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Circulating Gonadal and Adrenal Steroids in Amyotrophic Lateral Sclerosis: Possible Markers of Susceptibility and Outcome

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Key words
- gonadal steroids
- adrenal steroids
- motor neuron disease
- amyotrophic lateral sclerosis
- respiratory function
- hormones

Abstract

Although changes of circulating steroids have been reported in patients with sporadic amyotrophic lateral sclerosis (ALS), a full comparison of the adrenal and gonadal steroid profile between control subjects and ALS patients is lacking. Considering that respiratory failure is the most frequent cause of death in ALS, we looked into whether a relationship emerged between circulating steroids and respiratory parameters. Serum levels of adrenal and gonadal steroids were measured in 52 age- and gender-matched subjects (28 ALS and 24 controls) using radioimmunoassay techniques. We also evaluated respiratory parameters in ALS patients, including forced vital capacity (FVC), maximal inspiratory pressure (MIP), and maximal expiratory pressure (MEP). We found increased levels of testosterone in female ALS patients compared to healthy female subjects. Furthermore, control subjects showed a significant decline of testosterone, dehydroepiandrosterone and its sulfate, and a borderline decline of progesterone with increasing age. Instead, testosterone did not decline with increasing age in ALS patients. We also found that the dehydroepiandrosterone sulfate/cortisol ratio was positively associated with FVC, MIP, and MEP. Moreover, ALS patients showing higher testosterone levels and lower progesterone/free testosterone ratio presented a more rapid worsening of the monthly FVC. In conclusion, first our study revealed a differential steroid profile with age and gender in ALS patients relative to controls. Second, we demonstrated an association between some steroids and their ratios with respiratory function and disease progression. Thus, we hypothesize that the endogenous steroid profile could be a marker of susceptibility and prognosis in ALS patients.

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Abbreviations

ALS	Amyotrophic lateral sclerosis
CNS	Central nervous system
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
FT	Free testosterone
FVC	Forced vital capacity
HPA	Hypothalamic-pituitary-adrenal axis
MEP	Maximal expiratory pressure
MIP	Maximal inspiratory pressure
SHBG	Sex hormone-binding globulin
SOD1	Superoxide dismutase 1
TT	Total testosterone

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive, lethal, degenerative disorder of motor neurons. The hallmark of this disease is the selective death of motor neurons in the brain and spinal cord, leading to paralysis of voluntary muscles [1]. Respiratory impairment due to weakness of respiratory muscles is a major cause of morbidity and mortality in these patients [2]. Previous findings demonstrate that respiratory function is directly related to survival time [3].

Epidemiological studies have shown male predominance in ALS, suggesting the participation of hormonal factors in the development of the disease [4]. In this regard, changes of the hypothalamic-pituitary-adrenal (HPA) axis have been observed in ALS patients. Just as Patacchioli first reported a loss of the cortisol circadian rhythm [5], we have recently found increased serum cortisol levels in these patients suggesting HPA axis dysfunction [6]. Furthermore, in our results progesterone serum levels correlated positively with survival time, measured from the time of symptom onset [6]. In contrast, no correlation could be demonstrated between those factors and cortisol

levels. On the other hand, higher cortisol, lower dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) serum values with the consequent higher cortisol/DHEAS ratio have been found in chronic stress and neurodegenerative diseases other than ALS [7–9].

Changes in serum concentration of androgens and estrogens also occur in ALS and its animal models. Free testosterone levels were decreased in a group of ALS patients [10], whereas the finding of a low index-to-ring finger length ratio (2D:4D ratio) in ALS suggested that prenatal testosterone exposure might play a role in motor neuronal vulnerability in adulthood [11, 12]. In the transgenic SOD1 model of familial ALS, ovariectomy leads to a significant acceleration of the disease, whereas estradiol treatment significantly delays disease progression [13].

The search for biomarkers useful for monitoring disease progression and assessing treatment effectiveness is particularly imperative in ALS [14–16]. Changes in circulating steroids throughout the course of ALS might influence the neuroprotective response against degenerative damage as well as respiratory function. The possibility that circulating steroids bear a relationship with respiratory parameters becomes plausible because steroids freely cross the blood brain barrier and interact with CNS areas involved in respiratory control [17, 18]. In this respect, the location of sex steroid receptors in respiratory motor neurons suggests the influence of these hormones in breathing [19]. Progesterone stimulates resting minute ventilation by acting on central respiratory regions such as the nucleus of the solitary tract, medulla oblongata, and hypothalamic nuclei structures [20–22]. Moreover, progesterone receptor immunoreactivity was detected in cervical spinal cord motor neurons, where the phrenic motor nucleus is located, from control subjects, ALS patients, and murine models of spinal cord injury [23, 24].

In the present work, ALS and control subjects were studied aiming to determine adrenal/gonadal steroid serum levels and the possible association of these measures with some respiratory parameters.

Materials and Methods

ALS patients and controls

ALS was diagnosed on the basis of the revised El Escorial criteria [25]. Only patients without a family history of ALS were selected. A total of 28 Caucasian patients with definite or probable ALS were serially recruited within the present study and followed over a period of 8 months. ALS patients with noninvasive ventilation support, gastrostomy or wheelchair-dependency were excluded from this study. Twenty-four healthy controls matched by age and gender were also recruited (**Table 1**). For the purposes of this study, all selected female patients and controls were postmenopausal and not receiving hormonal replacement therapy. Patients and controls under medications able to modify steroid metabolism or afflicted by inflammatory, endocrine or psychiatric disorders were excluded. The proportion of patients receiving riluzole was the same (82%) for both males and females. Patients were regularly followed in the outpatient clinic at 3-month intervals. Neurological functional assessment of the ALS group was recorded by employing the ALS Functional Rating Scale-Revised (ALSFRS-R) score [26]. We collected 3 data points for each patient: 1) the time of blood sampling (FVC_0 and ALSFRS-R); 2) 3 months before (FVC_1), and 3) 3 months after (FVC_2) blood extraction. At blood sample collection, the mean ± SEM of the ALSFRS-R value of selected ALS patients was 38 ± 1.2, and the FVC% was 65.5 ± 5.3 (**Table 1**). The study was approved by the Ethics Committee of the Ramos Mejia Hospital. All subjects gave written consent to vein-puncture and were informed about the aim of the study.

Table 1 Clinical background of ALS patients and control subjects.

	Control	ALS
Mean age (years)	53 ± 2.1	52 ± 2.4
Men/women	14/10	17/11
Bulbar/spinal		6/22
Symptom duration (months)		18 ± 2.7
Disease duration (months)		12 ± 3.3
FVC%*		65.5 ± 5.3
ALSFRS-R (points)*		38 ± 1.2

Data were expressed as mean ± SEM. FVC: Forced vital capacity. ALSFRS-R: ALS Functional Rating Scale-Revised

*Corresponds to values obtained at the time of blood sample collection

Steroid measurements

Serum steroid levels in ALS and controls were analyzed according to age and gender. Serum samples were obtained after blood collection by vein-puncture, immediately centrifuged, frozen in small aliquots and stored at −80 °C. All samples were withdrawn between 10:00 AM–12:00 PM, because of the circadian rhythm variations of their concentrations [27]. We used a radioimmunometric assay (Coat-A-Count Kit, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) for the quantitative determination of serum levels of progesterone (unit: ng/ml; detection limit: 0.1 ng/ml; coefficient of variation intra-assay < 8.8 and coefficient of variation inter-assay < 9.7), cortisol (unit: µg/dl; detection limit: 1 µg/dl; coefficient of variation intra-assay < 5.1 and coefficient of variation inter-assay < 6.4), estradiol (unit: pg/ml; detection limit: 20 pg/ml; coefficient of variation intra-assay < 7 and coefficient of variation inter-assay < 8.1), total testosterone (TT) (unit: ng/dl; detection limit: 4 ng/dl; coefficient of variation intra-assay < 8 and coefficient of variation inter-assay < 7.3), and DHEAS (unit: ng/ml; detection limit: 50 ng/ml; coefficient of variation intra-assay < 5.3 and coefficient of variation inter-assay < 11). For the quantitative measurement of DHEA, we used a radioimmunoassay kit (Immunotech, Prague, Czech Republic; unit: ng/ml; detection limit: 0.009 ng/ml; coefficient of variation intra-assay < 3.8 and coefficient of variation inter-assay < 8.6). As TT concentrations are affected by alterations in SHBG [28], we determined both forms of circulating testosterone: TT and free testosterone (FT). FT values were obtained by calculation from TT, albumin, and SHBG concentrations as validated by a previous report [29]. The SHBG determinations were assayed by using an immunoradiometric assay kit (IRMA-Count Siemens Healthcare Diagnostics, Los Angeles, CA, USA; unit: nmol/l; detection limit: 0.04 nmol/l; coefficient of variation intra-assay < 5.3 and coefficient of variation inter-assay < 8.5). Serum DHEAS/cortisol and progesterone/FT ratios were calculated in all subjects. The DHEAS/cortisol ratio corresponds to a parameter modified by age and age-related disorders, while the ratio of progesterone/FT is influenced by age and also by gender [30].

Evaluation of respiratory status

The state of respiratory function was evaluated in each patient at the 3 data points expressed above: FVC_1%, FVC_0%, and FVC_2%.

Table 2 Steroid serum levels in controls and ALS patients of both genders.

	Control		ALS		Control vs. ALS p-value	
	Male	Female	Male	Female	Male (p)	Female (p)
Total testosterone (ng/dl)	38.98 ± 3.7	1.42 ± 0.2	48.26 ± 7.1	3.57 ± 0.57	NS	**<0.01****
Free testosterone (ng/dl)	6.29 ± 0.7	0.13 ± 0.02	7.06 ± 0.9	0.43 ± 0.08	NS	**<0.01****
DHEA (ng/ml)	12.1 ± 1.6	9.04 ± 2.06	13.8 ± 1.8	7.86 ± 1.3	NS	NS
DHEAS (ng/ml)	1194 ± 179	430 ± 92	1281 ± 196	520 ± 95	NS	NS
Estradiol (pg/ml)	13 ± 2.2	2.1 ± 0.5	16.3 ± 2.2	4.41 ± 1.2	NS	NS
Cortisol (µg/dl)	11.9 ± 1.97	11 ± 2.3	17.44 ± 2.5	16.17 ± 2	NS	NS
Progesterone (ng/ml)	0.47 ± 0.04	0.26 ± 0.07	0.68 ± 0.08	0.37 ± 0.06	**0.05**	NS

Steroid hormones were tested in controls and ALS patients according to gender. For statistical analysis, we used two-tailed Mann-Whitney test. NS: Nonsignificant. p-Values lower than 0.05 were considered significant

Both total testosterone and free testosterone were increased in females ALS patients in comparison to controls (**$p < 0.01$). A trend toward significantly higher progesterone levels were found in male ALS patients than male controls ($p = 0.05$). Results were expressed as mean ± SEM. ALS: Amyotrophic Lateral Sclerosis; DHEA: Dehydroepiandrosterone; DHEAS: Dehydroepiandrosterone sulfate

Respiratory muscle function was assessed by measurement of the predicted FVC% and the maximum pressures measured at the mouth: the obtained parameters were a percentage of the predicted MIP and MEP. All the respiratory function tests were performed in a sitting position. FVC was measured employing a dry wedge spirometer (Vitalograph ALPHA, Ennis, Ireland). MIP and MEP values were achieved using a respiratory pressure meter (MicroRPM, CareFusion, San Diego, CA, USA). FVC, MIP, and MEP were determined with a pressure transducer, flanged mouthpiece and nose clip with attention being paid to obtaining a good lip seal. All patients had to repeat each maneuver 3 times, with the best result being recorded and employed for analysis. FVC% was expressed as the deficit with respect to the normal minimal predicted value for matched age, height, smoking status, and gender. The American Thoracic Society predictive spirometry values were used as the normal standards [31,32]. Outcome measures included the rate of variation of FVC%, 3 months before (FVC_1) and 3 months after (FVC_2) blood sample collection [33]. Monthly decline of FVC% was calculated as follows: FVC_2–FVC_1/time in months between FVC_1 and FVC_2. Previous reports [34] have defined a ≥ 30% annual decline of FVC as a rapid worsening of this parameter. Hence, we considered patients with a ≥ 2.5% monthly decline of FVC as showing a rapid progression. Patient groups with FVC% < and ≥ 2.5% had a similar functional status with a mean ± SEM ALSFRS-R value of 38 ± 1.6 and 35.6 ± 3.4, respectively (p = NS).

Statistical analysis

For continuous variables, Wilcoxon rank-sum and Mann-Whitney nonparametric tests were used for comparison of means in univariate analysis. Spearman Rho correlation analysis was employed to compare steroid levels with increasing age and respiratory parameters. In the figures, the linear regression line, the Spearman correlation coefficient (R_{Rho}) and the respective Rho correlation p-values are given. p-Values lower than 0.05 were considered significant and results were expressed as mean ± SEM. Analyses were carried out using R-project statistical pack [35].

Results

Univariate analysis of gonadal and adrenal steroids between ALS patients and controls

In our cohort, we first analyzed endogenous steroid levels in ALS patients and healthy controls according to gender. When comparing serum estradiol, DHEA, DHEAS, and cortisol, no differences were found between female or male patients and their respective controls (**Table 2**). However, levels of TT and FT were significantly higher in female ALS patients compared to female controls ($p < 0.01$, **Table 2**). We also found a borderline increased level of progesterone in male ALS patients compared to their controls ($p = 0.05$). No differences were observed in SHBG serum levels for both ALS female and male patients and their respective healthy controls.

Binary correlation between steroid levels and age in ALS patients and controls

We further explored the relationship between steroid serum levels and age. Spearman correlation analysis revealed a negative R_{Rho} for all steroids, indicating a normal decline of their levels with age in control subjects. Controls showed a significant decline in TT, FT, DHEA, DHEAS, and borderline for progesterone (**Fig. 1a–e**, $R_{Rho}: -0.5$, $p < 0.05$; $R_{Rho}: -0.5$, $p < 0.05$; $R_{Rho}: -0.55$, $p < 0.01$; $R_{Rho}: -0.61$, $p < 0.01$, and $R_{Rho}: -0.48$, $p = 0.07$, respectively). ALS patients also showed a significant age-associated decline for some but not all steroids (**Fig. 1**). For instance, progesterone, DHEAS and, less noticeably, DHEA declined with age ($R_{Rho}: -0.47$, $p < 0.05$; $R_{Rho}: -0.49$, $p < 0.05$, and $R_{Rho}: -0.38$, $p = 0.07$; **Fig. 1c–e**). In contrast, TT and FT levels did not significantly decline with increasing age in ALS patients as they did in controls. Moreover, neither cortisol nor estradiol concentrations showed an age-dependent decrease.

Respiratory functional parameters and steroid profile in ALS patients

To establish whether a relationship exists between the steroid profile and the respiratory parameters in ALS patients, we performed a correlation analysis between FVC%, MIP%, and MEP% at the time of blood collection and the concentration of all measured steroids and their ratios. As a result, we found a significant positive correlation between DHEAS levels ($R_{Rho} = 0.40$, $p < 0.05$) or DHEAS/cortisol ratio and FVC% (**Fig. 2a**: $R_{Rho} = 0.41$, $p < 0.05$). Moreover, the DHEAS/cortisol ratio, a useful value that decreases with age or stress conditions [30], was the only parameter demonstrating a positive correlation with MIP% (**Fig. 2b**: $R_{Rho} = 0.60$, $p < 0.05$) and MEP% (**Fig. 2c**: $R_{Rho} = 0.53$, $p < 0.05$). Serum cortisol, estradiol, progesterone, and testosterone levels or the ratio of progesterone/FT, a value affected by age and gender, bared no relationship with functional respiratory parameters at sample collection time.

The relationship between steroids and the progression of respiratory impairment was also evaluated. Serum FT and estradiol

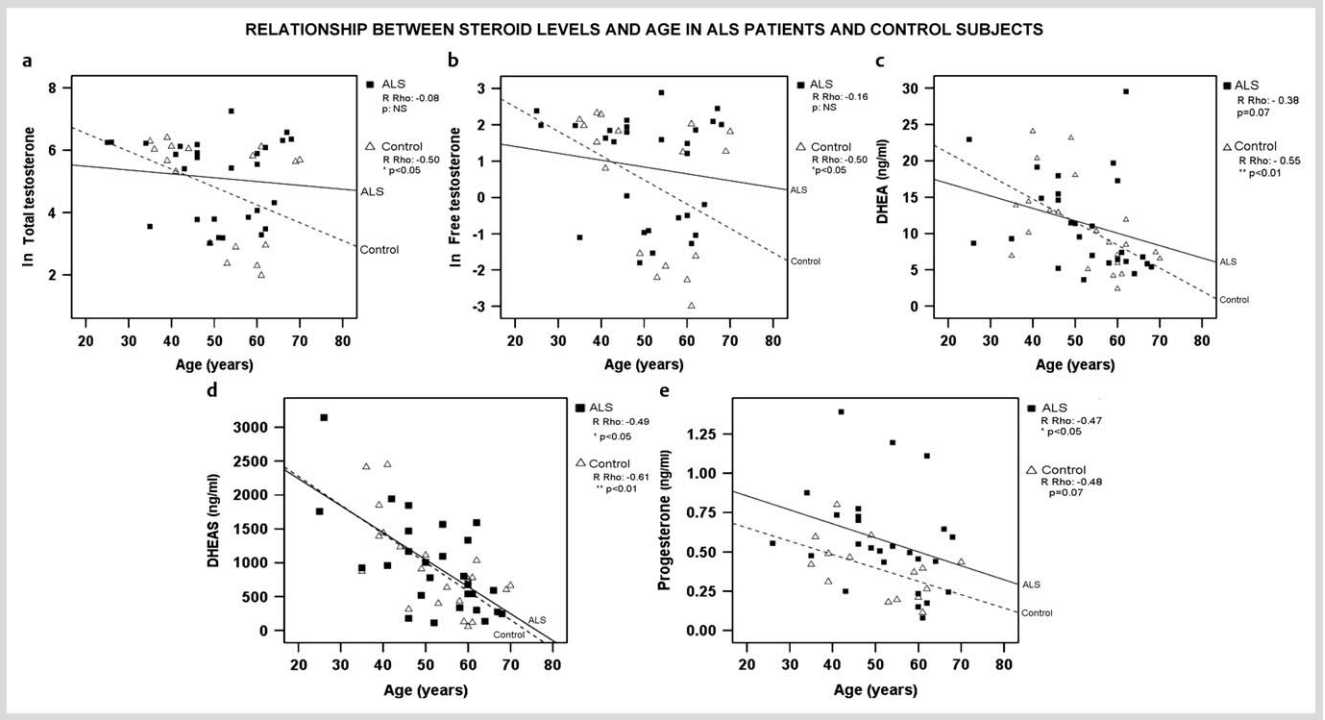

Fig. 1 Relationship between steroid serum levels and age (years) in ALS patients and control subjects. **a** Total testosterone (ng/dl), **b** Free testosterone (ng/dl), **c** Dehydroepiandrosterone (DHEA) (ng/ml), **d** Dehydroepiandrosterone sulfate (DHEAS) (ng/ml), and **e** Progesterone (ng/ml). Total testosterone and free testosterone concentrations have been transformed using the logarithm function. Spearman correlation analysis was employed to analyze steroid levels with increasing age. Shown are the linear regression line as well as the Spearman rank correlation coefficient (R Rho) and its p-value.

showed a significant negative relationship with the rate of FVC% decline in our ALS cohort (**Fig. 3a**: $R_{Rho} = -0.48$, $p < 0.05$, r^2: 0.26, FVC% decline = −0.85 + (−0.29) FT; **Fig. 3b**: $R_{Rho} = -0.49$, $p < 0.05$, r^2: 0.31, FVC% decline = −0.27 + (−0.15) estradiol, respectively). These results suggest that higher circulating levels of FT or estradiol were associated to a greater loss of FVC% points per month (i.e., negative values of FVC decline). Likewise, we also demonstrated a positive association between the ratio of progesterone/FT and FVC% declination (**Fig. 3c**: $R_{Rho} = 0.64$, r^2: 0.42, FVC% declination = −0.43 + 1.33 progesterone/FT; $p < 0.01$. For instance, no fall of FVC (i.e., FVC decline with positive values or 0) correlated with increased values of progesterone/FT ratio. No significant relationship was found between the DHEAS/cortisol ratio or the other measured circulating steroids to the rate of FVC% decline. Considering that estradiol synthesis depends on testosterone production in men and postmenopausal women [36], we investigated the eventual association between these 2 parameters. Interestingly, estradiol circulating levels were positively correlated with testosterone serum levels both in controls (R_{Rho}: 0.69, $p < 0.01$) and patients (R_{Rho}: 0.65, $p < 0.0001$, r^2: 0.53, estradiol = 5.52 + 0.02 TT), showing that androgens may possibly convert into estrogens in our population of ALS patients.

Steroids in patients with rapid worsening of FVC

Considering the behavior of steroid levels and the ratios that showed significant correlations with the FVC% fall, we analyzed their values according to the rapid worsening of FVC (≥ 2.5% per month). As a result, levels of TT and FT were significantly higher in patients with a monthly FVC decline ≥ 2.5% than in the group with < 2.5% ($p < 0.05$ for both hormones, **Fig. 4a, b**). Contrarily, there was no difference in levels of progesterone, cortisol, DHEAS in patients with rapid or slow worsening of their FVC. From all the ratios analyzed, only the progesterone/FT ratio showed a significantly higher value in the slow progression group than in the rapid FVC decay group ($p < 0.01$, **Fig. 4c**).

Discussion

Our present study has demonstrated that steroids in ALS patients bear a differential profile in relation to age, gender and some parameters of respiratory function. These findings seem to suggest that certain steroids may convey a "protective" role while others might exert a negative influence on disease susceptibility and progression.

The presence of gender differences in serum steroid levels in ALS patients and controls was demonstrated by this investigation. Indeed, postmenopausal female patients showed significantly higher TT and FT serum levels compared to postmenopausal female controls. The results shown in this study demonstrated no significant differences in SHBG serum concentration between patients and control subjects. Therefore, the androgen build-up found in female ALS patients may not correspond to an altered production of SHBG. On the other hand, decreased aromatization of testosterone at extragonadal sites, (i.e., adipose tissue, osteoblasts, endothelium, aortic smooth muscle cells, and the brain) could determine the androgen elevation in ALS patients. For instance, the hippocampal expression of aromatase is lowered in Alzheimer's dementia and other neurodegenerative diseases [37,38]. However, estradiol serum concentration was similar in female ALS patients and female controls, suggesting that the mechanisms involved in the elevated TT or FT levels do

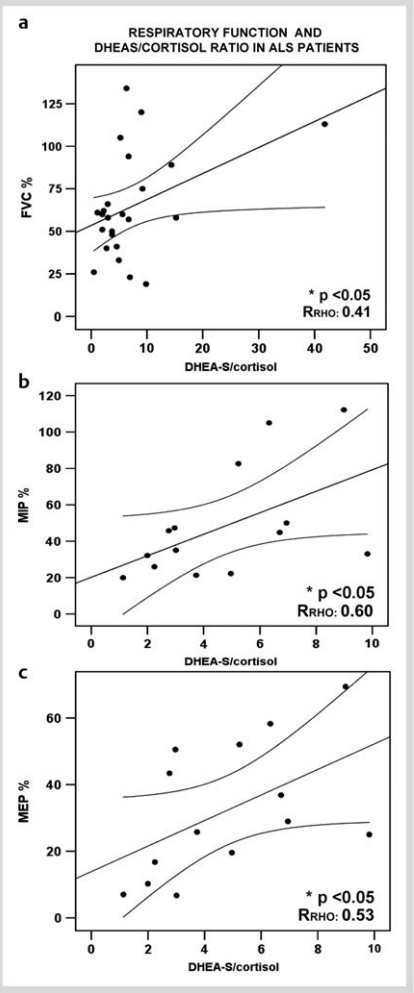

Fig. 2 Correlation between DHEAS/cortisol ratio and **a** FVC%, **b** MIP%, **c** MEP% in ALS patients. The linear regression lines as well as the Spearman's rank correlation coefficient(s) (R_{Rho}) and p-value are shown. From all the steroids analyzed, only significant correlations were drawn. Upper and lower lines represent the 95% confidence interval of the mean. FVC: Forced vital capacity, MIP: Maximal inspiratory pressure. MEP: Maximal expiratory pressure. DHEAS: Dehydroepiandrosterone sulfate. Note that from a total of 28 patients, 24 were tested with FVC%, 14 were tested with MIP and MEP%.

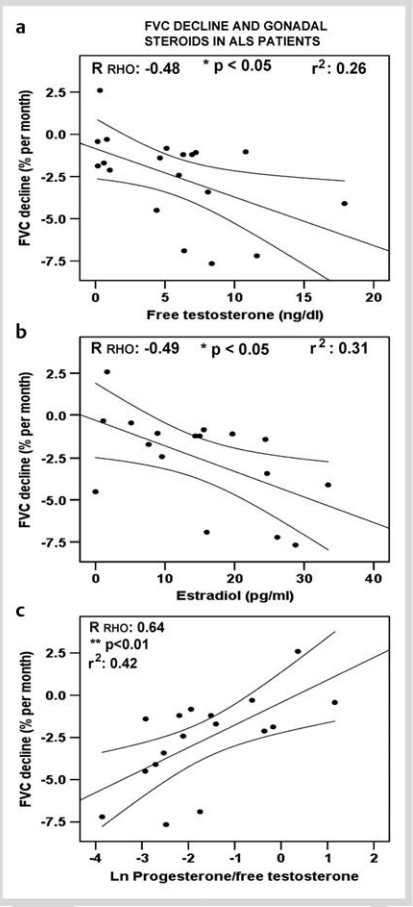

Fig. 3 Interrelationship between FVC% decline and **a** free testosterone serum concentration; **b** estradiol, and **c** progesterone/free testosterone ratio in ALS patients. Free testosterone and estradiol concentration units are given in ng/dl and pg/ml, respectively. FVC% decline was calculated as follows: the percentage that was lost every month using the first (FVC_1) and subsequent (FVC_2) measurements of these capacities and the time elapsed between them (FVC_2–FVC_1/time in months between FVC_1 and FVC_2). The linear regression lines as well as the Spearman's rank correlation coefficient (R_{Rho}) and p-value are shown. Upper and lower lines represent the 95% confidence interval of the mean. In the lower plot, the progesterone/testosterone ratio has been transformed using the logarithm function. FVC: Forced vital capacity.

not depend on the aromatization process. In fact, a positive relationship between testosterone and estradiol serum levels was found in ALS patients.

A different explanation involving high testosterone levels could be advanced by the androgen insensitivity hypothesis. Increased prenatal testosterone levels, as assessed by the presence of a low index-to-ring finger length ratio, have recently been proposed to be a risk factor for motor neuronal vulnerability [11]. High intrauterine testosterone levels might also reflect pre-existing androgen insensitivity. Indeed, testosterone is known to mediate motor neuron survival after binding to the classic androgen receptor [39]. A clear example of malfunction of the androgen receptor is the motor neuron disease called X-linked spinobulbar muscular atrophy (Kennedy's disease). Our finding of higher TT and FT levels in ALS patients might signal some form of androgen insensitivity. This could also explain why levels of TT and FT did not fall with age in ALS patients, whereas they did in controls.

In terms of respiratory function, we found that DHEAS serum levels and the ratio of DHEAS/cortisol were positively associated with FVC%, MIP% and MEP%. DHEA and its sulfate form, DHEAS, are neuroactive steroids, which downregulate the stress system. It has been suggested that the changes of the ratio of DHEAS and cortisol may reflect enhanced vulnerability to psychopathology. In particular, a significantly lower DHEAS/cortisol ratio has been associated with psychiatric disorders like depression [30] and age-related primary degenerative CNS disorders such as Alzheimer's dementia [40]. In humans, glial and neuronal cells of the CNS synthesize de novo or metabolize steroids like DHEA and DHEAS [18]. At this level, DHEAS functions as a negative modulator of the GABA(a) receptor. One study indicates that DHEAS suppresses the inhibition of respiratory frequency caused by GABA(a) receptor activation, hence posing a stimulatory effect [41]. Conversely, plasma DHEAS levels are reduced in patients with severe obstructive sleep apnea syndrome, whereas improvement of DHEAS concentration was observed following continuous positive airway treatment [42]. These results suggest that this steroid could work as a good biochemical sensor of respiratory function.

Our results also showed that cortisol – as a part of the DHEAS/cortisol ratio – correlated negatively with all measured respiratory parameters. Abnormalities at several levels of the HPA axis, which may promote glucocorticoid-dependent neurodegeneration, have been reported in patients with ALS [5,6] and Alzheimer's disease [43]. However, it is unknown whether endogenous cortisol levels can influence ALS progression or respiratory status. Along this line, the increments of adrenal corticosterone reported in models of ALS, that is, the Wobbler [44] and the $SOD1^{G93A}$ transgenic mouse [45], have shown to accelerate paralysis and decrease survival, suggesting that glucocorticoids may negatively impact progression in those models.

Our study also indicated that FT correlated with FVC% decay, indicating that higher levels of the male sex hormone in patients were associated with a greater loss of FVC% points per month. This could be due to the known negative influence of testosterone on respiratory capacity as occurs in sleep disorders [19]. Interestingly, elevated serum levels of estradiol were also nega-

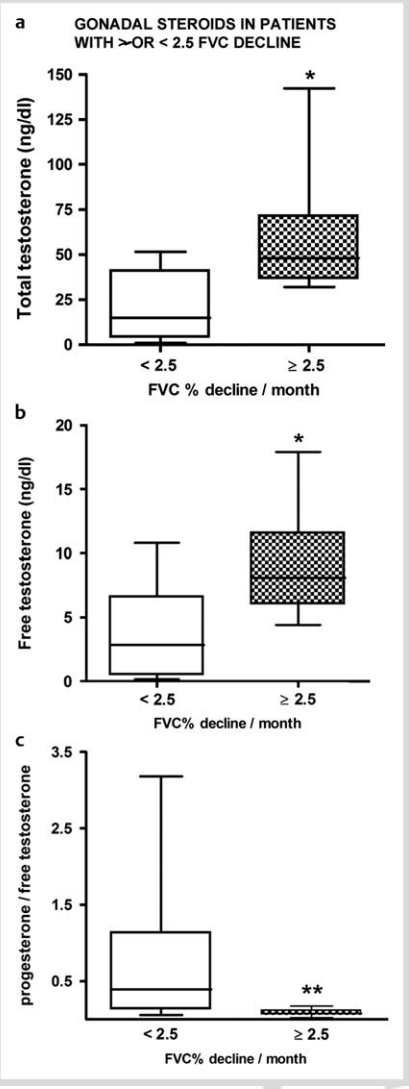

Fig. 4 Levels of total testosterone **a** and free testosterone in serum **b** and progesterone/free testosterone ratio **c** in patients with FVC decline/month < and ≥ 2.5. Patients with FVC decline/month ≥ 2.5 showed increased levels of total testosterone and free testosterone and decreased progesterone/free testosterone ratio than those patients with FVC decline per month < 2.5 (**a**, $^{*}p<0.05$; **b**, $^{*}p<0.05$; **c**, $^{**}p<0.01$, respectively). Results in the box represent the median, 25th and 75th percentiles. Whiskers correspond to the highest and the lowest values of the 95% confidence interval of the mean. For statistical analysis, we used two-tailed Mann-Whitney test.

tively correlated with the FVC% fall. In our cohort, composed of men and postmenopausal women, circulating estradiol could derive from peripheral aromatization of androgens [36]. In fact, we have demonstrated a positive correlation between estradiol and FT serum concentration in both healthy subjects and ALS patients.

Regarding FVC% progression, we observed increased ratios of progesterone/FT positively associated with a lesser decline or a slow worsening of FVC% per month. For many years, hyperventilation in human pregnancy has been associated with elevated progesterone levels. In the luteal phase of the menstrual cycle in which progesterone levels are high, hyperventilation lowers the upper airway resistance and decreases the end-tidal pressure of CO_2 [46,47]. Hence, it is possible that neuroactive progesterone in combination with the effects of testosterone could influence respiratory capacity and the rate of FVC declination in ALS patients.

Importantly, ALS is known to have a greater incidence in men than in women across all age groups [48]. However, the male/female gender ratio is lower in older patients (ratio of 3.98/1 for onset at age < 49 years and 1.63/1 for onset at age > 55 years), suggesting either younger male or older postmenopausal female patients have increased disease susceptibility [48]. Interestingly, we found significantly higher levels of testosterone in female postmenopausal patients, and no decline with increasing age in either gender, compared to controls. Indeed, a reduction in the ratio of progesterone/FT naturally occurs in men and postmenopausal women. In this respect, progesterone levels are lower in postmenopausal women and men than those found in females during the peak of the menstrual cycle. In these groups, circulating progesterone derives from the adrenal glands (both sexes) or the testes [24]. Androgen plasma levels progressively decline with age; however, after menopause the ovary appears to contribute a higher proportion of circulating testosterone [28].

In brief, circulating testosterone and the calculation of progesterone/FT and DHEAS/cortisol ratios could become valuable indexes of disease susceptibility and outcome that are modulated by age and gender. In this study, testosterone levels and the ratios of DHEAS/cortisol and progesterone/FT were associated to respiratory function in ALS patients. This is an important issue, because there is no available information regarding the role of endogenous steroid levels of the gonadal/adrenal axis on neither the susceptibility status of ALS patients nor the progression of the disease. In this regard, the search for biomarkers in ALS seems imperative. Further studies are needed to disclose the role of different steroid hormones on ALS patients.

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Conflict of Interest

The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

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