Cell Physiology* 2007 **293** C313–C320. (doi:10.1152/ajpcell.00573.2006)
- Minetto MA, Qaisar R, Agoni V, Motta G, Longa E, Miotti D, Pellegrino MA & Bottinelli R. Quantitative and qualitative

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- adaptations of muscle fibers to glucocorticoids. *Muscle and Nerve* 2015 **52** 631–639. (doi:10.1002/mus.24572)
- 20 Miller BS, Ignatoski KM, Daignault S, Lindland C, Gauger PG, Doherty GM, Wang SC & University of Michigan Analytical Morphomics Group. A quantitative tool to assess degree of sarcopenia objectively in patients with hypercortisolism. *Surgery* 2011 **150** 1178–1185. (doi:10.1016/j.surg.2011.09.020)
 - 21 Wajchenberg BL, Bosco A, Marone MM, Levin S, Rocha M, Lerario AC, Nery M, Goldman J & Liberman B. 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. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 2791–2794. (doi:10.1210/jc.80.9.2791)
 - 22 Resmini E, Farkas C, Murillo B, Barahona MJ, Santos A, Martinez-Momblan MA, Roig O, Ybarra J, Geli C & Webb SM. Body composition after endogenous (Cushing's syndrome) and exogenous (rheumatoid arthritis) exposure to glucocorticoids. *Hormone and Metabolic Research* 2010 **42** 613–618. (doi:10.1055/s-0030-1255032)
 - 23 Ragnarsson O, Glad CA, Berghthorsdottir R, Almqvist EG, Ekerstad E, Widell H, Wangberg B & Johannsson G. Body composition and bone mineral density in women with Cushing's syndrome in remission and the association with common genetic variants influencing glucocorticoid sensitivity. *European Journal of Endocrinology* 2015 **172** 1–10. (doi:10.1530/EJE-14-0747)
 - 24 Blanco C, Marazuela M, Flores J & Alvarez J. Severe respiratory failure secondary to Cushing's myopathy. *Journal of Endocrinological Investigation* 2001 **24** 618–621. (doi:10.1007/BF03343903)
 - 25 Khaleeli AA, Betteridge DJ, Edwards RH, Round JM & Ross EJ. Effect of treatment of Cushing's syndrome on skeletal muscle structure and function. *Clinical Endocrinology* 1983 **19** 547–556. (doi:10.1111/j.1365-2265.1983.tb00030.x)
 - 26 Dirks-Naylor AJ & Griffiths CL. Glucocorticoid-induced apoptosis and cellular mechanisms of myopathy. *Journal of Steroid Biochemistry and Molecular Biology* 2009 **117** 1–7. (doi:10.1016/j.jsbmb.2009.05.014)
 - 27 Magiakou MA, Mastorakos G, Gomez MT, Rose SR & Chrousos GP. Suppressed spontaneous and stimulated growth hormone secretion in patients with Cushing's disease before and after surgical cure. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 131–137. (doi:10.1210/jc.78.1.131)
 - 28 Odagiri E, Yamanaka Y, Ishiwatari N, Jibiki K, Demura R, Demura H, Suda T & Shizume K. Studies on pituitary-gonadal function in patients with Cushing's syndrome. *Endocrinologia Japonica* 1988 **35** 421–427. (doi:10.1507/endocrj1954.35.421)
 - 29 Carroll PV, Monson JP, Grossman AB, Besser GM, Plowman PN, Afshar F & Savage MO. Successful treatment of childhood-onset Cushing's disease is associated with persistent reduction in growth hormone secretion. *Clinical Endocrinology* 2004 **60** 169–174. (doi:10.1046/j.1365-2265.2003.01922.x)
 - 30 Cuneo RC, Salomon F, Wiles CM & Sonksen PH. Skeletal muscle performance in adults with growth hormone deficiency. *Hormone Research* 1990 **33** (Supplement 4) 55–60. (doi:10.1159/000181585)
 - 31 Fournier M, Huang ZS, Li H, Da X, Cercek B & Lewis MI. Insulin-like growth factor I prevents corticosteroid-induced diaphragm muscle atrophy in emphysematous hamsters. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology* 2003 **285** R34–R43. (doi:10.1152/ajpregu.00177.2002)
 - 32 Johannsson G, Sunnerhagen KS & Svensson J. Baseline characteristics and the effects of two years of growth hormone replacement therapy in adults with growth hormone deficiency previously treated for Cushing's disease. *Clinical Endocrinology* 2004 **60** 550–559. (doi:10.1111/j.1365-2265.2004.02018.x)
 - 33 Bhattacharyya A, Kaushal K, Tymms DJ & Davis JR. Steroid withdrawal syndrome after successful treatment of Cushing's syndrome: a reminder. *European Journal of Endocrinology* 2005 **153** 207–210. (doi:10.1530/eje.1.01953)
 - 34 Lee MC, Lee JS, Lee MJ, Lee JH & Kim HI. Fas mediates apoptosis in steroid-induced myopathy of rats. *Neuropathology and Applied Neurobiology* 2001 **27** 396–402. (doi:10.1046/j.1365-2990.2001.00344.x)
 - 35 Leong DP, Teo KK, Rangarajan S, Lopez-Jaramillo P, Avezum A Jr, Orlandini A, Seron P, Ahmed SH, Rosengren A, Kelishadi R *et al.* Prognostic value of grip strength: findings from the Prospective Urban Rural Epidemiology (PURE) study. *Lancet* 2015 **386** 266–273. (doi:10.1016/S0140-6736(14)62000-6)
 - 36 Group SR, Wright JT Jr, Williamson JD, Whelton PK, Snyder JK, Sink KM, Rocco MV, Reboussin DM, Rahman M, Oparil S *et al.* A randomized trial of intensive versus standard blood-pressure control. *New England Journal of Medicine* 2015 **373** 2103–2116. (doi:10.1056/NEJMoa1511939)
 - 37 Rantanen T, Masaki K, He Q, Ross GW, Willcox BJ & White L. Midlife muscle strength and human longevity up to age 100 years: a 44-year prospective study among a decedent cohort. *Age* 2012 **34** 563–570. (doi:10.1007/s11357-011-9256-y)
 - 38 Clayton RN. Mortality in Cushing's disease. *Neuroendocrinology* 2010 **92** (Supplement 1) 71–76. (doi:10.1159/000315813)
 - 39 Cohen S, Nathan JA & Goldberg AL. Muscle wasting in disease: molecular mechanisms and promising therapies. *Nature Reviews Drug Discovery* 2015 **14** 58–74. (doi:10.1038/nrd4467)
 - 40 Uchikawa K, Takahashi H, Hase K, Masakado Y & Liu M. Strenuous exercise-induced alterations of muscle fiber cross-sectional area and fiber-type distribution in steroid myopathy rats. *American Journal of Physical Medicine and Rehabilitation* 2008 **87** 126–133. (doi:10.1097/PHM.0b013e31815869d0)

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