Thigh Muscle Fat Infiltration Is Associated With Impaired Physical Performance Despite Remission in Cushing's Syndrome

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Context: Muscle weakness is common in patients with Cushing's syndrome (CS) and may persist after the resolution of hypercortisolism. Intramuscular fatty infiltration has been associated with the deterioration of muscle performance in several conditions.

Objectives: To quantify the degree of fatty infiltration in the thigh muscles of "cured" CS patients and evaluate the relationship between intramuscular fatty infiltration and physical performance.

Design: This was a cross-sectional study.

Setting: Tertiary referral center.

Patients: Thirty-six women with CS in remission, and 36 controls matched for age, BMI, menopausal status, and level of physical activity.

Main Outcome Measures: 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.

Conclusions: Thigh muscle fatty infiltration is increased in "cured" CS patients and is associated with poorer muscle performance. Future studies are needed to establish therapeutic strategies to improve muscle weakness in these patients. **(*J Clin Endocrinol Metab* 105: 1–11, 2020)**

Key Words: Cushing's syndrome, muscle, fatty infiltration, muscle performance

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Glucocorticoid excess in Cushing's syndrome (CS) is associated with proximal myopathy, which leads

to muscle weakness that mainly affects the lower limbs (1). While up to 70% of patients with endogenous hypercortisolism self-reported muscle weakness at diagnosis, a decrease in both hand grip strength and proximal muscle functionality was described in CS patients during the active phase of the disease as compared with obese controls (2, 3). Recent data analyzed from the German Cushing's Registry demonstrated that muscle function impairment persists even long after hormone normalization in "cured" CS patients (3).

Growing evidence 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 (4–9). Although the increased amount of fatty infiltration in the muscle may be a marker of the progressive loss of skeletal muscle mass described in these conditions, both in vitro and in vivo studies suggest that fatty infiltration of skeletal muscle may also be an important contributor to structural and metabolic changes ultimately affecting muscle performance (9–13).

Cortisol excess in CS is associated with fat partitioning, consistent with glucocorticoids being prominent regulators of body fat metabolism and regional distribution (14–16). 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 (16). Interestingly enough, Geer et al showed that whole-body intermuscular fat, defined as the adipose tissue located between muscle groups and beneath the muscle fascia, did not change in patients with Cushing's disease (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 (17). No studies have evaluated the intramuscular accumulation of fat in CS patients thus far. Whether there is fatty infiltration in the muscles of CS patients after long-term remission and whether this is associated with poor muscle function is currently unknown.

In the last years, quantitative muscle MRI has been used to quantify fatty infiltration in the skeletal muscle of patients with several muscle disorders (18). The Dixon technique is a robust chemical-shift imaging application producing water- and fat-only images from different echo acquisitions (19). Of all the MRI-sequences available, Dixon is the most widely used in quantitative muscle MRI, due to its excellent accuracy for intramuscular fat quantification, expressed as percentages of fat fraction (FF), in comparison to T1-weighted MRI, spectroscopy, and histology examination (20–22). Moreover, Dixon-assessed FF has proven to be a reliable outcome measure that is related to muscle function parameters in several neuromuscular disorders (7, 23, 24).

Given that previous exposure to cortisol excess is associated with irreversible changes in several tissues, such as bone and brain, leading to residual morbidity in "cured" CS patients, and that glucocorticoids are important modulators of fat accumulation in muscle, we hypothesized that muscle architecture remains impaired long-term after normalization of cortisol levels, due to increased fatty infiltration (25–28). Thus, the aims of this study were (1) to quantify the FF, a parameter indicating the degree of intramuscular fatty infiltration, in the thigh muscles of patients with CS, long-term after successful control of hypercortisolism, using 3-point Dixon imaging on MRI, and (2) to evaluate the relationship between fatty infiltration and muscle performance on function testing.

Materials and Methods

Subjects

We studied 36 women with CS in remission. Patients below the age of 65 years, who had been in remission for a minimum of 3 years, were consecutively included in our study during their follow-up visits at the endocrine clinic of the Hospital Sant Pau, Barcelona, Spain.

Diagnosis of CS was made after clinical, biochemical, and radiological evaluations, based on internationally agreed guidelines (29). All patients had abnormal values on at least two of the following tests: elevated UFC, late-night salivary or serum cortisol, 1 mg overnight dexamethasone suppression test (ODST), or 48-hour 2 mg/day low-dose dexamethasone suppression test (LDDST). Twenty-eight patients had CD due to a microadenoma (n = 25) or a macroadenoma (n = 3). The remaining 8 patients had an adrenal adenoma. The median duration of hypercortisolism was 33 (24) months and was defined as the time elapsed from the initial symptoms, as referred by patients, and final diagnosis of CS.

Thirty patients (83%) received preoperative treatment with steroidogenesis inhibitors to control clinical symptoms of hypercortisolism. All the CD patients underwent transsphenoidal surgery (TSS) a median of 154 (117) months previously, and 7 of them (19%) also received radiotherapy a median of 142 (108) months after unsuccessful surgery (n = 1) or relapse (n = 6). All the patients diagnosed with an adrenal adenoma underwent adrenalectomy a median of 100 months (51) months, previously. Mean (±SD) time of remission, defined as the time elapsed from diagnostic confirmation of remission to study entry, was 13 ± 7 years, [median, 13(8); and range, 3 to 204 months]. Cushing's syndrome was considered in remission if either adrenal insufficiency was demonstrated (basal morning cortisol <171 nmol/L [<6.2µg/dL] and/or undetectable 24-hour free urinary cortisol) or morning cortisol suppression <50nmol/L (<1.8µg/dL) after 1 mg dexamethasone overnight was observed.

Thirty patients (83%) had received hydrocortisone (HC) replacement (between 10 and 20 mg does per day) for a median of 19 (21) months after surgery. Median time free from

HC replacement was 84 (104) months. At study entry, 3 patients were still taking HC at a stable dose of 20 mg per day and mean (± SD) duration of treatment was 148 ± 35 months.

At study entry, all the subjects were re-evaluated for possible pituitary insufficiency. Three patients had growth hormone deficiency (GHD), 2 of whom were replaced with recombinant human GH (mean duration of treatment [± SD] 138 ± 42 months).

Twenty-one women (58%) were postmenopausal. Mean (± SD) duration of menopause was 96 ± 61 months. No patients were taking estrogen/progesterone hormone replacement at study entry. No patients with pituitary-dependent CS developed gonadotropin deficiency after surgery. Five (14%) were hypothyroid (3 due to TSH deficiency and 2 due to primary hypothyroidism) and all of them were on stable doses (65 ± 22 µg/day) of L-thyroxine replacement (mean duration of treatment [±SD] 127 ± 32 months).

Patients were informed about the study during their follow-up visit at our clinic. If they agreed to participate, they were asked to sign the consent.

For each patient, female blood donors matched for age and body mass index (BMI) at the time of the study were identified and recruited upon their consent to participate. They were subsequently selected and matched to the patients based on their menopausal status and degree of physical activity.

During the study visit (day 1), data collection and a medical history review and physical examination were performed. A blood sample for study purposes was also drawn on the same day. Subjects underwent MRI and physical performance evaluation on day 2 and day 3, respectively.

Exclusion criteria were: subjects older than 65 years, active disease, inflammatory disorders, diabetes mellitus, kidney or liver dysfunctions, malignancies, neurological dysfunctions, documented physical disability or motility limitations, and treatment with local or systemic glucocorticoids during the previous year.

All subjects gave full, written consent and the study was approved by the ethics committee of our institution (IIB-CEIC).

Degree of physical activity

Patients were asked to complete the International Physical Activity Questionnaire (IPAQ) (30), which classify subjects based on 3 categories of physical activity: low, moderate, and high. High physical activity indicated (1) vigorous-intensity activity on at least 3 days (20 minute minimum, achieving a minimum total physical activity of at least 1500 metabolic equivalents (MET)–minutes/week, or (2) 7 or more days of any combination of walking, moderate-intensity, or vigorous-intensity activities, achieving a minimum total physical activity of at least 3000 MET-minutes/week. Moderate physical activity indicated (1) 3 or more days of vigorous-intensity activity of at least 20 minutes per day, or (2) 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day, or (3) 5 or more days of any combination of walking, moderate-intensity, or vigorous-intensity activities, achieving a minimum total physical activity of at least 600 MET-minutes/week. Low physical activity indicated that physical activity was not moderate or vigorous. We classified 22 patients as having a low physical activity level and 14 as having a moderate physical activity level. No patients had a high level of physical activity.

Determination of CK activity

Creatine kinase activity was measured using the standardized reverse reaction (creatine phosphate and adenosine triphosphate [ATP]) and activation by N-acetylcysteine, using commercial reagent (Alinity; Abbott GmbH &Co, Wiesbaden, Germany). Inter- and intra-assay coefficients of variations were below 2% and the normal range for healthy women was 29–168 U/L.

Physical performance

During the study visit, both patients and controls underwent the following tests under the supervision of a physical medicine specialist (H.B.) and blinded to study groups:

- Gait speed (GS) is a test involving an automatic process that simultaneously controls and supports balance and rhythmic activity. It was defined as the length of the 6-meter (m) walking course divided by the time it took subjects to walk the course at their usual pace, scored in m/seconds (m/s). Lower numbers indicate a more severe impairment of an individual's functional capacity.
- Timed up and go (TUG) requires the subject to stand up from a chair, walk a distance of 3 meters, turn around, and return and sit down again, scored in seconds. A longer time (s) indicates more severe impairment of postural stability, gait, stride length, and sway.
- For the 30-second chair stand, participants were asked to (1) sit in the middle of the chair, place their hands on the opposite shoulder, crossed at the wrists, against their chest; (2) keep their feet flat on the floor and back straight; (3) On the word "go," rise to a full standing position, and then sit down again; (4) repeat this for 30 seconds. The number of times that participants were able to come from sitting to a full standing position in 30 seconds was recorded (number of times). Lower numbers indicate a more severe impairment of strength in the lower limbs and balance.

Muscle strength

Grip strength was measured 3 times with a JAMAR handheld dynamometer (Patterson Medical, Nottinghamshire, United Kingdom) on both hands in a standardized manner, scored in Kg. The average of 3 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 analyzed in the dominant hand and corrected for age and gender according to the manufacturer's information (normalized grip strength).

Muscle imaging

All patients were examined in a 1.5T MR system (1.5T Achieva DStream; Philips Healthcare, Best, The Netherlands) at HSCSP, as previously described (31). We used the same positioning protocol for all patients: a supine position with

the legs stretched out. Images were analyzed by a single expert (A.A.) who was blinded to study groups.

Axial 2D Dixon FFE was performed on the pelvis and thighs by using a 32-elements body coil with the following parameters: TR/TE = 5.78/1.8, 4 ms, flip angle = 15°, field of view (FOV) = 520 x 340 x 300 mm, voxel size = 1 x 1 x 3 mm for thighs, and FOV = 520 x 320 x 200 mm, and voxel size = 1.3 x 1.7 x 5 mm for pelvis. This Dixon sequence follows a 7-peak fat modeling and provides separate images for fat and water. Total acquisition time was 45 minutes per patient.

The Dixon MR images obtained were analyzed using a PRIDE tool developed by Philips (Philips Research Image Development Environment). Regions of Interest (ROIs) were manually drawn on 2 compartments of the thighs. The anterior compartment encompassed the *rectus femoris*, *vastus intermedius*, *vastus lateralis*, and *vastus medialis* muscles, while the posterior compartment encompassed the *adductor magnus*, *semitendinosus, semimembranosus*, and the long and short head of the *biceps femoris*. For every ROI, the total area and area covered by fat were calculated automatically using the PRIDE tool. The FF coefficient was defined as fat/(fat+water), where fat and water were the image intensity values over the ROI for the fat and water Dixon images, respectively. From those 2 parameters, and assuming that water content corresponds mainly to muscle, fat and muscle area were estimated. Cumulative values across all slices were also computed and, as a final index, muscle FF was calculated as follows: FF = (muscle fat area x 100)/muscle area. Once we obtained the values from every muscle, we calculated the thigh FF as follows: thigh FF = (sum of fat area of all thigh muscles x 100)/sum of muscle area of all thigh muscles).

Statistical analysis

Quantitative outcomes are presented as mean ± SD (Gaussian distribution) or median (interquartile range, non-Gaussian distribution), and qualitative outcomes are expressed as percentages. Data distribution was analyzed using the Kolmogorov–Smirnov test. Comparisons between 2 groups were performed using the independent samples t-test or the Mann–Whitney U test, depending on the distribution, and between 3 groups using ANOVA followed by the Bonferroni test as a post hoc test or a Kruskal–Wallis H test, depending on data distribution.

Correlations were assessed using the Pearson's correlation coefficient or Spearman rank order depending on whether the data were normally distributed. Stepwise regression modeling was performed to evaluate factors potentially influencing physical performance, including age, BMI, menopausal status, fatty infiltration, and muscle volume.

Analyses were performed using SPSS 21.0 statistical package for Window (SPSS Inc, Chicago, Illinois). Tests were two-tailed, and a $P < 0.05$ was considered significant.

Results

General characteristics, muscle fatty infiltration, and muscle volume

General characteristics of subjects included are shown in Table 1. Cushing's syndrome patients showed greater muscle FF in both the anterior and posterior compartment of the thigh as compared with 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) (Fig. 1 and 2). Cushing's syndrome patients also had greater muscle FF in the combined anterior and posterior compartments as compared with controls (20.6 ± 4.8% vs. 17.9 ± 4.7%, $P = 0.027$) (Fig. 1). Patients with CS had similar lean muscle volume as compared with controls (13198 ± 1879 cm^3 vs. 13589 ± 1901cm (3); $P = 0.24$). When only premenopausal women were analyzed, FF in the anterior compartment was still greater in CS patients as compared with controls (16 ± 4 vs. 13 ± 2.3; $P = 0.027$) (Table 2). When only postmenopausal women were analyzed, FF in the posterior compartment was still significantly greater in CS patients as compared with controls (24.9 ± 5.4 vs. 21.2 ± 4.4; $P = 0.022$) (Table 3).

We did not find any associations between duration of hypercortisolism and muscle fatty infiltration or muscle volume (P = n.s.). After excluding both patients with growth hormone (GH) deficiency and the 2 who were on hydrocortisone replacement, significant greater FF in all compartments persisted.

Muscle performance and strength

Results of muscle performance testing in CS patients and healthy controls are shown in Table 1 and Fig. 2. Time needed to complete the TUG test was longer in CS patients as compared with controls (6.7 ± 1.6 vs. 5.9 ± 1.5 s; $P = 0.023$), suggesting an impaired static and dynamic balance. Performance in this test was still significantly poorer in CS patients as compared with controls when including only postmenopausal women (7.2 ± 1.8 vs. 6.3 ± 1.6; $P = 0.033$) (Table 3)

The performance on the 30-second chair stand test was more impaired in CS patients as compared with controls, as indicated by the lower average number of times the former stood from a chair during 30 seconds (14.6 ± 4.1 vs. 17.6 ± 5; $P = 0.011$). This finding suggests impaired strength in lower limbs of CS patients. Performance on this test remained significantly more impaired in either premenopausal or postmenopausal CS women as compared with their counterpart in the control group (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) (Tables 2 and 3).

Patients with CS showed a slower velocity in their usual pace in comparison to controls (1.1 ± 0.2s/m vs. 1.2 ± 0.2s/m; $P = 0.034$) on GS test, suggesting lower functional capacity. We did not find any associations between duration of hypercortisolism and muscle performance or strength (P = n.s.). After excluding both

Table 1. Baseline characteristics and physical performance in 36 patients with Cushing's syndrome (CS) and 36 age- and BMI-matched controls

	CS	Controls	*P*-value
Age (years)	51(15)	50 (20)	0.715
BMI (Kg/m^2)	26.6 ± 3.7	26 ± 4.39	0.575
Time of remission (years)	13 (8)	–	–
Pituitary adenoma	28 (78%)	–	–
Adrenal adenoma	8 (22%)	–	–
Hydrocortisone substitution	3 (8%)	–	–
Menopause	21 (58%)	21 (58%)	–
Duration of menopause (months)	96 ± 61	102 ± 62	0.835
GH substitution	2 (5%)	–	–
CK (U/L)	91 (56)	100.4 (82)	0.414
Gait speed (m/s)	1.1 ± 0.2	1.2 ± 0.2	**0.034**
Time up and go (TUG) (s)	6.7 ± 1.6	5.9 ± 1.6	**0.023**
30-second chair stand (number of times)	14.7 ± 4.1	17.7 ± 5	**0.008**
Hand grip strength in dominant hand (Kg)	21.3 (3.6)	21.7 (6.2)	0.452

Normal range for CK was 29-168 U/L. Values are expressed as mean ± SD or median (interquartile range) depending on the distribution.
Abbreviations: BMI, body mass index; CK, creatinkinase; GH, growth hormone; MRI, magnetic resonance imaging.

patients with GH deficiency and the 2 who were on hydrocortisone replacement, physical performance was still poorer in patients on all tests.

Association between FF and muscle function

Correlations between FF and muscle performance testing in CS patients are shown in Table 4. In patients, greater mean FF in the anterior compartment was negatively associated with performance on TUG (r = 0.504, *P* = 0.003), 30-second chair stand (r = -0.425, *P* = 0.014), and GS (r = -0.373, *P* = 0.033). In a multiple linear regression model, FF in the anterior compartment predicted the performance on TUG (ß 0.504, *P* = 0.003) after adjusting for age, BMI, menopause, and muscle volume in CS patients.

Greater mean FF in the posterior compartment was negatively associated with performance on TUG (r = 0.653, *P* < 0.001), 30-second chair stand (r = -0.531, *P* = 0.001), and GS (r = -0.484, *P* = 0.004) in CS patients. In a multiple regression model, FF in the posterior compartment predicted TUG (ß 0.635, *P* < 0.001) and GS (ß -0.484, *P* = 0.004) after adjusting for age, BMI, menopause, and muscle volume in patients.

Greater mean FF in the combined anterior and posterior compartments was negatively associated with performance on TUG (r = 0.629, *P* < 0.001), 30-second chair stand (r = -0.524, *P* < 0.002), and GS (r = -0.461, *P* = 0.007) in CS patients. In a multiple linear regression model, FF(%) in the combined anterior and posterior compartments predicted performance on TUG (ß 0.626, *P* < 0.0001) and GS (ß -0.461, *P* = 0.007) after adjusting for age, BMI, menopause, and muscle volume in patients. After excluding both patients with GH deficiency and the 2 who were on hydrocortisone replacement, all the significant associations persisted.

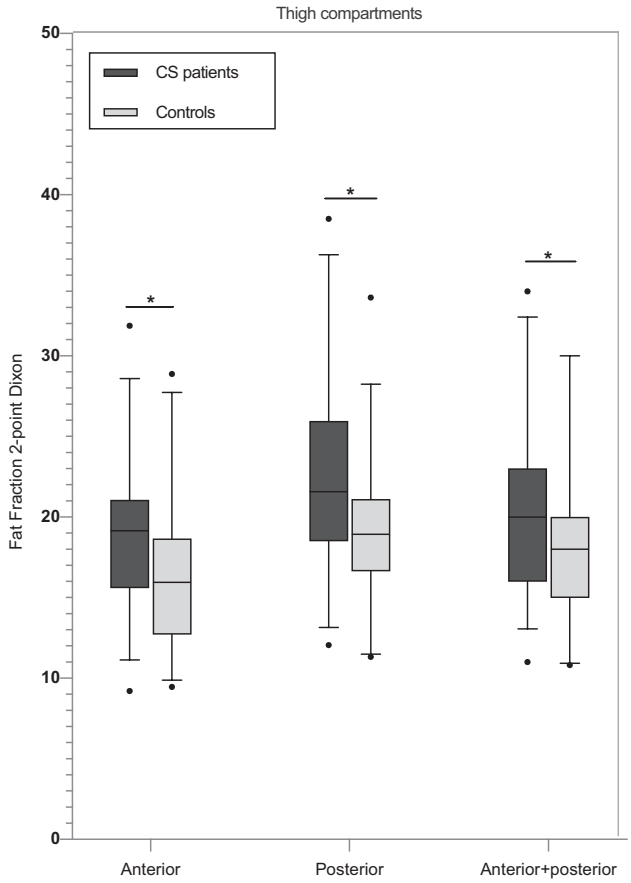

Figure 1. Comparison of fatty infiltration FF in thigh compartments between groups. Abbreviations: Abterior+Posterior, combined anterior and posterior compartments; CS, Cushing's syndrome patients. *A paired t-test was used according to statistical distribution of groups. *P* < .05.

In controls, FF in the anterior compartment was negatively associated with poor performance on the 30-second chair stand (r = -0.598, *P* < 0.001). Increased FF in the posterior compartment was negatively associated with poor performance on the 30-second

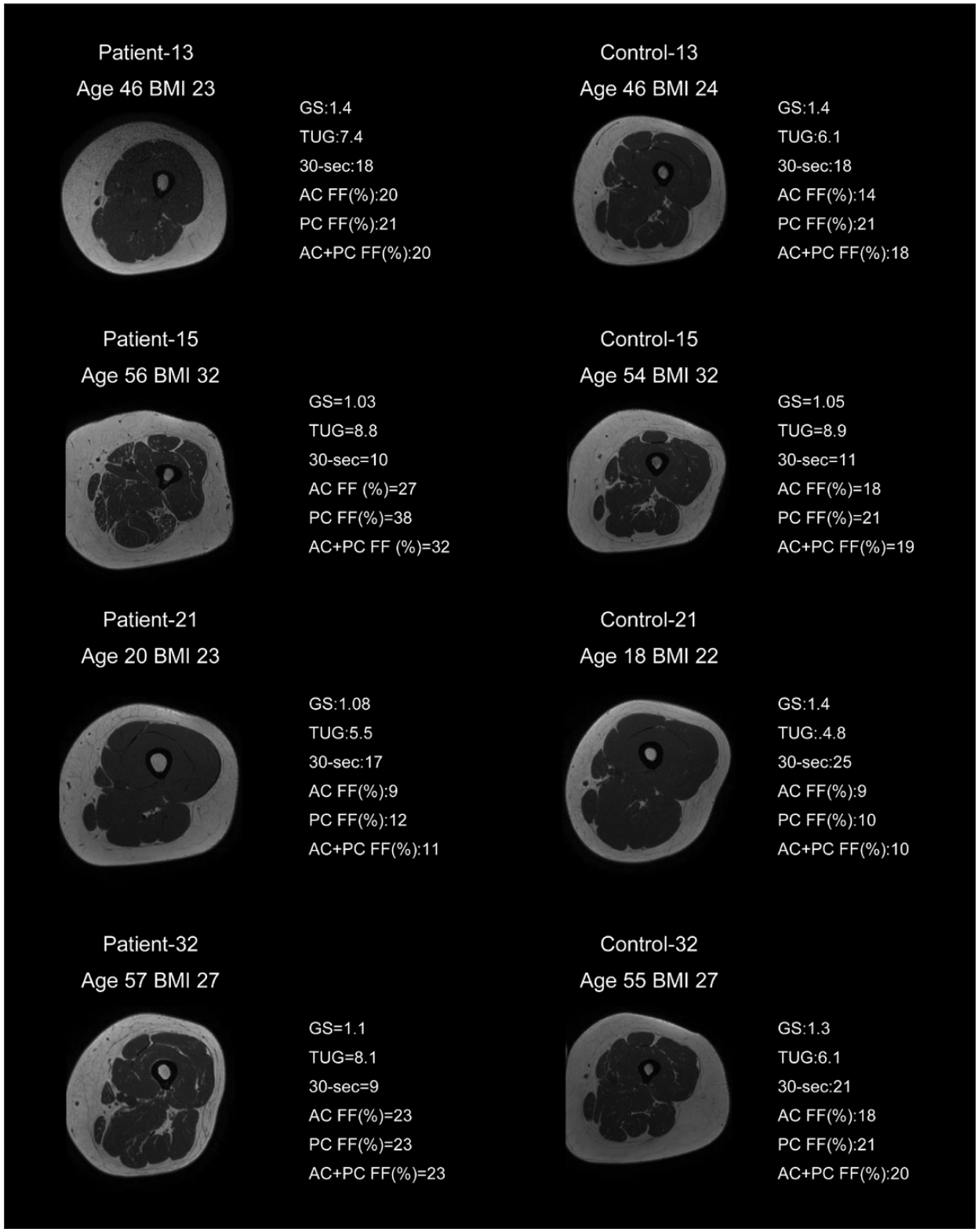

Figure 2. Fatty infiltration (FF) and functional tests in Cushing's syndrome patients vs their age- and BMI matched controls. Abbreviations: AC FF, anterior compartment fat fraction; AC+PC FF, combined anterior and posterior compartments fat fraction; BMI, body mass index; FF, fat fraction; GS, gait speed; PC FF, posterior compartment fat fraction; TUG, timed up and go; 30-sec, 30-second chair-stand.

chair stand (r = -0.562, P = 0.001). Increased FF in the combined anterior and posterior compartments was negatively associated with poor performance on the 30-second chair stand (r = -0.619, P < 0.001). In controls, no predictors of performance on the 30-second chair stand were identified in a multiple regression model, including BMI, age, menopause, muscle volume, and FF.

Table 2. Fat fraction (FF) in thigh compartments, performance and muscle strength parameters in 15 premenopausal patients with Cushing's syndrome (CS) and 15 controls

	Cushing's syndrome	Controls	*P*-value
Gait speed (m/s)	1.2 ± 0.2	1.3 ± 0.1	0.23
Time up and go (s)	5.9 ± 1	5 ± 1	0.065
30-Second chair stand (number of times)	17.2 ± 4	21.3 ± 4	**0.021**
nHGS (S.D.)	−1.7 ± 0.8	−1.3 ± 0.7	0.167
FF anterior compartment (%)	16 ± 4	13 ± 2.3	**0.027**
FF posterior compartment (%)	18.3 ± 3.9	16.2 ± 3.4	0.165
FF combined anterior and posterior compartments (%)	17 ± 3.7	14.6 ± 2.6	0.06
MRI lean mass volume (cm^3)	14 703 ± 1767	14 721 ± 1535	0.977

Abbreviations: FF, fat fraction; MRI, magnetic resonance imaging; nHGS, normalized hand grip strength.

Table 3. Fat fraction (FF) in thigh compartments, performance and muscle strength parameters in 21 postmenopausal patients with Cushing's syndrome (CS) and 21 age- and BMI-matched controls

	CS	Controls	*P*-value
Gait speed (m/s)	1 ± 0.1	1.2 ± 0.2	0.111
Time up and go (s)	7.2 ± 1.8	6.3 ± 1.6	**0.033**
30-second chair stand (number of times)	13.2 ± 3.4	15.4 ± 3.9	**0.033**
nHGS (S.D.)	−0.8 ± 0.7	−0.9 ± 0.7	0.820
FF anterior compartment (%)	20.5 ± 4.4	18.4 ± 4.8	0.147
FF posterior compartment (%)	24.9 ± 5.4	21.2 ± 4.5	**0.022**
FF combined anterior and posterior compartments (%)	22.7 ± 4.5	20.2 ± 4.4	0.078
MRI lean mass volume (cm^3)	12308 ± 27	13037 ± 1643	0.114

Abbreviations: FF, fat fraction; MRI, magnetic resonance imaging; nHGS, normalized hand grip strength.

Table 4. Correlation between the fat fraction (FF) in the anterior, posterior, and combined anterior and posterior compartments of the thigh measured by 2-point Dixon vs. gait speed, time up and go, and 30-second chair stand in patients

	Gait Speed (m/s)		Time Up and Go (s)		30-Second Chair Stand (Number of Times)	
FF (%)	**R**	***P*-value**	**R**	***P*-value**	**R**	***P*-value**
Anterior compartment	−0.373	**0.033**	0.504	**0.003**	−0.425	**0.014**
Posterior compartment	−0.484	**0.004**	0.653	**<0.001**	−0.531	**0.001**
Combined anterior and posterior compartments	−0.461	**0.007**	0.629	**<0.001**	−0.524	**0.002**

Abbreviation: FF, fat fraction.

Discussion

We have shown that female patients with CS in long-term remission have increased intramuscular fatty infiltration in both the anterior and posterior compartments of the thigh, and this is associated with impaired performance on functional tests which assess individual functional capacity, balance, and strength (32–35). 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, and menopausal status (36).

"Cured" CS patients frequently complain of fatigability at follow-up visits. Indeed, Berr et al showed that the performance on the chair rising test, which was more impaired in active CS as compared with controls, did not improve 6 months after successful treatment of hypercortisolism (3). 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 (3).

Muscle weakness, mostly involving the lower limbs, is one of the commonest complications of chronic exposure to cortisol excess in CS (2). Steroid-induced myopathy, which is characterized by the preferential atrophy of type IIa, fast-twitch muscle fibers is partly related to the well-known antianabolic, proteolytic, and proapoptotic effects of glucocorticoids, resulting in the breakdown of the contractile proteins and suppression of myofibrillar protein synthesis (1, 37). 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 a decrease in circulating muscle protein levels (38). 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 (15, 16) 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 (3). 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 1 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 (39–41). Burt el al documented the restoration of protein synthesis 13 months after successful treatment of CS along with a significant increase in percentage lean body mass, suggesting that changes in muscle mass may, at least in part, be reversible following hormone normalization (42). Future pathologic studies evaluating muscle composition after long-term remission are needed to confirm these findings. Our results support the hypothesis that the sustained alteration of physical performance in "cured" CS may be associated with the deterioration of muscle quality due to intramuscular fat accumulation rather than to decreased muscle mass (43).

This is consistent with previous observations in human aging (4, 5, 44). In a large cohort of elderly people followed-up over 3 years, a progressive decline of muscle strength was reported, which was only minimally paralleled by concomitant loss of muscle mass (44). 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 (45). 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 (46). An inverse association between the degree of skeletal muscle fatty infiltration and physical performance has also been described in patients with neuromuscular disorders (6, 7, 31, 47–50). 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 (51). In vitro studies showed that intramuscular secretion of proinflammatory adipokines blunts the expression of contractile proteins in myotubes, impairs myoblast differentiation, and promotes myofiber atrophy (12, 13, 51). Moreover, fatty infiltration of muscle is associated with insulin resistance, which may determine glycolytic disturbances and reduced cellular uptake of glucose, ultimately leading to a reduced ATP pool and impaired myofibril contractile function (52, 53). Fatty infiltration may also induce direct mechanical alteration in the muscle. Yoshida et al demonstrated that the presence of noncontractile 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 (54).

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 (16, 40). Persistent elevation of both DXA-measured total fat and trunk fat was documented in CS patients after long-term remission as compared with controls (40). Noteworthy, Geer et al found that the intermuscular adipose tissue, as measured by whole-body semiquantitative 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 (16). We used the Dixon technique to quantify, for the first time in CS, the intramuscular fat, which is the adipose tissue located inside the muscle and surrounding the muscle fibers. We postulated that this fat depot may be persistently elevated in women previously exposed to overt hypercortisolism, possibly perpetuating functional impairment after a "cure" (7, 21–24, 55).

However, the cross-sectional design of our study prevents from inferring causality based on our data. Further studies are needed to increase our understanding of the pathogenic mechanisms linking muscle function and intramuscular fat.

Pathways whereby glucocorticoids regulate fat metabolism and mediate specific fat accumulation in muscles are complex and not completely understood. Glucocorticoids are known to enhance both the conversion of preadipocytes to adipocytes and

the differentiation of the bone marrow mesenchymal stem cells towards the adipocyte lineage (56). While dexamethasone administration to mice enhanced adiposity and insulin resistance in the 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 (28, 57). The 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 the 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 (58). 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 (59). In agreement with this, when we analyzed patients and controls separately according to the menopausal status, 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 (25, 60).

The small sample size is a limitation of the study as is 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 (2). Also, we have only included patients in long-term remission. Future studies are needed to prospectively evaluate structural and functional changes in the muscle of CS patients before and after surgical correction of cortisol excess.

A strength of this study is the low prevalence of hypopituitarism in our patients. 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. Although we have only included female patients in order to analyze a more homogeneous sample, intergender differences in muscle structure and function may exist and, therefore, future studies should also evaluate these parameters in men (61).

In conclusion, we have demonstrated that 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. We have also demonstrated an independent association between fatty infiltration and impairment of performance on several function tests in "cured" CS patients. Our results suggest that clinicians should be aware of this sustained deterioration of muscle health and specifically address this problem during follow-up. Future studies are needed to establish the most effective therapeutic strategies to improve muscle weakness in these patients.

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