Vitamin D is Not a Protective Factor in ALS

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SUMMARY

Aims: Vitamin D deficiency has been associated with poorer prognosis in ALS. Better understanding of the role of vitamin D in ALS is needed to determine whether trials of systematic supplementation are justified. Our aim was to report vitamin D levels during the course of ALS and to evaluate its relationship with clinical parameters at diagnosis and with disease progression. **Methods:** We prospectively collected vitamin D serum concentrations from 125 consecutive ALS patients. Cox proportional hazard models analyzed the relationship between vitamin D concentrations, clinical parameters, and survival. **Results:** The mean vitamin D concentration was below our laboratory's lower limit of normal ($P < 0.0001$) and did not change during the course of the disease. The concentrations were higher in patients with bulbar onset ($P = 0.003$) and were negatively associated with body mass index (BMI) ($P = 0.0095$). Models with ALSFRS-R (ALS Functional Rating Scale-Revised) and BMI as a covariates showed that vitamin D concentrations predicted worse prognosis. **Conclusion:** The distribution of vitamin D concentrations in our cohort was consistent with previous reports. Surprisingly, we noted a negative effect of higher vitamin D levels on prognosis in ALS. More detailed research is warranted to determine whether manipulation of vitamin D could be beneficial to patients.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by dropout of upper and lower motor neurons in the brain and spinal cord. Genetic factors, including mutations of the *C9orf72*, *SOD1* (superoxide dismutase 1), *TARDPB*, and *FUS* (Fused in sarcoma) genes, explain nearly 50% of familial cases (FALS) [1]. A complex interaction of genetic and environmental influences may underlie sporadic forms (sALS). A series of events including oxidative stress, glutamate-mediated excitotoxicity, oxidative stress, mitochondrial dysfunction, and protein misfolding appear to have important roles in cell death [2].

Most studies of vitamin D have been related to bone metabolism, cellular and immune function, or brain development [3]. High levels of vitamin D may be protective for several neurological diseases, including Alzheimer's [4] and Parkinson's [5] diseases, schizophrenia [6], multiple sclerosis [7], depression [8], and ALS [9]. The nuclear hormone receptor for vitamin D (VDR) and the 1- α hydroxylase that enables the formation of active vitamin D are present in neurons and glial cells [10,11]. Vitamin D may

influence gene expression in ALS brain and muscle through effects on Toll-like receptors and calcium-binding proteins and may influence cellular-signaling mechanisms such as those elicited by glutamate, reactive oxygen species, or nitric oxide synthase [12–16].

The precise role of vitamin D deficiency in neurological conditions or circumstantial is debated. Lower vitamin D levels predicted faster progression in 94 patients with ALS [10]. Some studies suggest that vitamin D could ameliorate pathological mechanisms [15] and that supplementation could slow the disease course [12,16]. On the other hand, reports from a high copy SOD1 G93A-mutated transgenic mouse model of ALS suggest that administration of vitamin D could alter dopaminergic activity in the brain [16], which may be toxic to female pups [14] and, when deficient, may delay onset and attenuate early disease severity [17]. In one study, vitamin D supplementation in rodents did not produce beneficial effects on age of onset or disease duration [15]. A better understanding of the role vitamin D in neurodegeneration is needed before consideration is given to clinical trials.

The aim of this study was to prospectively report changes in vitamin D levels during the course of ALS and to evaluate the relationship between vitamin D levels and clinical as well as biological parameters at diagnosis and over time.

Material and Methods

Enrolled Patients

We enrolled 147 consecutive patients at the ALS Center in Tours from March 2012 to February 2014, who had vitamin D concentration measurements, at least during the diagnosis examination. All had sporadic disease and met diagnostic criteria for definite or probable ALS based on the El Escorial World Federation diagnostic criteria [18]. All patients were treated with riluzole from the date of diagnosis. We excluded 22 patients, who took nutritional supplementation (whatever, both, the type of supplementation and the etiology of the decision to supplement; Table S1), and analyzed the remaining 125 patients.

Clinical Data

The patients were diagnosed and followed in the ALS French center of Tours, where all clinical and biological data were centralized. For each patient, we obtained clinical data including information on diagnosis, gender, current age, site of onset (limb or bulbar), age at onset, and disease duration. Bulbar onset was defined as first symptom of dysphagia, dysphonia, or dysarthria. Limb onset was defined as symptoms first occurring in the limbs. The age at onset was taken as the time at which motor weakness was first noted by the patient. Disease duration was expressed as the time from first motor symptom to death or tracheostomy. We collected ALS Functional Rating Scale-Revised (ALSFRS-R) scores and values for forced vital capacity (FVC; expressed as percent predicted) at diagnosis and at each follow-up visit (every 3 months). We calculated the variation in ALSFRS-R (var_ALSFRS), FVC (var_FVC), and weight (var_weight) as the percent of the variations weighted by the time between the two most distant evaluations. We also collected data on bioelectric impedance (BIA) that is an easy, quick, and noninvasive method to measure body composition (e.g., FFMI: free fat mass index). This technique is based on the following principle: electric current flows at different rates through the body depending upon its composition. This method was performed using a Bodystat Quadscan® (Douglas, Isle of Man, British Isles) instrument with surface electrodes (Contrôle graphique médical®, Brie-Comte-Robert, France) according to standard and previously described methods [19].

Biological Data

We drew blood for laboratory studies at diagnosis. The following biological variables are part of the routine biochemical workup of patients at our center: cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and LDL/HDL ratio, and were analyzed using a AU2700™ chemistry analyzer (Beckman Coulter®, Fullerton, CA, USA), and 25-OH vitamin D was analyzed by competitive chemiluminescence immunoassay using IDS-iSYS™ analyzer (IDS, Boldon, UK; accredited method).

The biological variables were assessed during each consultation, every 3–6 months, in the ALS center. Five time points following diagnosis were included in this study: <12 months, 12–18 months, 18–24 months, 24–30 months and >30 months.

Signed consent was not required for these biochemical tests (lipids, vitamin D) because they were part of usual clinical care.

Statistical Analysis

First, a paired student's *t*-test showed no significant variation in vitamin D levels over the 30 months (<12 months, 12–18 months, 18–24 months, 24–30 months, and >30 months) study period, so we calculated the median vitamin D level for each patient and went on to use this value for all subsequent analyses.

Pearson's test evaluated the association between vitamin D levels and lipid parameters. Student's *t*-test or Wilcoxon test assessed the relationship between vitamin D levels and clinical parameters, including BIA variables.

We also performed an univariate survival analysis to evaluate the influence of biochemical and clinical parameters on disease duration (Kaplan Meier or Cox proportional hazard model, based on the type of model). After a collinearity check, multivariate Cox proportional hazard models explored factors significantly associated with survival. *P* values <0.05 were considered significant.

JMP statistical software version. 7.0.2 (SAS Institute, Cary, NC, USA) performed the statistical analyses.

Results

Subjects

Table 1 shows the characteristics of the 125 patients at baseline as well as parameters of disease progression. BIA data were obtained for 73.6% of the cohort. The Table S1 showed that supplemented patients had lower ALSFRS (*P* = 0.007) and FVC (*P* = 0.02) than nonsupplemented patients, showing a worst clinical status.

Evolution of Vitamin D Levels

Blood levels of vitamin D ranged from 49.5 nmol/L to 62.3 nmol/L over 30 months and did not differ across the 5 time categories (*P* > 0.08, cutoff of the *P* value based on Bonferroni's correction: 0.01, Table 2). The median vitamin D level over 30 months was 50.3 (min: 15; max: 136.5 or range 15–136.5) nmol/L. The mean vitamin D concentration of 50.75 [49.5–55.9] nmol/L was below the lower limit of normal for our laboratory (75 nmol/L; *P* < 0.0001). We also justified the exclusion of supplemented patients who had higher vitamin D levels (Table S1, *P* < 0.0001).

Relationship Between Vitamin D Levels and Biological or Clinical Parameters

There was no association (not shown) between the levels of vitamin D and cholesterol (6.1 [5.8–6.4] mmol/L), triglycerides (1.7 [1.5–1.9] mmol/L), HDL cholesterol (1.6 [1.5–1.7] mmol/L), LDL cholesterol (3.8 [3.6–4.0] mmol/L), and HDL/LDL ratio (0.46 [0.41–0.50]) (*P* > 0.37 for each parameter).

Table 1 Main characteristics of patients, with demographical and clinical parameters at diagnosis and parameters of disease progression

Parameters at diagnosis	
Sex, n (%)	
Male	63 (50.4)
Female	62 (49.6)
Site at onset, n (%)	
Spinal	81 (64.8)
Upper	29 (23.2)
Lower	52 (41.6)
Bulbar	44 (34.2)
Age (years), mean (IC 95%)	65 (63–67)
ALSFRS-R, mean (IC 95%)	34.4 (33.0–35.7)
FVC (%), mean (IC 95%)	80.5 (75.4–85.6)
BMI (kg/m ²), mean (IC 95%)	24.2 (23.5–24.8)
FFMI (kg/m ²), mean (IC 95%)	16.1 (15.4–16.7)
Basal metabolism (Kcal/day), mean (IC 95%)	1324.2 (1278.1–1370.2)
Total metabolism (Kcal/day), mean (IC 95%)	1959.5 (1884.9–2034.1)
Parameters of disease progression, mean (IC 95%)	
var_weight	0.09 (0.02–0.16)
var_ALSFRS	0.28 (0.19–0.34)
var_FVC	0.46 (–0.11–1.02)
Disease duration (months)	31.8 (27.9–35.7)

Table 2 Evolution of vitamin D levels over 30 months after the diagnosis. Paired Student's *t*-test, cutoff of *P* value = 0.01

	Mean [IC 95%]	<i>P</i> value
Vitamin D levels (nmol/L)		
0–12 months	56.2 [48.8–63.5]	>0.08
12–18 months	62.3 [54.4–70.1]	
18–24 months	55.4 [48.2–62.6]	
24–30 months	53.0 [41.8–64.3]	
>30 months	49.5 [41.3–57.6]	

The median vitamin D level was higher in the bulbar than the spinal subgroup (62.4 [55.3–69.5] nmol/L vs. 49.2 [44.0–54.3] nmol/L, *P* = 0.003) (Figure 1) and higher in women (50.1 [52.6–65.6] nmol/L) than men (48.6 [43.2–54.0] nmol/L) (*P* = 0.024, not shown). Vitamin D levels were negatively associated with BMI (*P* = 0.0095), basal metabolism (*P* = 0.0085), and FFMI (*P* = 0.0334) (Figure 2).

Relationship Between Disease Progression and Clinical and Biological Parameters

There was a trend toward greater decline in ALSFRS-R in patients with higher levels of vitamin D (*P* = 0.06, not shown). The HDL/LDL ratio at diagnosis was positively correlated with weight loss over the course of the disease (*P* = 0.0016, not shown). Patients with bulbar onset had greater weight loss than those with limb onset (*P* = 0.01, not shown). We did not observe significant relation between the analyzed parameters and var_FVC (not shown).

Figure 1 Vitamin D concentrations according to the site at onset. Patients with bulbar onset have higher vitamin D levels (*P* = 0.003).

Site of onset and age at onset were not associated with the survival in this cohort (not shown).

Among the parameters associated with survival (Table 3), we found ALSFRS-R at diagnosis (*P* = 0.01) and vitamin D levels (*P* = 0.003 using the Cox model and *P* = 0.0022 using Kaplan–Meier analysis based on the median level of vitamin D, Figure 3). After checking collinearity of the variables, we noted that ALSFRS-R was significantly associated with FVC at diagnosis (*P* = 0.006), BMI (*P* = 0.0095), basal metabolism (*P* = 0.009), and total metabolism (*P* = 0.004). Thus, we included ALSFRS-R at diagnosis and vitamin D levels and then stratified the analysis by BMI (above and below the median = 23.6 kg/m²) in multivariate analysis. Cox proportional hazards modeling showed that vitamin D was a prognostic factor (*P* = 0.047 and *P* = 0.0098 in the subgroups above and below the median BMI, respectively, Table 3) when controlling for ALSFRS-R. ALSFRS-R was a significant predictor of prognosis only in the subgroup of patients with low BMI (*P* = 0.0031).

The Cox analysis including ALSFRS-R at diagnosis and stratified by gender showed that vitamin D was a significant predictor of prognosis in women (*P* = 0.023) but not men (*P* = 0.16; data not shown).

Discussion

This study focused on variations in vitamin D levels over the course of ALS to help clarify the relationship between vitamin D and biological as well as clinical disease characteristics.

Two main findings can be summarized about vitamin D in ALS:

- 1 Vitamin D levels were stable over the course of the disease, a finding that is consistent with a recent 3-year longitudinal study (mean vitamin D level at the first test in that study = 51.7 ± 24 nmol/L and at the final examination = 56.7 ± 24.7) [20].
- 2 The distribution of vitamin D values was also consistent with reports from other laboratories [9,21]. Hypovitaminosis D is usually classified as follows: <12.5 nmol/L = severe deficiency; 12.5–25 nmol/L = moderate deficiency; 25–50 nmol/L = mild deficiency; and >50 nmol/L = sufficient [22,23]. Recent recommendations suggest a minimal serum 25-OH vitamin D level of 75 nmol/L in fragile elderly subjects [24].

Figure 2 Inverse correlation between vitamin D levels and **(A)** BMI ($P = 0.0095$), **(B)** FFMI ($P = 0.0334$), **(C)** basal metabolism ($P = 0.0085$).

Table 3 Survival analysis (Cox model) including univariate analysis and multivariate analysis with respective hazard ratio. Med: median of vitamin D level = 23.6 nmol/L

Variables	Univariate analysis		Multivariate analysis			
	P value	Hazard ratio	BMI < Med		BMI > Med	
			P value	Hazard ratio	P value	Hazard ratio
ALSFRS at diagnosis	0.029	0.32 [0.11–0.89]	0.0031	0.13 [0.03–0.49]	0.75	
FVC (%)	0.019	0.26 [0.08–0.81]				
Vitamin D levels (nmol/L)	0.003	6.28 [1.88–19.42]	0.047	6.6 [1.03–35.80]	0.0098	6.5 [1.56–28.18]
BMI (kg/m^2)	0.0095	0.16 [0.03–0.64]				
Basal metabolism (Kcal/day)	0.005	0.15 [0.03–0.58]				
Total metabolism (Kcal/day)	0.002	0.13 [0.03–0.49]				

Bold values indicate statistically significant P value < 0.05.

Figure 3 Kaplan–Meier curve according to the median value of vitamin D concentrations. Red: patients with vitamin concentrations <50.2 nmol/L, blue: patients with vitamin D concentrations ≥50.2 nmol/L. **(A)** in the subgroup of patients with BMI <23.6 kg/m^2 , $P = 0.014$; **(B)** in the subgroup of patients with BMI ≥23.6 kg/m^2 , $P = 0.022$.

Other studies in ALS patients have also shown a higher frequency of vitamin D deficiency than in general populations worldwide (13% of ALS patients with levels <25 nmol/L compared to 6.7% of the general population) [25]. The published study considered as the most similar to ours, both in term of recruited ALS patients (clinical and demographical characteristics)

and size of the cohort is that of Camu *et al.* [9] Both cohorts had similar distribution of vitamin D concentrations (13% vs. 13.5% of patients with vitamin D levels <25 nmol/L, 66.6% vs. 70.2% with vitamin D levels between 25 and 75 nmol/L, and 13.6% vs. 16.2% with vitamin D levels >75 nmol/L in our cohort vs. the cohort of Camu *et al.* [9]).

Although ALS patients had not hypovitaminosis D, the concentrations were lower than that requested for elderly patients. This could be an indirect consequence of ALS, occurring through loss of mobility and insufficient exposure to sunlight for adequate synthesis, or to reduced intake as a result of dysphagia. No patient in our study was confined to bed; some participants needed wheelchairs for mobility, but all could leave the house regularly, so immobility does not completely explain our findings. Moreover, supplemented patients, because of malnutrition or poor intake, were excluded from this study. The targeted vitamin D concentrations requested for the elderly subjects [26] was not reached and may represent a risk factor in ALS, but the precise place vitamin D could hold in the pathogenesis or pathophysiology of ALS is unknown. The homogeneity of age and the small size of our cohort may explain that we observed no link between vitamin D levels and age.

As expected, we observed an inverse correlation between vitamin D levels and FFMI as well as BMI [27]. The relationship between concentrations of vitamin D and basal or total metabolism suggests a role for vitamin D homeostasis in global metabolism. Bias could also contribute to these findings: BMI, basal metabolism, and total metabolism were associated with survival and with each other together, limiting the scope of our survival findings. Malnutrition as quantified by BMI is a recognized marker of poor prognosis in ALS [28], so we controlled for the effects of nutritional status by stratifying the survival analysis on this parameter. ALSFRS-R score at diagnosis is also a known predictor of survival and so was included as a covariate in the multivariate survival analysis [29].

The survival analyses revealed a deleterious effect of “higher” vitamin D concentrations on the prognosis, independently of ALSFRS-R score at diagnosis and BMI, suggesting that this relation may be direct and independent of the nutritional status. This finding contrasts with many previous studies [9,25], including that of Camu *et al.* [9] who evaluated a similar cohort as ours. This apparent discrepancy could be due to numerous differences in the strategy of analysis: Camu *et al.* studied vitamin D levels both quantitatively and qualitatively, with class different from ours (<25; <75 and >75 nmol/L vs. < or >50 nmol/L), and they did not include nutritional parameters. Thus, the interpretation of both studies has to be made in the context of each design. All these results highlight that the role of vitamin D in ALS survival is not clearly defined and these findings need to be replicated.

The values of targeted vitamin D concentrations are established from protective role of vitamin D against neurodegeneration [30,31], through protecting on deleterious effects of inflammation, oxidative stress, calcium homeostasis deregulation, and protein synthesis [15,32–35], all mechanisms that contribute to the pathophysiology of ALS [3,13].

We emphasize that the so-called higher concentrations of vitamin D are still low compared to guidelines (only 13.6% of the ALS patients had levels >75 nmol/L), and studies in a subgroup of ALS patients with really high concentrations of vitamin D are needed to complete the interpretation and discussion.

This study has limitations. Our cohort may not be representative of the general ALS population. Vitamin D levels were higher in bulbar-onset patients, a finding that has not been previously reported. Some patients with the bulbar onset form had

dysphagia, but those who required nutritional supplementation were excluded from the study to avoid artificial elevations in vitamin D. In seeking to avoid bias caused by nutritional supplementation, we may have introduced a bias of another type by excluding those patients with the most severe ALS forms. The relatively reduced sample size lent low power to some analyses and made stratification impossible for some variable. Importantly, the narrow range of vitamin D concentrations is also a limit to such studies. Even if the interindividual variability of vitamin D concentrations is superior to the analytical variability, these data should be considered cautiously.

There may be gender-specific effects of vitamin D, which could be due to polymorphic variants in the VDR (Vitamin D receptor) gene [4], but our cohort was too small to stratify on gender. A toxic effect of vitamin D in female rodents with significantly reduced food intake has been reported [36], possibly due to synergy between vitamin D3 and estrogen [37] through downregulation of *CYP24A1*, the calcitriol deactivating enzyme. The deleterious effect of higher vitamin D principally noted in women in our cohort appears coherent with the literature, but reasons underlying higher concentrations in women than men are unclear.

Vitamin D concentrations have to be carefully controlled. Indeed, vitamin D deficiency may be associated with delay in disease onset and reduced disease severity early in the ALS murine model [17]. The same observation has been made in multiple sclerosis and merits further exploration [38]. The management of vitamin D concentrations may be delicate as articles reported benefits of vitamin D on muscle strength in both peer-reviewed journals and public health literature, and other showed that supplementation in humans does not positively affect muscle strength in patients with vitamin D levels >25 nmol/L [39,40].

It is not possible, to date, to establish a specific relationship between vitamin D supplementation and survival. No rigorous studies have so far evaluated the role of vitamin D supplementation and established a target range of vitamin D in ALS. Guidelines come from general nutritional recommendations and on the systematic supplementation to compensate for inadequate consumption of vitamin D-rich foods and prevent osteoporosis. Additional studies are needed to establish the role of vitamin D in ALS pathophysiology and explore a possible place for replacement in the disease.

Conclusion

Our study adds to the growing literature on the relationship between neurological disorders and hypovitaminosis D. In this study, there was a negative effect of higher vitamin D levels on the course of ALS, a finding at odds with some previous studies. The mechanisms underlying the relationship between vitamin D and ALS are still obscure; well-designed prospective studies are needed to better understand the involvement of vitamin D in ALS. Future research could explore the role vitamin D plays in ALS physiopathology with concurrent examination of oxidative stress, calcium concentration, and intracellular calcium buffering. Better understanding of hypovitaminosis D in ALS is needed at the bench prior to embarking on humans trials of supplementation.

Conflict of Interest

The authors declare no conflict of interest. Dr Blasco, Miss Madji Hounoum, Dr Dufour-Rainfray, Mr Patin, Pr Maillot, Dr Beltran, Pr Gordon, Pr Andres, and Pr Corcia have no disclosures to report.

References

- Leblond CS, Kaneb HM, Dion PA, Rouleau GA. Dissection of genetic factors associated with amyotrophic lateral sclerosis. *Exp Neurol* 2014;**262**(Pt B):91–101.
- Shaw PJ. Molecular and cellular pathways of neurodegeneration in motor neurone disease. *J Neurol Neurosurg Psychiatry* 2005;**76**:1046–1057.
- Kesby JP, Eyles DW, Burne TH, McGrath JJ. The effects of vitamin D on brain development and adult brain function. *Mol Cell Endocrinol* 2011;**347**:121–127.
- Annweiler C, Llewellyn DJ, Beauchet O. Low serum vitamin D concentrations in Alzheimer's disease: a systematic review and meta-analysis. *J Alzheimers Dis* 2013;**33**:659–674.
- Vinh Quoc Luong K, Thi Hoang Nguyen L. Vitamin D and Parkinson's disease. *J Neurosci Res* 2012;**90**:2227–2236.
- Belvederi Murri M, Respino M, Masotti M, et al. Vitamin D and psychosis: mini meta-analysis. *Schizophr Res* 2013;**150**:235–239.
- Ascherio A, Munger KL, White R, et al. Vitamin D as an early predictor of multiple sclerosis activity and progression. *JAMA Neurol* 2014;**71**:306–314.
- Spedding S. Vitamin D and depression: a systematic review and meta-analysis comparing studies with and without biological flaws. *Nutrients* 2014;**6**:1501–1518.
- Camu W, Tremblier B, Plasset C, et al. Vitamin D confers protection to motoneurons and is a prognostic factor of amyotrophic lateral sclerosis. *Neurobiol Aging* 2014;**35**:1198–1205.
- Zehnder D, Bland R, Williams MC, et al. Extrarenal expression of 25-hydroxyvitamin d(3)-1 alpha-hydroxylase. *J Clin Endocrinol Metab* 2001;**86**:888–894.
- Eyles DW, Smith S, Kinobe R, Hewison M, McGrath JJ. Distribution of the vitamin D receptor and 1 alpha-hydroxylase in human brain. *J Chem Neuroanat* 2005;**29**:21–30.
- Shen L. Further support for vitamin D supplement in delaying the progression of ALS. *Med Hypotheses* 2011;**77**:698.
- Karam C, Scelsa SN. Can vitamin D delay the progression of ALS? *Med Hypotheses* 2011;**76**:643–645.
- Gianforaro A, Solomon JA, Hamadeh MJ. Vitamin D(3) at 50x AI attenuates the decline in paw grip endurance, but not disease outcomes, in the G93A mouse model of ALS, and is toxic in females. *PLoS ONE* 2013;**8**:e30243.
- Gianforaro A, Hamadeh MJ. Vitamin D as a potential therapy in amyotrophic lateral sclerosis. *CNS Neurosci Ther* 2014;**20**:101–111.
- Smith MP, Fletcher-Turner A, Yurek DM, Cass WA. Calcitriol protection against dopamine loss induced by intracerebroventricular administration of 6-hydroxydopamine. *Neurochem Res* 2006;**31**:533–539.
- Solomon JA, Gianforaro A, Hamadeh MJ. Vitamin D3 deficiency differentially affects functional and disease outcomes in the G93A mouse model of amyotrophic lateral sclerosis. *PLoS ONE* 2011;**6**:e29354.
- Brooks BR. Functional scales: summary. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2002;**3**(Suppl 1):S13–S18.
- Roubeau V, Blasco H, Maillot F, Corcia P, Praline J. Nutritional assessment of amyotrophic lateral sclerosis in routine practice: value of weighing and bioelectrical impedance analysis. *Muscle Nerve* 2014;**51**:479–484.
- Saliba W, Barnett O, Stein N, Kershenbaum A, Rennert G. The longitudinal variability of serum 25(OH)D levels. *Eur J Intern Med* 2012;**23**:e106–e111.
- Bendik I, Friedel A, Roos FF, Weber P, Eggersdorfer M. Vitamin D: a critical and essential micronutrient for human health. *Front Physiol* 2014;**5**:248.
- Bischoff-Ferrari HA. Optimal serum 25-hydroxyvitamin D levels for multiple health outcomes. *Adv Exp Med Biol* 2014;**810**:500–525.
- Saliba W, Barnett-Griness O, Rennert G. The relationship between obesity and the increase in serum 25(OH)D levels in response to vitamin D supplementation. *Osteoporos Int* 2013;**24**:1447–1454.
- Rizzoli R, Boonen S, Brandi ML, et al. Vitamin D supplementation in elderly or postmenopausal women: a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Curr Med Res Opin* 2013;**29**:305–313.
- Karam C, Barrett MJ, Imperato T, MacGowan DJ, Scelsa S. Vitamin D deficiency and its supplementation in patients with amyotrophic lateral sclerosis. *J Clin Neurosci* 2013;**20**:1550–1553.
- Pludowski P, Grant WB, Bhattoa HP, et al. Vitamin d status in central europe. *Int J Endocrinol* 2014;**2014**:589587.
- Pathak K, Soares MJ, Calton EK, Zhao Y, Hallett J. Vitamin D supplementation and body weight status: a systematic review and meta-analysis of randomized controlled trials. *Obes Rev* 2014;**15**:528–537.
- Ngo ST, Steyn FJ, McCombe PA. Body mass index and dietary intervention: implications for prognosis of amyotrophic lateral sclerosis. *J Neurol Sci* 2014;**340**:5–12.
- Creemers H, Grupstra H, Nolle F, Van den Berg LH, Beelen A. Prognostic factors for the course of functional status of patients with ALS: a systematic review. *J Neurol* 2014;**261**:7564.
- Annweiler C, Beauchet O. Vitamin D-mentia: randomized clinical trials should be the next step. *Neuroepidemiology* 2011;**37**:249–258.
- Annweiler C, Beauchet O. Possibility of a new anti-alzheimer's disease pharmaceutical composition combining memantine and vitamin D. *Drugs Aging* 2012;**29**:81–91.
- Moore ME, Piazza A, McCartney Y, Lynch MA. Evidence that vitamin D3 reverses age-related inflammatory changes in the rat hippocampus. *Biochem Soc Trans* 2005;**33**:573–577.
- Brewer LD, Thibault V, Chen KC, Langub MC, Landfield PW, Porter NM. Vitamin D hormone confers neuroprotection in parallel with downregulation of L-type calcium channel expression in hippocampal neurons. *J Neurosci* 2001;**21**:98–108.
- Cantorna MT. Vitamin D, multiple sclerosis and inflammatory bowel disease. *Arch Biochem Biophys* 2012;**523**:103–106.
- Salles J, Chanet A, Giraudet C, et al. 1,25(OH)2-vitamin D3 enhances the stimulating effect of leucine and insulin on protein synthesis rate through Akt/PKB and mTOR mediated pathways in murine C2C12 skeletal myotubes. *Mol Nutr Food Res* 2013;**57**:2137–2146.
- Gianforaro A, Hamadeh MJ. Dietary vitamin D3 supplementation at 10x the adequate intake improves functional capacity in the G93A transgenic mouse model of ALS, a pilot study. *CNS Neurosci Ther* 2012;**18**:547–557.
- Nashold FE, Spach KM, Spanier JA, Hayes CE. Estrogen controls vitamin D3-mediated resistance to experimental autoimmune encephalomyelitis by controlling vitamin D3 metabolism and receptor expression. *J Immunol* 2009;**183**:3672–3681.
- DeLuca HF, Plum LA. Vitamin D deficiency diminishes the severity and delays onset of experimental autoimmune encephalomyelitis. *Arch Biochem Biophys* 2011;**513**:140–143.
- Stockton KA, Mengersen K, Paratz JD, Kandiah D, Bennell KL. Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporos Int* 2011;**22**:859–871.
- Holick MF. Vitamin D: the other steroid hormone for muscle function and strength. *Menopause* 2009;**16**:1077–1078.

Supporting Information

The following supplementary material is available for this article:

Table S1 Characteristics of patients with and without supplementation. Patients with supplementation had significant lower ALS-FRS ($P = 0.007$) and FVC ($P = 0.02$) and higher vitamin D levels ($P < 0.001$).