Metabolic Alterations in Patients with Amyotrophic Lateral Sclerosis

Mark R. Janse van Mantgem

UMC Utrecht Brain Center

METABOLIC ALTERATIONS IN PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

Mark R. Janse van Mantgem

Metabolic Alterations in Patients with Amyotrophic Lateral Sclerosis

The Netherlands ALS Foundation funded this work.

Cover by: Annette Broers, Instagram @paintings_by_annette
Printed by: ProefschriftMaken | www.proefschriftmaken.nl
ISBN: 978-90-393-7663-8

Metabolic Alterations in Patients with Amyotrophic Lateral Sclerosis

Metabole veranderingen in patiënten met Amyotrofische Laterale Sclerose

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de
rector magnificus, prof. dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

dinsdag 14 mei 2024 des middags te 12.15 uur

door

Mark Robert Janse van Mantgem

geboren op 4 februari 1991
te Amstelveen

Promotor:

Prof. dr. L.H. van den Berg

Copromotoren:

Dr. R.P.A. van Eijk

Dr. S.T. Ngo

Beoordelingscommissie:

Prof. dr. R.A.H. Adan

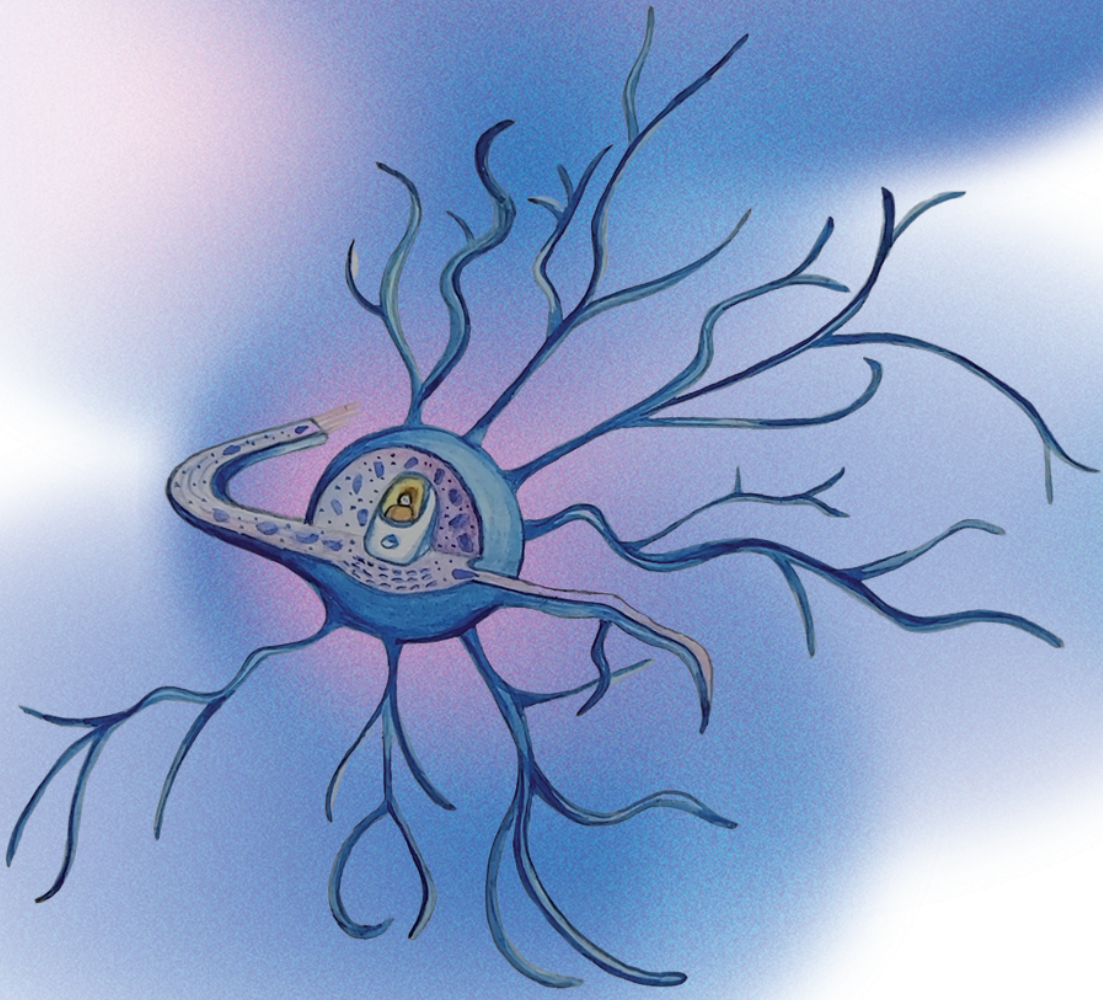
Prof. dr. B. van Engelen

Prof. dr. L.J. Kappelle

Prof. dr. N.C. Notermans

Prof. dr. F.L.J. Visseren (voorzitter)

CONTENT



Chapter 1

General Introduction

AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease, which causes irreversible damage to lower (LMN) and upper motor neurons (UMN) in the brain and spinal cord.[1–3] The lifetime risk of developing ALS in the Western world is 1 in 400.[4] It can occur at any age, with a peak incidence between the ages 50 and 75 years.[5] The cause can be designated as genetic in up to 10% of the cases.[6] When patients present at the outpatient clinic, they may have a variety of symptoms – from mild to severe – in the bulbar or spinal region, or both. Overall, patients develop progressive muscle weakness, eventually resulting in respiratory failure, which in turn causes death.[1,2] After symptom onset, median survival time is approximately three years,[7] but large differences between subgroups are seen.[8] The heterogeneous presentation makes diagnosis of ALS challenging for neurologists. With the exception of Riluzole – a drug with glutamate antagonist activity which extends survival by approximately three to six months – no other treatment for ALS is currently available in Europe.[9]

ALS is the most common form of the motor neuron diseases (MND). Two other, even rarer variants within the MND spectrum, are progressive muscle atrophy (PMA) and primary lateral sclerosis (PLS). The three subtypes have been shown to share common pathological features,[10] but, in contrast to ALS, in PMA only the LMN are affected, and in PLS only the UMN.[11–13] Moreover, the disease course of PMA is fairly similar to that of ALS,[11] while patients with PLS show a slower progression with a median survival time of more than 15 years.[14] Identifying specific biomarkers to differentiate between MND-subtypes and to differentiate within the heterogeneous syndrome of ALS, might lead to improvements in individual care, such as better counseling of patients and family relatives about disease course, prognosis, and survival. The aim is to start targeted therapy – which would seem to require an individualized approach due to the heterogeneous nature of the disease[15] – in order to interfere with the disease course.

BIOMARKERS OF METABOLISM

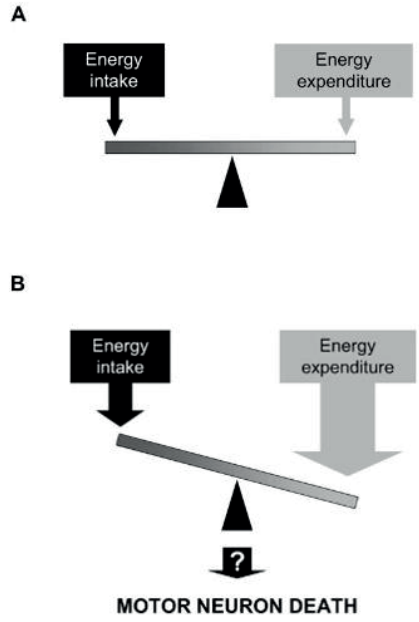
Mechanisms that underpin the pathogenesis of ALS are evolving and seem to differ between individuals.[3,15] In the last decade, an increasing consensus has developed that both genetic and environmental factors contribute to disease onset and progression.[3,15,16] Dysregulation of energy metabolism is one of the mechanisms that has been studied more extensively over the past decade as a factor contributing to the development of ALS.[17] This has resulted in a growing body of evidence that metabolic abnormalities correlate with the disease course, but also contribute to the individual's predisposition to developing ALS.[18–25] For instance, it was found that a higher pre-symptomatic body mass index (BMI) reduces the risk of ALS by

up to 40%.[26] In line with this, increased subcutaneous fat mass[27] and higher levels of serum leptin[28] – causing an individual to feel satiated – have been shown to decrease mortality in ALS. Therefore, specific biomarkers might be indicative of a dysregulated energy metabolism, and, moreover, might predict disease progression and survival more accurately in patients with ALS. Also, they may present an opportunity to prevent disease onset.

Metabolic abnormalities

In healthy individuals, energy homeostasis is maintained by an adequate energy uptake and expenditure (**Figure 1, panel A**); this seems to be disturbed in patients with ALS (**Figure 1, panel B**).

Figure 1. Simplified schematic overview of energy imbalance in ALS.

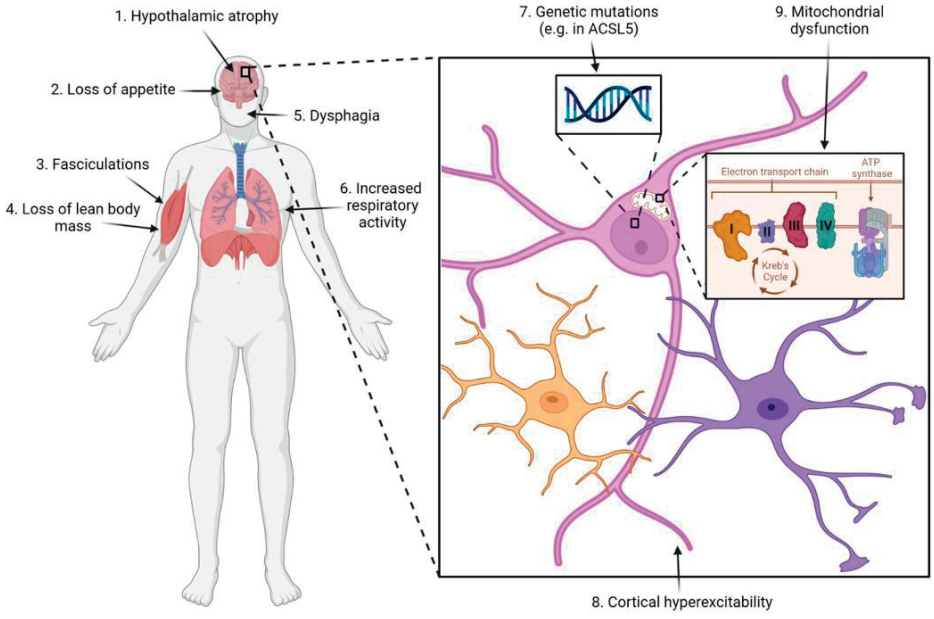


Panel A reflects a normal homeostasis between energy intake and expenditure in healthy individuals, whereas panel B reflects a dysregulated energy metabolism due to an increased energy expenditure.

A dysregulation in energy uptake might occur in patients with ALS due to bulbar muscle weaknesses, resulting in a reduced ability to chew, impaired mobility of the tongue, and dysphagia.[29,30] These factors are, therefore, a consequence of disease, while an imbalance in energy homeostasis is observed at or prior to diagnosis, suggesting a more causal relationship.[16,30,31] Unfortunately, the cellular mechanisms underlying this dysregulation in energy homeostasis are not known. It seems that both mitochondrial and glycolytic pathways are impaired and might result in motor neuron loss (**Figure 1**).[30,31] Potential causal pathways have been elucidated, such as hypothalamic dysfunction, caused by atrophy due to mo-

tor neuron degeneration,[32] mitochondrial dysfunction,[15] and genetic susceptibility,[18] and, moreover, seem to be affected by pre-symptomatic lifestyle.[16] An overview of the potential causes of developing an altered metabolic state is presented in **Figure 2**. These metabolic disturbances might result in an increased metabolic state (e.g. hypermetabolism), which has been shown to be a prognostically unfavorable phenomenon.[25] For this reason, there is an increasing interest in detecting metabolic changes.

Figure 2. Mechanisms potentially leading to an altered metabolic state in patients with ALS.
ALS.



Assessing metabolic abnormalities

Nowadays, we are able to determine a patient's metabolic state by assessing the resting energy expenditure (mREE).[33–35] The mREE, relative to the predicted REE (pREE), will result in a patient's metabolic index (MI), which defines a patient's metabolic state: hypo-, normo-, or hypermetabolic. However, the pREE can be determined by multiple pre-defined equations, none of which has been validated in patients with ALS.[36] Besides, almost all equations are dependent on components originating from total body composition, including fat-free mass (FFM) and fat mass (FM), which might be affected in patients with ALS.[27,37] This might explain the variety of findings regarding hypermetabolism.[22,31,38,39]

Moreover, the two techniques most commonly used in assessing FFM and FM, are: bioelectrical impedance analysis (BIA)[40] and air-displacement plethysmography (ADP).[41] These two techniques seem to generate adequate values in patients with ALS/MND.[42] However, neither

how these techniques compare nor the potential clinical consequence(s) of the two methods has been established in this population. While these issues need to be unraveled, in the meantime, patients and their relatives, are in need of better counseling on disease course, prognosis, and survival. Potential metabolic biomarkers which may determine the prognosis more accurately, are weight loss, creatinine and serum cholesterol levels at time of diagnosis. The main goal of unraveling the pathophysiology behind these metabolic changes is to be able, eventually, to interfere with these metabolic disturbances, ideally with medication. Therefore, it is necessary to both standardize the assessment of metabolic disturbances, and also determine the prognostic value of clinical markers related to metabolic changes. This thesis will contribute to demonstrating the importance of 1) assessing anthropometric measurements in patients with ALS, and 2) accuracy in determining the metabolic index.

OVERALL AIM AND THESIS OUTLINE

Considering the lack of standardization when assessing metabolic changes and, therefore, when determining their prognostic value in a clinical setting and their potential value as biomarker in clinical trials, it is essential to be consistent in the methodology applied in detecting these metabolic alterations. In the first chapters of this thesis (**chapters 2, 3, and 4**), we provide data on potential anthropometric biomarkers for predicting survival in patients with ALS. Weight loss (**chapter 2**) and serum cholesterol (**chapter 3**) have been of interest in various studies, however, with contradictory results. Therefore, we provide an overview of previous studies and, moreover, assess the prognostic value of these measurements at time of diagnosis, in large cohorts of patients with ALS. In addition, we show whether serum creatine levels are able to reflect fat-free mass loss during the disease course (**chapter 4**), which might be indicative of weight loss in patients in an advanced disease stage. This will help to optimize patient care.

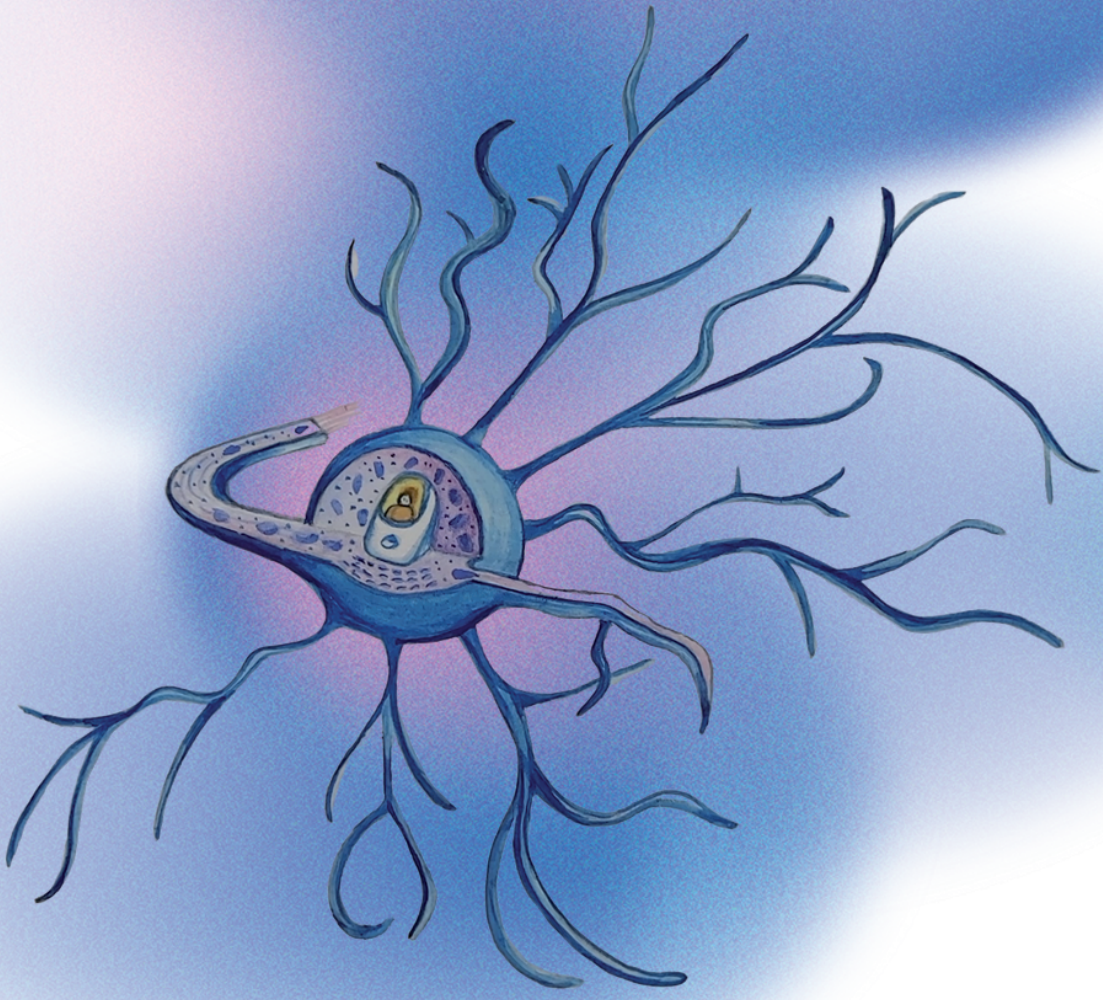
The last chapters of this thesis (**chapters 5, 6, and 7**) will evaluate the lack of standardization in assessing the metabolic index. We provide suggestions for using prediction equations in determining resting energy expenditure (**chapter 5**). Moreover, we will show that applying these equations depends on the appropriate determination of fat-free mass in patients with ALS (**chapter 6**). In addition, we assess the metabolic index in patients with ALS, PMA, and PLS to provide more clarity on the metabolic hypothesis in patients with ALS (**chapter 7**).

REFERENCES

1. Cleveland DW, Rothstein JD. From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. Nat Rev Neurosci 2001;2(11):806–819.
2. Mitchell JD, Borasio GD. Amyotrophic lateral sclerosis. Lancet 2007;369(9578):2031–2041.
3. van Es MA, Hardiman O, Chio A, et al. Amyotrophic lateral sclerosis. The Lancet 2017;390(10107):2084–2098.
4. Johnston CA, Stanton BR, Turner MR, et al. Amyotrophic lateral sclerosis in an urban setting: A population based study of inner city London. J Neurol 2006;253(12):1642–1643.
5. Huisman MHB, De Jong SW, Van Doormaal PTC, et al. Population based epidemiology of amyotrophic lateral sclerosis using capture-recapture methodology. J Neurol Neurosurg Psychiatry 2011;82(10):1165–1170.
6. Majoor-Krakauer D, Willems P, Hofman A. Genetic epidemiology of Amyotrophic Lateral Sclerosis. Clin Genet 2003;63:83–101.
7. Chiò A, Logroscino G, Hardiman O, et al. Prognostic factors in ALS: A critical review. Amyotrophic Lateral Sclerosis 2009;10(5–6):310–323.
8. Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol 2018;17(5):423–433.
9. Miller R, Mitchell J, Lyon M, Moore D. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders 2004;4(3):191–206.
10. Saberi S, Stauffer JE, Schulte DJ, Ravits J. Neuropathology of Amyotrophic Lateral Sclerosis and Its Variants. Neurol Clin 2015;33(4):855–876.
11. Visser J, Van Den Berg-Vos RM, Franssen H, et al. Disease Course and Prognostic Factors of Progressive Muscular Atrophy. Arch Neurol 2007;64(4):522–528.
12. Kim W-K, Liu X, Sandner J, et al. Study of 962 patients indicates progressive muscular atrophy is a form of ALS. Neurology 2009;73(20):1686–1692.
13. Turner MR, Barohn RJ, Corcia P, et al. Primary lateral sclerosis: Consensus diagnostic criteria. J Neurol Neurosurg Psychiatry 2020;91(4):373–377.
14. Chiò A, Calvo A, Moglia C, et al. Phenotypic heterogeneity of amyotrophic lateral sclerosis: A population based study. J Neurol Neurosurg Psychiatry 2011;82(7):740–746.
15. Hardiman O, Al-Chalabi A, Chio A, et al. Amyotrophic lateral sclerosis. Nat Rev Dis Primers 2017;3
16. Westeneng HJ, van Veenhuijzen K, van der Spek RA, et al. Associations between lifestyle and amyotrophic lateral sclerosis stratified by C9orf72 genotype: a longitudinal, population-based, case-control study. Lancet Neurol 2021;20(5):373–384.
17. De Aguilar JLG, Dupuis L, Oudart H, Loeffler JP. The metabolic hypothesis in amyotrophic lateral sclerosis: Insights from mutant Cu/Zn-superoxide dismutase mice. Biomedicine and Pharmacotherapy 2005;59(4):190–196.
18. Hop PJ, Zwamborn RAJ, Hannon E, et al. Genome-wide study of DNA methylation in Amyotrophic Lateral Sclerosis identifies differentially methylated loci and implicates metabolic, inflammatory and cholesterol pathways. medRxiv 2021;1–52.
19. Dupuis L, Pradat PF, Ludolph AC, Loeffler JP. Energy metabolism in amyotrophic lateral sclerosis. Lancet Neurol 2011;10(1):75–82.
20. Kirk SE, Tracey TJ, Steyn FJ, Ngo ST. Biomarkers of metabolism in amyotrophic lateral sclerosis. Front Neurol 2019;10(191):eCollection 2019.

21. Abdel-Khalik J, Yutuc E, Crick PJ, et al. Defective cholesterol metabolism in amyotrophic lateral sclerosis. J Lipid Res 2017;58(1):267–278.
22. Funalot B, Desport J, Sturtz F, et al. High metabolic level in patients with familial amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis 2009;10:113–117.
23. Tefera TW, Steyn FJ, Ngo ST, Borges K. CNS glucose metabolism in Amyotrophic Lateral Sclerosis: a therapeutic target? Cell Biosci 2021;11(1):1–17.
24. Chiang PM, Ling J, Jeong YH, Price DL, Aja SM, Wong PC. Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism. Proc Natl Acad Sci U S A 2010;107(37):16320–16324.
25. Steyn FJ, Ioannides ZA, Van Eijk RPA, et al. Hypermetabolism in ALS is associated with greater functional decline and shorter survival. J Neurol Neurosurg Psychiatry 2018;89(10):1016–1023.
26. Peter RS, Rosenbohm A, Dupuis L, et al. Life course body mass index and risk and prognosis of amyotrophic lateral sclerosis: results from the ALS registry Swabia. Eur J Epidemiol 2017;32(10):901–908.
27. Lindauer E, Dupuis L, Müller HP, Neumann H, Ludolph AC, Kassubek J. Adipose Tissue Distribution Predicts Survival in Amyotrophic Lateral Sclerosis. PLoS One 2013;8(6):6–12.
28. Nagel G, Peter RS, Rosenbohm A, et al. Adipokines, C-reactive protein and Amyotrophic Lateral Sclerosis - Results from a population- based ALS registry in Germany. Sci Rep 2017;7(1)
29. Ngo ST, van Eijk RPA, Chachay V, et al. Loss of appetite is associated with a loss of weight and fat mass in patients with amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2019;20(7–8):497–505.
30. Ludolph A, Dupuis L, Kasarskis E, Steyn F, Ngo S, McDermott C. Nutritional and metabolic factors in amyotrophic lateral sclerosis. Nat Rev Neurol 2023;(Online ahead of print)
31. Nelson AT, Trotti D. Altered Bioenergetics and Metabolic Homeostasis in Amyotrophic Lateral Sclerosis. Neurotherapeutics 2022;19(4):1102–1118.
32. Ahmed R, Steyn F, Dupuis L. Hypothalamus and weight loss in amyotrophic lateral sclerosis. Handb Clin Neurol 2021;180(C):327–338.
33. Nelson K, Weinsier R, Long C, Schutz Y. Prediction of resting energy expenditure from fat-free mass and fat mass. American Journal of Clinical Nutrition 1992;5(56):848–856.
34. Sherman MS, Pillai A, Jackson A, Heiman-Patterson T. Standard equations are not accurate in assessing resting energy expenditure in patients with amyotrophic lateral sclerosis. Journal of Parenteral and Enteral Nutrition 2004;28(6):442–446.
35. Jésus P, Marin B, Fayemendy P, et al. Resting energy expenditure equations in amyotrophic lateral sclerosis, creation of an ALS-specific equation. Clinical Nutrition 2019;38(4):1657–1665.
36. Flack KD, Siders WA, Johnson LA, Roemmich JN. Cross-Validation of Resting Metabolic Rate Prediction Equations. J Acad Nutr Diet 2016;116(9):1413–1422.
37. Tandan R, Levy EA, Howard DB, et al. Body composition in amyotrophic lateral sclerosis subjects and its effect on disease progression and survival. American Journal of Clinical Nutrition 2022;115(5):1378–1392.
38. Bouteloup C, Desport JC, Clavelou P, et al. Hypermetabolism in ALS patients : an early and persistent phenomenon. J Neurol 2009;256:1236–1242.
39. Desport J, Lacoste M, Preux P, Couratier P. Hypermetabolism in ALS : Correlations with Clinical and Paraclinical Parameters. Neurodegener Dis 2005;2(3–4):202–207.

40. Desport JC, Preux PM, Bouteloup-Demange C, et al. Validation of bioelectrical impedance analysis in patients with amyotrophic lateral sclerosis. American Journal of Clinical Nutrition 2003;77(5):1179–1185.
41. Fields DA, Goran MI, McCrory MA. Body-composition assessment via air-displacement plethysmography in adults and children: a review. Am J Clin Nutr 2002;75:453–467.
42. Von Hurst PR, Walsh DCI, Conlon CA, Ingram M, Kruger R, Stonehouse W. Validity and reliability of bioelectrical impedance analysis to estimate body fat percentage against air displacement plethysmography and dual-energy X-ray absorptiometry. Nutrition and Dietetics 2016;73(2):197–204.



Chapter 2

Prognostic Value of Weight Loss in Patients with Amyotrophic Lateral Sclerosis: a Population-Based Study

Mark R. Janse van Mantgem, M.D., Ruben P.A. van Eijk, M.D., PhD., Hannelore K. van der Burgh, MSc., Harold H.G. Tan, M.D., Henk-Jan Westeneng, M.D., Michael A. van Es, M.D., PhD, Jan H. Veldink, M.D., PhD, Leonard H. van den Berg, M.D., PhD.

Accepted at Journal of Neurology, Neurosurgery and Psychiatry

ABSTRACT

Objective: To determine the prevalence and prognostic value of weight loss (WL) prior to diagnosis in patients with amyotrophic lateral sclerosis (ALS).

Methods: We enrolled patients diagnosed with ALS between 2010 and 2018 in a population-based setting. At diagnosis, detailed information was obtained regarding the patient's disease characteristics, anthropological changes, ALS-related genotypes, and cognitive functioning. Complete survival data were obtained. Cox proportional hazard models were used to assess the association between WL and the risk of death during follow-up.

Results: The dataset comprised 2,420 patients of whom 67.5% reported WL at diagnosis. WL occurred in 71.8% of the bulbar-onset and in 64.2% of the spinal-onset patients; the mean loss of body weight was 6.9% (95% CI 6.8 – 6.9) and 5.5% (95% CI 5.5 – 5.6), respectively ($p < 0.001$). WL occurred in 35.1% of the patients without any symptom of dysphagia. WL is a strong independent predictor of survival, with a dose response relationship between the amount of WL and the risk of death: the risk of death during follow-up increased by 23% for every 10% increase in WL relative to bodyweight (HR 1.23, 95% CI 1.13 – 1.51, $p < 0.001$).

Conclusions: This population-based study shows that two-thirds of the patients with ALS have WL at diagnosis, which also occurs independent of dysphagia, and is related to survival. Our results suggest that WL is a multifactorial process that may differ from patient to patient. Gaining further insight in its underlying factors could prove essential for future therapeutic measures.

INTRODUCTION

In patients with amyotrophic lateral sclerosis (ALS), weight loss (WL) is an important clinical feature with a prevalence of 56%[1] to 62%.[2] WL has been related to functional and respiratory loss, contributing to both morbidity and mortality.[3] It follows that managing WL post-diagnosis may improve the prognosis of patients with ALS.

WL in patients with ALS may be multifactorial, with bulbar symptoms and/or dysphagia as leading cause. Interestingly, it may also occur independent of dysphagia as shown in a recent study.[2] Other mechanisms are, therefore, probably related to WL, which might explain the varying positive and negative results in studies on the beneficial effect of enteral nutrition.[4–7] Many studies focused on the prognostic effect of body mass index (BMI),[2,8–12] and WL[1,2,8,13–15] itself, and showed that patients with a lower BMI or WL at diagnosis have a poorer prognosis. Despite the value of these studies, they vary significantly in definition, methodology and/or results (table 1).[12,16] Consequently, the direct associations between survival, WL or BMI loss, their incremental value for prognostic tools such as the ENCALS survival model[17] or their relationship with the clinical phenotype, remains dubious.

In the present study, we, therefore, aim to determine the prognostic value of BMI and WL at time of diagnosis on survival in a large prospective population-based study of patients with ALS. In addition, we aim to assess the associations between WL and important clinical features, such as disease staging, dysphagia, site of symptom onset, cognition, and genetics.

METHODS

We conducted a prospective analysis of the national database of The Netherlands ALS Centre, Utrecht, The Netherlands. All patients with ALS in The Netherlands diagnosed with either possible, probable, probable laboratory-supported or definite ALS according to the revised El Escorial criteria (EEC)[18] are registered centrally at The Netherlands ALS Centre. The coverage rate of this population-based case-control study, named "Prospective ALS Study The Netherlands (PAN)" is approximately 81%:[19] mainly patients above 85 years old or those living near the border are being underreported. Complete case ascertainment was ensured by continuous recruitment through multiple sources: neurologists, rehabilitation physicians, the Dutch Neuromuscular Patient Association and our ALS centre website. The medical ethics committee and institutional board of the University Medical Centre Utrecht approved the protocol of this study.

Where possible, we collected clinical data at diagnosis or within a 3-month time-interval after diagnosis; otherwise data were recorded as missing. The definitions of the collected

Table 1. Literature overview of the prognostic value of pre-diagnostic WL and/or BMI loss in ALS patients

Author	Year	Study design	N	BMI or WL?	Multivariate analysis	HR (95% CI)	Interpretation of HR	Additional investigated factors in study
Limousin et al.	2010	Retrospective	63	Both	NR	NR	NA	Disease duration
Marin et al.	2011	Prospective	92	Both	ALSFRS at diagnosis, diagnostic delay, EEDC, FVC, MMT, BMI or WL	1.31 (1.08-1.60)	WL ≥ 5%	Site of disease onset
Shimizu et al.	2012	Prospective	77	BMI	Age at onset, FVC, BMI-RR	2.74 (1.47-5.13)	BMI-RR of > 2.5 kg/cm^2/y	FVC
Marin et al.	2016	Retrospective	261	WL	Age, ALSFRS-R, diagnostic delay, EEDC-R, site of onset, WL	1.45 (1.06-1.99)	WL >10%	None
Moglia et al.	2019	Retrospective	620	Both	Age at onset, ΔFRS, diagnostic delay, EEDC-R, COPD, WL slope	NR	NA	Dysphagia, site of disease onset
Present study	2019	Prospective	2,420	Both	Age at onset, ΔFRS, diagnostic delay, definite ALS[A], bulbar onset, FVC, presence of FTD, presence of *C9orf72* repeat expansion	1.23 (1.13-1.51)	WL per 10%	Disease severity, site of disease onset, dysphagia, *C9orf72* and *UNC13A* genotype, cognition, behaviour

Table summarizes the study design, number of patients with ALS, variables used in multivariate analysis and concomitant hazard ratio's (HR), and additional clinical characteristics in available studies on WL and/or BMI. Abbreviations: N = number of patients, BMI = body mass index, WL = weight loss, HR = hazard ratio, NR = not recorded, NA = not applicable, ALSFRS(-R) = ALS functional rating scale (- Revised), MMT = manual muscular testing, FVC = forced vital capacity, EEDC(-R) = El Escorial Diagnostic Criteria (– Revised), BMI-RR = BMI reduction rate, COPD = chronic obstructive pulmonary disease, ΔFRS = progression rate determined by: (48 – ALSFRS-R total score) / diagnostic delay, FTD = frontotemporal dementia. A = definite ALS according to the revised El Escorial criteria.

characteristics are specified below, and include: gender, age at diagnosis, age at onset, site of disease onset, date of symptom onset, diagnostic delay, presence of dysphagia, percentage of forced vital capacity (FVC) of predicted value, total score of the ALS functional rating scale – revised (ALSFRS-R), survival time, presence of *C9orf72* repeat expansion,[20] *UNC13A* genotype,[21] and height. Site of disease onset was divided into: bulbar, spinal, or thoracic/respiratory. Date of symptom onset was determined at the outpatient clinic as described in our Standard Operating Procedures (SOP) for Clinical Trials 2014: e.g. the date of symptom onset is determined in the middle of the month or the patient knows the exact date. Diagnostic delay was defined as the delay between date of diagnosis and date of symptom onset in months. We used item 3 of the ALSFRS-R questionnaire to assess the presence of dysphagia, defined by a score of ≤ 3. FVC is expressed as percentage of predicted. Survival time was defined as time between date of diagnosis and date of death or date last known alive. We used the municipal population register to check the date last known alive. This register is updated at quarterly intervals. Patients with more than 30 hexanucleotide repeats in the *C9orf72* gene were considered to be *C9orf72* carriers.[20] The genotype for the SNP, rs12608932, located in the *UNC13A* gene was divided into A/A, A/C and C/C.[21] Genetic data were handled anonymously, but we had to de-anonymize the data temporarily in order to match clinical data to genetic data. After linking these files, the data were re-anonymized according protocol.

Patients were classified into one of five prognostic subgroups according to the ENCALS survival model. This is an externally validated model for prediction of survival in patients with ALS. The model is described in more detail elsewhere.[17]

Cognitive and behavioural data

Cognitive and behavioural data were collected within two months of date of diagnosis. To assess cognitive impairment, we used three neuropsychological tests: the ALS-specific score from the 'Edinburgh Cognitive and Behavioural ALS Screen (ECAS),'[22,23] verbal fluency index (VFI) score,[24] and total score of the frontal assessment battery (FAB).[25] Prior to the development of the ECAS, VFI and FAB were used to screen for the presence of cognitive and/or behavioural changes. After the ECAS became available, it was used as the standard screening instrument. Patients were considered to have cognitive impairment if they scored abnormal on at least one of these tests. The ECAS ALS-specific score was defined as abnormal if a patient's residual score (i.e. observed score – expected score) was in the lowest 5.0% of the Dutch normative population, derived by using the percentile rank method on residuals.[23] VFI scores were transformed to standardized z-scores, and defined as abnormal if the score was below the fifth percentile.[24] Total FAB score of <13 was defined as abnormal.[25] To assess behavioural impairment, we used the ALS-Frontotemporal dementia-Questionnaire (ALS-FTD-Q)[26] and the ECAS behavioural scree.[22] An ALS-FTD-Q score of ≥22 points was defined

as abnormal or scoring two or more items on the ECAS behavioural screen.[27] Scoring apathy only on the ECAS behavioural screen was also defined as abnormal.

Weight loss at diagnosis

WL was determined at the outpatient clinic either by the nurse practitioner or medical doctor. All evaluations were made on the same day for every individual patient. However, if no WL data were available, we included WL data gathered from a questionnaire of the PAN within a 3-month time interval after date of diagnosis.[19] Medical correspondence was reviewed to gather remaining missing data.

We performed analyses with total and monthly absolute and relative WL. Total relative WL = (weight at diagnosis – weight at onset)/weight at onset. Monthly relative WL = (weight at diagnosis – weight at onset)/diagnostic delay. For illustrative purposes, we divided the amount of relative and absolute WL into four subgroups[4] of < 0%, 0% (no WL), <5%, 5-10%, ≥10% and < 0 kg, 0 kg (no WL), <5 kg, 5-10 kg, ≥10 kg, respectively. We calculated the BMI, or Quetelet index, at time of diagnosis according to the definition of BMI = weight/height^2, in kg/m^2.[28]

Statistical analysis

We performed our statistical analyses with RStudio (version 1.1.456, Rstudio: Integrated Development for R, Inc., Boston, USA, http://www.rstudio.com/). Missing data were accounted for by creating multiple imputed datasets (n=100) using predictive mean matching and bootstrapping, discarding the first 100 iterations (burn-in). Approximately 30.7% of missing data were accounted for by multiple imputations (MI). The imputation model contained all covariates; survival time was modelled using Nelson-Aalen estimates.[29] We used Rubin's rules to pool results across imputations.[30] Continuous variables were summarized as mean and standard deviation (SD), and categorical variables as frequency and proportion. To assess the effect of WL in different subgroups, we related WL to the following clinical characteristics or criteria: the revised EEC, ALSFRS-R score and ΔFRS (i.e. progression rate determined by: (48 – ALSFRS-R total score) / diagnostic delay),[31] King's clinical staging system,[32] prognostic subgroups,[17] *UNC13A* genotype, *C9orf72* repeat expansion, data on behavioural and cognitive impairment, site of disease onset and whether dysphagia was present. We determined the effect of these different clinical characteristics or criteria on WL by linear regression. Correlations of continuous variables were assessed using Pearson's correlation coefficient.

To assess the association between the risk of death and WL, we used Cox proportional hazard models. We adjusted all models for the eight clinical predictors from the ENCALS survival model,[17] namely: ΔFRS, diagnostic delay, age at onset, FVC, bulbar onset, definite ALS according to the revised EEC,[18] presence of frontotemporal dementia (FTD) and the presence of *C9orf72* repeat expansion.

Hazard ratios (HR) were obtained and reported with their 95% confidence intervals (95% CI). In order to evaluate the independent prognostic value of WL, we first created a model that contained the eight prognostic variables from the ENCALS survival model. Subsequently, we created a second model with an additional term for WL. To compare the two models, we determined the difference in likelihood ratios and Akaike's information criterion (AIC). Additionally, the concordance statistic (C-statistic) was determined to evaluate the improvement in apparent predictive performance. A similar strategy was used to evaluate the added value of dysphagia. Next, we used either quadratic or cubic transformations to assess non-linear associations between the risk of death during follow-up and WL. A similar strategy was used to obtain the independent prognostic value of BMI and/or dysphagia. In addition, we evaluated the interaction between dysphagia and WL. For illustrative purposes, we provide the Kaplan-Meier curves of categorized WL from time of symptom onset as well as from diagnosis. A p-value < 0.05 was considered statistically significant.

Data availability statement

All protocol, analyses, and anonymized data will be shared by request from any qualified investigator. We take full responsibility for the data, the analyses and interpretation, and the conduct of the research.

RESULTS

In total, we enrolled 2,420 patients with ALS with a total follow-up time of 3,773 person-years during which 1,836 deaths (75.9% of the patients) occurred. Included patients were diagnosed between 1 January 2010 and 23 May 2018. Overall median survival after diagnosis was 16.9 months (95% CI 16.2 – 17.5). Baseline characteristics of the cohort are listed in table 2. Differences between patients with and without missing data on WL are shown in supplement table 1.

Table 2. Baseline demographics and clinical characteristics at date of diagnosis

Characteristics	Overall (N = 2,420)
Males, no. (%)	1,380 (57.0)
Age at diagnosis, years	65.7 (10.9)
Diagnostic delay, months*	9.4 (6.1 – 15.5)
Dysphagia	
Yes (%)	1,068 (44.1)
No (%)	1,352 (55.9)
Site of disease onset	
Bulbar (%)	812 (33.5)
Spinal (%)	1,521 (62.9)
Thoracic/respiratory (%)	87 (3.6)
El Escorial	
Definite ALS (%)	576 (23.8)
Probable ALS (%)	882 (36.4)
Probable ALS lab-supported (%)	447 (18.5)
Possible ALS (%)	515 (21.3)
FVC, %	85.9 (27.5)
ALSFRS-R score	38.6 (8.6)
ΔFRS*	0.66 (0.33 – 1.59)
C9orf72 status	
Repeat (%)	209 (8.6)
Normal (%)	2211 (91.4)
UNC13A genotype	
A/A (%)	945 (39.0)
A/C (%)	1,064 (44.0)
C/C (%)	411 (17.0)
Cognitive impairment	
Yes (%)	481 (19.9)
No (%)	1,939 (80.1)
Behavioural impairment	
Yes (%)	751 (31.0)
No (%)	1,669 (69.0)
Weight at diagnosis, kg	73.5 (17.4)
Weight loss data	
Total absolute weight loss, kg	5.1 (8.6)
Total relative weight loss, %	6.2 (9.7)
Absolute weight loss per month, kg	0.60 (1.2)
Relative weight loss per month, %	0.72 (1.4)
BMI at diagnosis, kg/m^2	24.6 (5.2)

The dataset represents mean (SD), unless indicated otherwise, over 100 imputations. * = Data are median values with 25%-75% inter-quartile range (IQR).

Abbreviations: ALS = amyotrophic lateral sclerosis, ALSFRS-R = amyotrophic lateral sclerosis functional rating scale revised, ΔFRS = progression rate determined by: (48 – ALSFRS-R total score) / diagnostic delay, FVC = forced vital capacity. BMI = body mass index.

Baseline characteristics and weight loss

Of all patients, 3.2% were underweight (i.e. BMI < 18.5) and 8.1% obese (i.e. BMI ≥ 30.0) at diagnosis.[28] At diagnosis, 67.5% of all patients reported WL; in 50.4% WL was ≥ 5% and in 26.5% ≥ 10%. Approximately one-third of the patients – in different clinical stages – had not lost any weight at time of diagnosis. Patients with bulbar, spinal or thoracic/respiratory onset reported WL in 71.8%, 64.2% and 86.6% of the cases, respectively ($p < 0.001$). Mean relative WL in these subgroups was 6.9% (95% CI 6.8 – 6.9), 5.5% (95% CI 5.5 – 5.6), and 11.0% (95% CI 10.8 – 11.2), respectively ($p < 0.001$). Dysphagia was present in 44.1% of the patients. At diagnosis, WL occurred in 73.5% of those with and 62.8% of those without dysphagia, with a mean WL of 7.5% (95% CI 7.5 – 7.6) and 5.1% (95% CI 5.1 – 5.2), respectively ($p < 0.001$). This suggests that WL is not solely related to dysphagia. Results were similar for non-imputed data.

Figure 1 shows the relationship between relative WL and various clinical aspects of ALS. The revised EEC, total ALSFRS-R score, King's staging, and prognostic subgroups[17] were related to WL (all $p < 0.001$). Interestingly, no statistical differences in cognitive and behavioural impairment or *C9orf72* and *UNC13A* risk genotypes were associated with WL. This could be due to the amount of missing data in these variables.

Figure 1. Relative weight loss stratified by clinical, genetic, behavioural and cognitive data

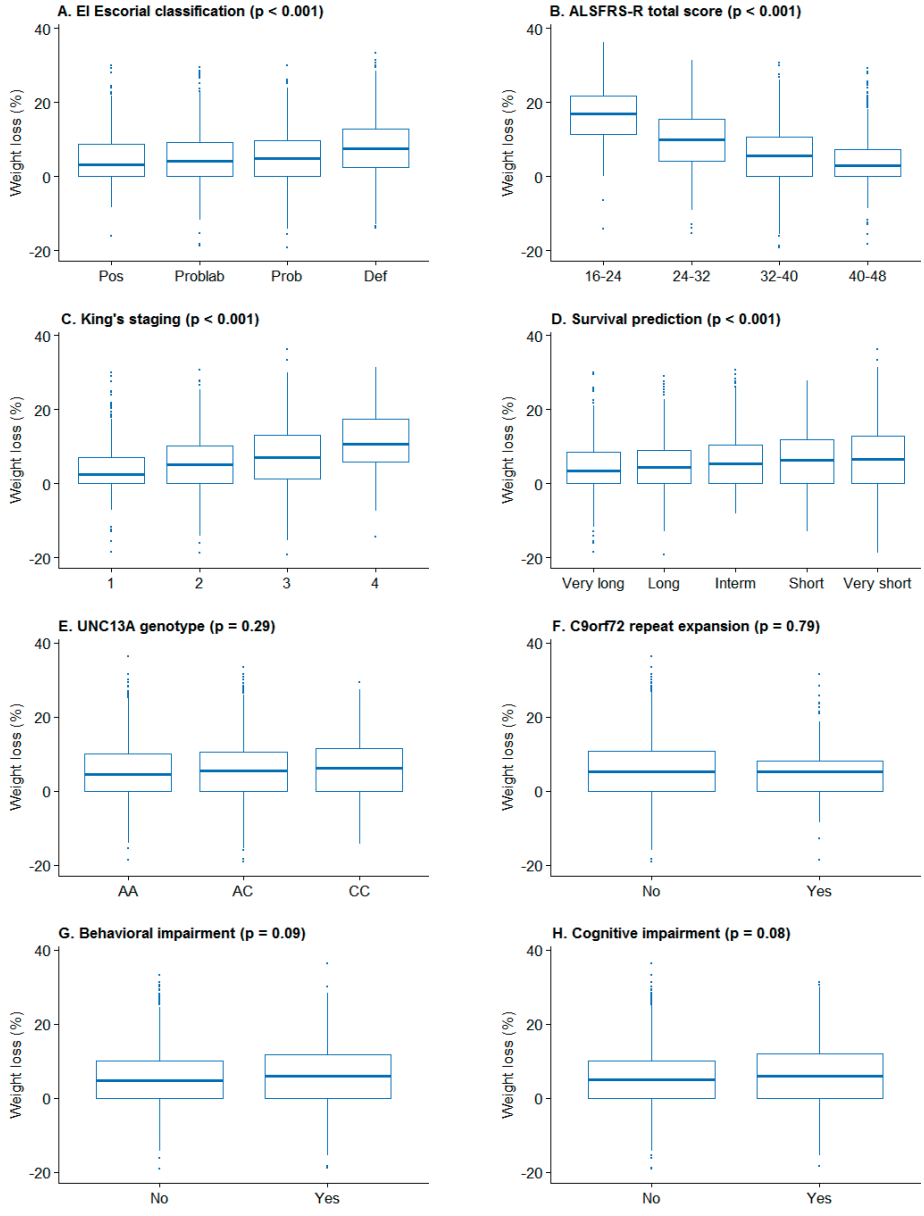


Figure 1. Boxplots are summarizing relative weight loss subdivided into (A) El Escorial classification, (B) total ALSFRS-R score, (C) King's clinical stage, (D) prognostic subgroups as defined by Westeneng et. Al,[17] I *UNC13A* genotype, (F) *C9orf72* repeat expansion, (G) Behavioural impairment and (H) Cognitive impairment. Abbreviations: Def = Definite ALS, Prob = probable ALS, Problab = Probable ALS laboratory supported, Pos = possible ALS; Very long = predicted very long, Long = predicted very long, Interm = predicted intermediate, Short = predicted short, Very short = predicted very short survival according to the ENCALS survival model; AA = *UNC13A* A/A genotype, AC = *UNC13A* A/C genotype, CC = *UNC13A* C/C genotype. P values are based on the likelihood ratio test.

Table 3 shows the number of patients tested for each cognitive or behavioural test. No differences in WL between male and female patients were found ($p = 0.41$). Pearson's r between WL and FVC was -0.32 (95% CI -0.36 – -0.29, $p < 0.001$), between WL and ALSFRS-R total score -0.37 (95% CI -0.40 – -0.34, $p < 0.001$), and between WL slope and ΔFRS 0.37 (95% CI 0.33 – 0.40, $p < 0.001$).

Table 3. Overview of the used screening instruments to assess cognitive and behavioural impairment

Screening instrument	Data available	Data not available
Cognitive impairment		
ECAS, ALS specific score (%)	452 (18.7)	1,968 (81.3)
VFI (%)	13 (0.5)	2,407 (99.5)
FAB (%)	906 (37.4)	1,514 (62.6)
Behavioural impairment		
ALS-FTD-Q (%)	878 (36.3)	1,542 (63.7)
ECAS behavioural screen (%)	154 (6.4)	2,266 (93.6)

The dataset represents total number (n) and n/N (%). The data fulfilled the criteria to be collected within two months of date of diagnosis. Abbreviations: ECAS = Edinburgh cognitive and behavioural ALS screen, ALS = amyotrophic lateral sclerosis, VFI = verbal fluency index, FAB = frontal assessment battery, ALS-FTD-Q = ALS-frontotemporal dementia-questionnaire.

Survival and weight loss

The adjusted HR for absolute WL in kg was 1.03 (95% CI 1.02 – 1.04, $p < 0.001$), indicating that with each additional kilo of WL the risk of dying during follow-up increases by 3%. Similarly, BMI had an HR of 0.96 (95% CI 0.94 – 0.99, $p = 0.005$), indicating that a lower BMI at diagnosis increases the risk of death. For illustrative purposes we categorized relative and absolute WL into four categories (figure 2).

Median survival after diagnosis with and without WL was 14.7 (95% CI 13.9 – 15.7) *vs* 22.5 months (95% CI 20.5 – 24.7), respectively, resulting in an adjusted HR of no WL *vs* WL of 1.40 (95% CI 1.23 – 1.60, $p < 0.001$). In figure 3, we illustrate a strong dose-response relationship between relative WL (figure 3A) and the risk of death during follow-up ($p < 0.001$). Gaining weight prior to diagnosis lowers the risk of death during follow-up. A similar association was observed for BMI, however, BMI might be a nonlinear entity (figure 3B).

Figure 2. Time to event analysis according to relative and absolute weight loss

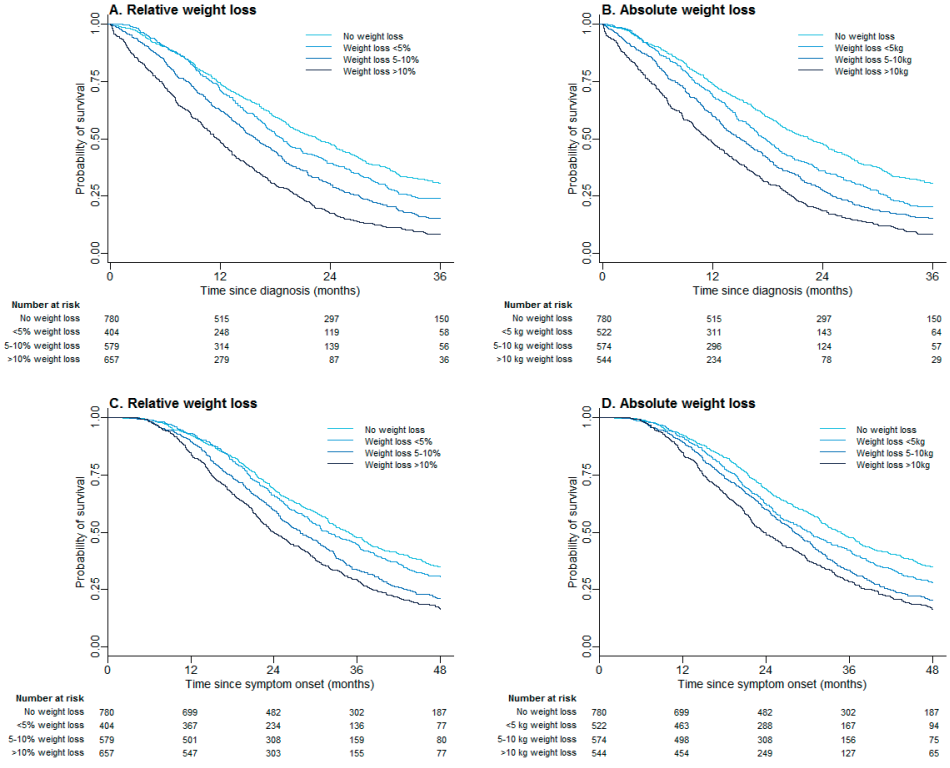


Figure 2. Panels A and B are the observed Kaplan-Meier curves of the overall survival pattern since date of diagnosis, whereas panels C and D show the overall survival pattern since disease onset. Relative weight loss (WL) was calculated by dividing the observed WL at diagnosis by weight at onset.

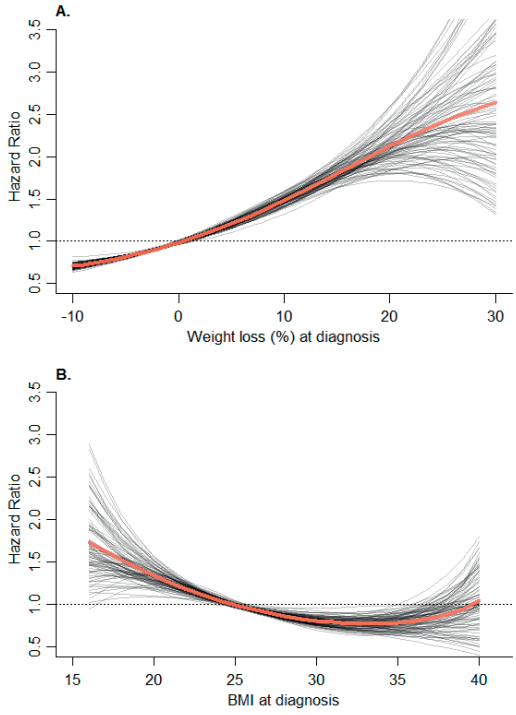


Figure 3. Dose-response relationship between weight loss and risk of death during follow-up

Cox proportional hazard model of the relationship between weight loss (WL) and risk of death, adjusted for the predicted prognosis,[17]. The dark coloured lines represent the modelled relationship in every imputed dataset and reflect the uncertainty of the mean effect in the data. The red line represents the pooled relationship over imputed datasets.

Panel A shows the hazard risk ratio of increased relative WL at diagnosis on survival ($p < 0.001$). Panel B summarizes the relationship between risk of death and BMI at diagnosis ($p = 0.005$).

As shown in **figure 4**, site of symptom onset and dysphagia were related to WL. A significant difference in survival was observed in spinal-onset patients depending on whether or not they had experienced WL (log-rank $p < 0.001$), as well as in patients with bulbar onset with and without WL (log-rank $p < 0.001$). Interestingly, the effect of WL on survival did not differ significantly between patients with or without dysphagia ($p = 0.46$), nor between bulbar and spinal onset patients ($p = 0.63$). This effect of WL on survival did neither differ between male and female patients ($p = 0.57$) (*not shown*).

Figure 4. Effect of weight loss stratified by site of symptom onset and the presence of dysphagia

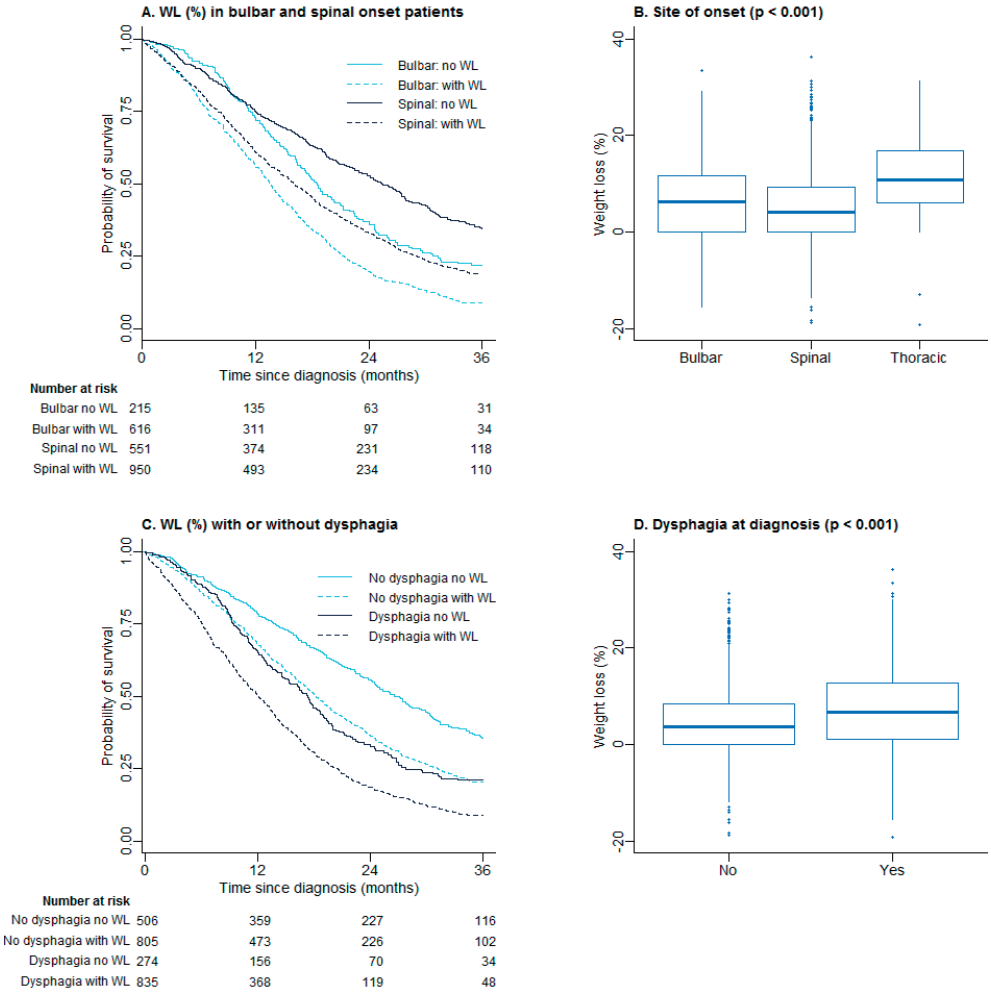


Figure 4. Boxplots are summarizing relative weight loss subdivided into (B) site of symptom onset and (D) whether dysphagia was present at diagnosis in patients with ALS. P-values are based on the likelihood ratio test. Panels A and C are the observed Kaplan-Meier curves of the overall survival pattern. Abbreviations: WL = weight loss.

The independent prognostic value of weight loss

Finally, we obtained the best fitting model in our multivariate analysis with relative WL compared to absolute WL (AIC 23,997 vs. 24,003, respectively), albeit the difference was minimal. Table 3 shows the effect of adding relative WL to the eight covariates from the ENCALS survival model.[17] Model 1 includes these eight covariates only; model 2 includes the covariates of model 1, but WL has been added. A decrease in AIC (24,031 *vs.* 23,997, *p*

< 0.001), and a slight increase in C-statistics (79.41*vs.* 79.50) was achieved when adding WL to the original eight covariates from the ENCALS survival model (table 3). When we used dysphagia instead of relative WL as covariate in our multivariate model, the model did not improve, and C-statistic decreased to 79.36. The effect sizes of the original eight predictors remained practically unchanged – and are statistically significant – when WL was added. This may suggest that relative WL carries unique information about the prognosis of patients with ALS.

Table 3. Weight loss in a multivariate model

Covariate	Model 1		Model 2	
	HR (95% CI)	*p-value*	*HR (95% CI)*	*p-value*
Bulbar onset	1.19 (1.07 – 1.33)	0.002	1.18 (1.06 – 1.32)	0.003
FTD	1.29 (1.02 – 1.63)	0.032	1.30 (1.03 – 1.64)	0.026
Definite ALS	1.43 (1.25 – 1.64)	< 0.001	1.36 (1.18 – 1.57)	< 0.001
Diagnostic delay (months)*	0.21 (0.17 – 0.25)	< 0.001	0.19 (0.15 – 0.23)	< 0.001
FVC*	1.28 (1.18 – 1.38)	< 0.001	1.23 (1.14 – 1.34)	< 0.001
ΔFRS*	0.51 (0.43 – 0.61)	< 0.001	0.55 (0.46 – 0.65)	< 0.001
C9orf72	1.19 (0.98 – 1.44)	0.08	1.20 (0.99 – 1.46)	0.06
Age at onset (years)*	1.03 (1.03 – 1.04)	< 0.001	1.03 (1.03 – 1.04)	< 0.001
Total WL (per 10%)			1.23 (1.13 – 1.51)	< 0.001
C-statistic	79.41		79.50	
AIC	24,031		23,997	

Data are HRs with corresponding p-values, and determined with a cox proportional hazards model. P-values indicate if a factor fits into the model.
Model 1 = model with the eight covariates from the ENCALS survival model. Model 2 = addition of relative WL to Model 1.
Abbreviations: HR = hazard ratio, FTD = frontotemporal dementia, FVC = forced vital capacity, ΔFRS = progression rate determined by: (48 – ALSFRS-R total score at diagnosis) / diagnostic delay, WL = weight loss, AIC = Akaike's information criterion. * Factors are converted as described by Westeneng et al.[17]

DISCUSSION

In this prospective, population-based study, we showed that WL occurs in approximate two-thirds of the patients with ALS at the time of diagnosis, irrespective of the presence of bulbar symptoms. WL appeared to be an independent predictor of survival with a dose-response relationship between the relative amount of WL and risk of death. These results suggest that – when taking WL into account – the patient's prognosis might be predicted more accurately. Gaining further insight into the underlying factors causing WL could prove essential for future therapeutic measures, because it may be a multifactorial process that could differ from patient to patient.

The underlying cause of WL was indicated to be multifactorial by our finding that WL also occurs in 31.4% of the patients without dysphagia or other bulbar symptoms. Bulbar symptoms may result in a decreased food intake and, therefore, to WL. Another important factor contributing to WL at diagnosis is muscle wasting by loss of motor neurons.[33,34] A previous study has shown that, on a population level, primarily fat-free mass (i.e. muscle mass) declines over time, whereas the mean fat mass remains relatively stable.[34] This is supported by our finding that the extent of functional loss – a surrogate of muscle strength – correlates significantly with WL at diagnosis. However, this correlation does not account for all variation in WL, which may suggest distinct disease mechanisms. Moreover, the reported values are population averages, and there may be a considerable amount of between-patient variability. Other causing factors have been described, such as an altered metabolic state,[33,35-37] an altered olfaction and gustation,[35] the loss of appetite,[34] secondary factors, and cognitive disturbances.[4] Note that we did not find a significant association between cognitive disturbances and WL. A hypermetabolic state has been reported in 41%[36] to 55%[37] of the patients with ALS, and may result in nutritional imbalance, and, consequently, in WL.[33] However, the evidence of a hypermetabolic state on WL at diagnosis is varying. This is mainly due to different methodologies or might be effected by dietary interventions during study procedures.[36–38] An altered olfaction, gustation,[35] and the loss of appetite,[34,39] may lead to a changed dietary pattern and lower energy intake. Loss of appetite is reported in 29%[34] to 47%[39] of the patients with ALS. Secondary factors, for example, loss of autonomy due to disease progression or side-effects of medication may result in WL.[4] Thus, as the cause of WL most likely varies from individual to individual, it would seem important in future studies to: (1) determine the change in body composition – by, for example, air displacement plethysmography or bioelectrical impedance analysis – rather than total WL, and (2) obtain detailed insight in the patient's metabolic balance, food intake, activity level, metabolic and/or hormonal balance.[40]

Indeed, this multifactorial process of WL suggests that therapeutic interventions may also have to be individually tailored to each underlying factor. For this reason, it may be debatable whether patients with ALS require enteral feeding, and if they do, whether they need the same food composition or intervention. Moreover, we have shown that a group of patients across all clinical stages, as demonstrated by the King's staging and ALSFRS-R score, does not lose weight (figure 1). This implies the need for individually tailored interventions, because not every intervention might effectively slow WL across all patients with ALS. To illustrate, if WL is mainly due to muscle wasting, enteral nutrition may not be effective, whereas an increased caloric intake or administration of food supplements may ameliorate WL due to, for example, a hypermetabolic state. This was recently confirmed in a large clinical trial, where high-caloric intake does only prolonged survival in patients with a fast-progressing sub-type, underscoring the need for personalized nutritional interventions.[41]

BMI has mainly been assessed as categorical variable according to the definition of the WHO.[28] This may have resulted in an underestimation of the impact of WL. Moreover, a large heterogeneity is seen in studies that assessed BMI (table 1)[12,16] and the use of BMI might not be an accurate measure to estimate the amount of fat mass in patients with ALS.[42] In 2011 and 2016 Marin et al. were able to show an increased risk of death when WL of 5% or 10%, respectively, occurred.[13,14] They were not able to reproduce the increased death risk of 5% WL in their 2016 study, but our study confirms that any reported WL at diagnosis results in a worse prognosis with a clear dose-response relationship. Recently, Moglia et al[2] recommended stratifying patients in clinical trials according to the presence of dysphagia at time of enrolment, instead of stratifying patients based on their site of symptom onset. We believe that stratification based on multivariate prediction models, such as the ENCALS survival model,[17] seems more appropriate, because dysphagia or site of symptom onset are only a part of the prognostic determinants.[43] Moreover, concerning our data, the ENCALS survival model did not improve when we added dysphagia as covariate, but it did when we added total relative WL. Larger gains could be achieved in the future, because more clinical variables have shown their relevance in predicting survival in patients with ALS (e.g. neurofilaments[44] or plasma creatinine[45]). This underscores the need to update, modify and validate our current prediction tools.

The main strengths of this prospective study are the population-based design, the excellent follow-up on survival time, and the large sample size, allowing assessment of the independent prognostic value of WL. Moreover, we handled WL as a continuous variable, and consequently showed that any WL is an important independent predictor of survival, albeit with a strong dose-response relationship. This enabled us to investigate the additional value of WL at diagnosis to the overall prediction accuracy of the ENCALS survival model[17] (table 3).The slight improvement might be caused by collinearity of WL with other predictors in the model, such as diagnostic delay and ΔFRS. However, WL – and all other predictors – remained significant within model 2, showing that WL is an independent predictor of survival, and is not collinear with the other predictors. Therefore, this could result in better prognostication for our patients and more efficient patient selection for clinical trials.[46] Finally, in our study we were able to associate WL with relevant and frequently used clinical parameters, such as: El Escorial classification, King's staging, relation with ALSFRS-R score, ΔFRS, site of symptom onset, and dysphagia. As a result, clinicians may be able to use our results easily.

The main limitation of this study is the missing information on causative factors related to WL, as previously described,[33–37,39] and the assessment of total WL rather than a subdivision in loss of fat and fat-free mass. Consequently, we were only able to reliably estimate the total prevalence of WL in patients with ALS. Longitudinal data on more detailed parameters may help significantly to unravel the sources of WL, estimate their contribution to the total WL and, in the end, improve the delivery of tailored care to the individual patient. Furthermore,

data on several clinically important parameters were missing from our study. By performing multiple imputations of the missing values,[29,47] we were able to retain all cases in our database and, therefore, preventing selection bias, but possibly at the cost of a decrease in precision. Nevertheless, due to the large sample size, we were able to accurately estimate population-averaged associations between most patient characteristics and WL. However, large amounts of cognitive data were missing and our study may have lacked power in estimating the association with WL. Similarly, although we were able to assess the relation of WL with *C9orf72* and *UNC13A*, it could be of interest for future studies to further explore relationships with other ALS-related genes, such as *SOD1*, *FUS* or *TARDBP*.

In conclusion, this study shows that WL at time of diagnosis has a significant prognostic value for survival in patients with ALS. The severity of WL is related to a higher risk of death and is not solely due to the presence of bulbar symptoms or dysphagia. Multifactorial causes are involved in the genesis of WL, and sorting out these causes in every individual patient with ALS might result in well-tailored care, and improved therapeutic strategies.

REFERENCES

1. Körner S, Hendricks M, Kollewe K, et al. Weight loss, dysphagia and supplement intake in patients with amyotrophic lateral sclerosis (ALS): Impact on quality of life and therapeutic options. BMC Neurology 2013;13
2. Moglia C, Calvo A, Grassano M, et al. Early weight loss in amyotrophic lateral sclerosis : outcome relevance and clinical correlates in a population-based cohort. J Neurol Neurosurg Psychiatry 2019;90(6):666–673.
3. Kasarskis J. Nutritional status of patients with amyotrophic lateral sclerosis: relation to the proximity of death. The American Journal of clinical nutrition 1996;63(1):130–137.
4. Greenwood DI. Nutrition management of amyotrophic lateral sclerosis. Nutrition in Clinical Practice 2013;28(3):392–399.
5. Cui F, Sun L, Xiong J, Li J, Zhao Y, Huang X. Therapeutic effects of percutaneous endoscopic gastrostomy on survival in patients with amyotrophic lateral sclerosis: A meta-analysis. PloS ONE 2018;13(2):1–13.
6. Kellogg J, Bottman L, Arra EJ, Selkirk SM, Kozlowski F. Nutrition management methods effective in increasing weight, survival time and functional status in ALS patients: a systematic review. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 2018;19(1–2):7–11.
7. Fasano A, Fini N, Ferraro D, et al. Percutaneous endoscopic gastrostomy, body weight loss and survival in amyotrophic lateral sclerosis: a population-based registry study. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 2017;18(3–4):233–242.
8. Limousin N, Blasco H, Corcia P, et al. Malnutrition at the time of diagnosis is associated with a shorter disease duration in ALS. Journal of the Neurological Sciences 2010;297(1–2):36–39.
9. Desport JC, Preux PM, Truong TC, Vallat JM, Sautereau D, Couratier P. Nutritional status is a prognostic factor for survival in ALS patients. Neurology 1999;53(5):1059–1063.
10. Jawaid A, Murthy SB, Wilson AM, et al. A decrease in body mass index is associated with faster progression of motor symptoms and shorter survival in ALS. Amyotrophic Lateral Sclerosis 2010;11(6):542–548.
11. Paganoni SMP, Jing Deng M, Matthew Jaffa B, Cudkowicz ME, Wills A-M. Body mass index, not dyslipidemia, is an independent predictor of survival in amyotrophic lateral sclerosis. Muscle Nerve 2011;44(1):20–24.
12. Ning P, Yang B, Li S, Mu X, Shen Q, Xu Y. Systematic review of the prognostic role of body mass index in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 2019;20(5–6):356–367.
13. Marin B, Desport JC, Kajeu P, et al. Alteration of nutritional status at diagnosis is a prognostic factor for survival of amyotrophic lateral sclerosis patients. J Neurol Neurosurg Psychiatry 2011;82:628–634.
14. Marin B, Arcuti S, Jesus P, et al. Population-based evidence that survival in amyotrophic lateral sclerosis is related to weight loss at diagnosis. Neurodegenerative Diseases 2016;16(3–4):225–234.
15. Shimizu T, Nakayama Y, Matsuda C, et al. Prognostic significance of body weight variation after diagnosis in ALS: a single-centre prospective cohort study. Journal of Neurology 2019;266(6):1412–1420.
16. Dardiotis E, Siokas V, Sokratous M, et al. Body mass index and survival from amyotrophic lateral sclerosis. Neurology 2018;8(5):1–8.

17. Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a ersonalized prediction model. The Lancet Neurology 2018;17(5):423–433.
18. Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis 2000;1(5):293–299.
19. Huisman MHB, De Jong SW, Van Doormaal PTC, et al. Population based epidemiology of amyotrophic lateral sclerosis using capture-recapture methodology. Journal of Neurology, Neurosurgery and Psychiatry 2011;82(10):1165–1170.
20. Cooper-Knock J, Hewitt C, Highley JR, et al. Clinico-pathological features in amyotrophic lateral sclerosis with expansions in C9ORF72. Brain 2012;135(3):751–764.
21. Diekstra FP, van Vught PWJ, van Rheenen W, et al. UNC13A is a modifier of survival in amyotrophic lateral sclerosis. Neurobiology of Aging 2012;33(3):630.e3-630.e8.
22. Abrahams S, Newton J, Niven E, Foley J, Bak TH. Screening for cognition and behaviour changes in ALS. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 2014;15(1–2):9–14.
23. Bakker LA, Schröder CD, Spreij LA, et al. Derivation of norms for the Dutch version of the Edinburgh cognitive and behavioral ALS screen. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 2019;1–2:19–27.
24. Beeldman E, Jaeger B, Raaphorst J, et al. The verbal fluency index : Dutch normative data for cognitive testing in ALS. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 2014;5(6):388–391.
25. Terada T, Miyata J, Obi T, et al. Frontal assessment battery and frontal atrophy in amyotrophic lateral sclerosis. Brain and Behavior 2017;7(6):1–9.
26. Raaphorst J, Beeldman E, Schmand B, et al. A new screening tool for behavioral disturbances in ALS. Neurology 2012;79(13):1377–1383.
27. Strong MJ, Abrahams S, Goldstein LH, et al. Amyotrophic lateral sclerosis – frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 2017;18(3–4):153–174.
28. World Health Organization regional office for Europe. Nutrition. Body Mass Index – BMI. 2018;(accessed 05-11-2018)
29. White I, Royston P. Imputing missing covariate values for the Coxmodel. Statist. Med. 2009;28(10):1982–1998.
30. Rubin D, Schenker N. Multiple imputation in health-care databases: an overview and some applications. Stat. Med. 1991;10(4):585–598.
31. Kimura F, Fujimura C, Ishida S, et al. Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. Neurology 2006;66(2):265–267.
32. Roche JC, Rojas-Garcia R, Scott KM, et al. A proposed staging system for amyotrophic lateral sclerosis. Brain 2012;135(3):847–852.
33. Dupuis L, Pradat PF, Ludolph AC, Loeffler JP. Energy metabolism in amyotrophic lateral sclerosis. The Lancet Neurology 2011;10(1):75–82.
34. Ngo ST, van Eijk RPA, Chachay V, et al. Loss of appetite is associated with a loss of weight and fat mass in patients with amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 2019;0:1–9.
35. Viguera C, Wang J, Mosmiller E, Cerezo A, Maragakis NJ. Olfactory dysfunction in amyotrophic lateral sclerosis. Annals of Clinical and Translational Neurology 2018;5(8):976–981.

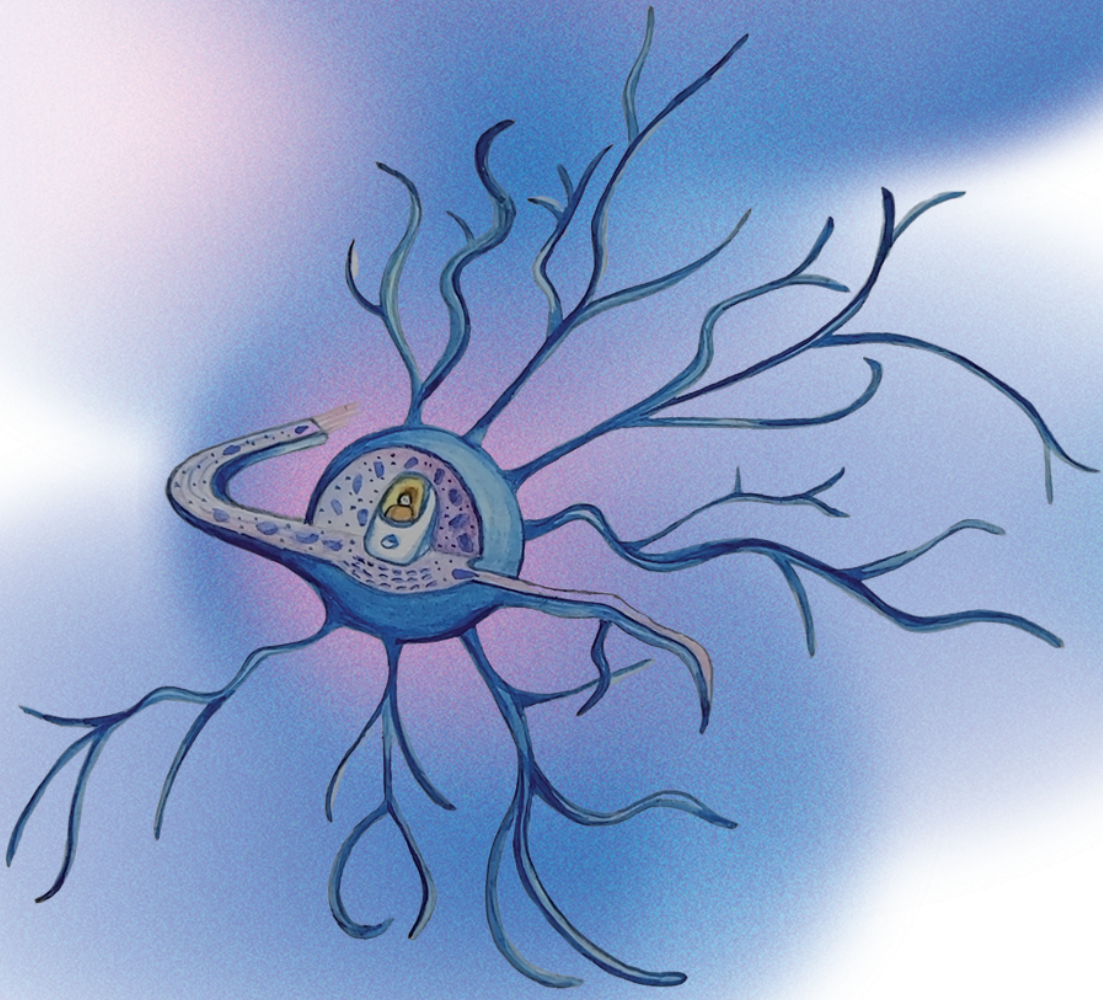
36. Steyn FJ, Ioannides ZA, Van Eijk RPA, et al. Hypermetabolism in ALS is associated with greater functional decline and shorter survival. Journal of Neurology, Neurosurgery and Psychiatry 2018;89:1016–1023.
37. Jésus P, Fayemendy P, Nicol M, et al. Hypermetabolism is a deleterious prognostic factor in patients with amyotrophic lateral sclerosis. European Journal of Neurology 2018;25(1):97–104.
38. Bouteloup C, Desport JC, Clavelou P, et al. Hypermetabolism in ALS patients : an early and persistent phenomenon. Journal of Neurology 2009;256:1236–1242.
39. Holm T, Maier A, Wicks P, et al. Severe Loss of Appetite in Amyotrophic Lateral Sclerosis Patients: Online Self-Assessment Study. Interactive Journal of Medical Research 2013;2(1):1–10.
40. Ngo ST, Steyn FJ, Huang L, et al. Altered expression of metabolic proteins and adipokines in patients with amyotrophic lateral sclerosis. Journal of the Neurological Sciences 2015;357(1–2):22–27.
41. Ludolph AC, Dorst J, Dreyhaupt J, et al. Effect of High-Caloric Nutrition on Survival in Amyotrophic Lateral Sclerosis. Annals of Neurology 2020;87(2):206–216.
42. Ioannides ZA, Steyn FJ, Henderson RD, Mccombe PA, Ngo ST. Anthropometric measures are not accurate predictors of fat mass in ALS. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration 2017;18(7–8):486–491.
43. Eijk RPA Van, Westeneng H, Nikolakopoulos S, Verhagen IE. Refining eligibility criteria for amyotrophic lateral sclerosis clinical trials. Neurology 2019;92(5):451–460.
44. Lu CH, Petzold A, Topping J, et al. Plasma neurofilament heavy chain levels and disease progression in amyotrophic lateral sclerosis: Insights from a longitudinal study. Journal of Neurology, Neurosurgery and Psychiatry 2015;86(5):565–573.
45. Van Eijk RPA, Eijkemans MJC, Ferguson TA, Nikolakopoulos S, Veldink JH, Van Den Berg LH. Monitoring disease progression with plasma creatinine in amyotrophic lateral sclerosis clinical trials. Journal of Neurology, Neurosurgery and Psychiatry 2018;89(2):156–161.
46. Eijk RPA Van, Westeneng H, Nikolakopoulos S, Verhagen IE. Refining eligibility criteria for amyotrophic lateral sclerosis clinical trials. Neurology 2019;92(5):451–460.
47. Eekhout I, De Vet HCW, Twisk JWR, Brand JPL, De Boer MR, Heymans MW. Missing data in a multi-item instrument were best handled by multiple imputation at the item score level. Journal of Clinical Epidemiology 2014;67(3):335–342.

Supplement table 1. Overview of baseline demographics and clinical characteristics between patients with and without missing data on weight loss at date of diagnosis

Characteristics	Weight loss not missing (N = 1,406)	Weight loss missing (N = 1,014)	p-value
Gender			0.85
Male (%)	799 (56.8)	581 (57.3)	
Female (%)	607 (43.2)	433 (42.7)	
Age at diagnosis (years)	65.9 (10.7)	65.5 (11.2)	0.38
Diagnostic delay (months)*	9.4 (6.0 – 15.4)	9.7 (6.1 – 15.5)	0.03
Dysphagia			< 0.001
Yes (%)	509 (36.2)	151 (14.9)	
No (%)	623 (44.3)	292 (28.8)	
Unknown (%)	274 (19.5)	571 (56.3)	
Site of disease onset			0.03
Bulbar (%)	396 (28.2)	244 (24.1)	
Spinal (%)	672 (47.8)	538 (53.1)	
Thoracic/respiratory (%)	42 (3.0)	27 (2.7)	
Unknown (%)	296 (21.1)	205 (20.2)	
FVC (%)	88.3 (21.8)	88.3 (22.2)	0.96
ALSFRS-R score	39.6 (5.2)	39.8 (4.7)	
ALSFRS-R slope*	0.71 (0.39 – 1.38)	0.65 (0.33 – 1.11)	0.12
C9orf72 status			0.99
Repeat (%)	80 (8.5)	69 (8.6)	
Normal (%)	866 (91.5)	734 (91.4)	
Unknown (%)	460 (32.7)	211 (20.8)	
UNC13A genotype			0.56
AA (%)	412 (38.6)	282 (40.3)	
AC (%)	473 (44.3)	302 (43.2)	
CC (%)	182 (17.1)	115 (16.5)	
Unknown	339 (24.1)	315 (31.1)	
Cognitive impairment			0.63
Yes (%)	150 (10.7)	38 (3.7)	
No (%)	640 (45.5)	182 (17.9)	
Unknown (%)	616 (43.8)	794 (78.3)	
Behavioural impairment			0.76
Yes (%)	223 (15.9)	54 (3.8)	
No (%)	508 (36.1)	132 (13.0)	
Unknown (%)	675 (48.0)	828 (81.2)	

The dataset represents mean (SD) or n/N (%). *Data are median values with 25%-75% inter-quartile range (IQR). Abbreviations: ALS = amyotrophic lateral sclerosis, ALSFRS-R = amyotrophic lateral sclerosis functional rating scale revised, FVC = forced vital capacity.

P values are indicating if there are differences between patients with missing data on weight loss (WL) and patients without missing data on WL at diagnosis. Data are recorded as missing when no data was available or the data was not gathered within two months after date of diagnosis. P values are independent t-tests for continuous variables and Chi-square tests for categorical variables.



Chapter 3

The Association between Serum Lipids and Survival in Patients with Amyotrophic Lateral Sclerosis: a Meta-Analysis and Population-Based Study

Mark R. Janse van Mantgem, M.D., Wouter van Rheenen, M.D., PhD., Anemone V. Hackeng, BSc., Michael A. van Es, M.D., PhD, Jan H. Veldink, M.D., PhD, Leonard H. van den Berg, M.D., PhD*, Ruben P.A. van Eijk, M.D., PhD*

***Equal contribution**

Accepted at Neurology

ABSTRACT

Objective: To explore the association between lipids, polygenic profile scores (PPS) for biomarkers of lipid metabolism, markers of disease severity, and survival in patients with Amyotrophic Lateral Sclerosis (ALS).

Methods: We meta-analyzed the current literature on the prognostic value of lipids in patients with ALS. Subsequently, we evaluated the relationship between lipid levels at diagnosis, clinical disease stage and survival in all consecutive patients diagnosed in The Netherlands. We determined the hazard ratio of each lipid for overall survival, defined as death from any cause. A subset of patients was matched to a previous Genome Wide Association Study (GWAS); data were used to calculate PPS for biomarkers of lipid metabolism, and to determine the association between observed lipid levels at diagnosis and survival.

Results: Meta-analysis of four studies indicated that none of the biomarkers of the lipid metabolism were statistically significantly associated with overall survival; there was, however, considerable heterogeneity between study results. Using individual patient data (N = 1,324), we found that increased HDL-cholesterol was associated with poorer survival (HR of 1.33 (95% CI 1.14 to 1.55, $p < 0.001$)). The correlation between BMI and HDL-cholesterol (Pearson's r -0.26, 95% CI -0.32 to -0.20) was negative, and between BMI and triglycerides positive (Pearson's r 0.18, 95% CI 0.12 to 0.24). Serum concentrations of total cholesterol and LDL-cholesterol were lower in more advanced clinical stages (both $p < 0.001$). PPS for biomarkers of lipid metabolism explained 1.2% to 13.1% of their variance at diagnosis. None of the PPS were significantly associated with survival (all $p > 0.50$).

Conclusions: Lipids may contain valuable information about disease severity and prognosis, but their main value may be driven as a consequence of disease progression. Our results underscore that gaining further insight into lipid metabolism and longitudinal data on serum concentrations of the lipid profile could improve the monitoring of patients and potentially further disentangle ALS pathogenesis.

INTRODUCTION

Lipids act as structural components of neuronal membranes, signaling molecules and energy substrates required for normal functioning of neurons.[1] Although the exact pathophysiological mechanisms underlying Amyotrophic Lateral Sclerosis (ALS) are unknown,[2] it is likely that the origins of the condition lie in a multi-step process,[3] followed by intra-neuronal disease propagation, altered neuronal metabolism, and ultimately neuronal death. Dysregulated energy metabolism is a consequence of this process,[4] which also affects biomarkers of the lipid metabolism, such as cholesterol, its carriers (i.e. LDL-, and HDL-cholesterol) and triglycerides. Albeit little is known about changes in the preclinical stage, two recent studies comprising a Mendelian randomized study,[5] and a prospective cohort study of over 500,000 people,[6] related premorbid metabolic changes to the risk of ALS.

The association between biomarkers of lipid metabolism, prognosis and disease progression after disease onset have proven more difficult to characterize. Though high lipid levels have been shown to increase metabolic stress,[7–9] and potentially lead to a more aggressive disease course,[2] some studies have suggested that abnormal lipid levels may actually be beneficial to the patient's prognosis.[10–14] Elucidating the interplay between clinical phenotype and lipid metabolism may reveal potential therapeutic interventions, and better address the mixed results from dietary interventions obtained thus far.[15,16] In this study, therefore, we aim to summarize the current literature, and to explore the relationships between lipids, ALS survival, polygenic profile scores for lipid levels, and markers of disease progression in a large population-based study, in order to address the disparate data in the literature.

METHODS

A two-step approach was employed: first, we conducted a systematic review to summarize and meta-analyze the current literature on the prognostic value of biomarkers of lipid metabolism in patients with ALS. Second, we assessed the prognostic value of lipids in a large population-based cohort study, explored their relationship with disease severity, and assessed the causal association between polygenic profile scores and survival after disease onset. Throughout the text we define 'biomarkers of lipid metabolism' as an umbrella term for: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG).

Systematic review

Search and study selection

We conducted the systematic search in four literature databases: PubMed, EMBASE, DARE, and the Cochrane Library; the study protocol for the systematic review can be found in

the supplementary material (**Supplement document 1**: Systematic Review Protocol). Additional forms or information, such as data collection forms, can be provided on request. The primary purpose of the meta-analyses was to provide an explanatory summary of the current literature. All databases were last searched in June 2022. Search terms included the MeSH-terms: 'Amyotrophic Lateral Sclerosis', 'Motor Neuron Disease', 'Cholesterol', 'Cholesterol, LDL', 'Cholesterol, HDL', 'Triglyceride', 'Lipid', 'Prognosis', 'Survival', 'Mortality', 'Kaplan-Meier estimate' and 'Proportional Hazard Models'. Studies were selected on the basis of the following inclusion criteria: (1) Participants diagnosed with ALS according to the revised El Escorial criteria (EEC);[17] (2) Reporting of at least one of the following measurements: TC, HDL-C, LDL-C or TG, obtained after symptom onset; (3) Reporting of survival time and hazard ratio (HR); (4) Written in English or Dutch. Study eligibility was not based on sample size. All articles were screened independently by two reviewers for title and abstract (M.J.v.M. and A.H.). In- and excluded articles were discussed; if no consensus was reached, a third reviewer was consulted (R.P.A.v.E.).

Data collection and meta-analysis

For each included study, we extracted the following variables: author, publication year, country, number of participants, and statistical analysis parameters (i.e., covariates, hazard ratio (HR) and 95% confidence interval). We used the Quality in Prognosis Studies (QUIPS) tool to determine the quality and risk of bias of the included articles.[18] Studies that provided a HR for at least one, non-dichotomized, biomarker of the lipid metabolism were included in the meta-analysis. Standardized HRs (SE) were back-transformed to mmol/L by dividing by the study standard deviation; if studies reported biomarkers of lipid metabolism in mg/dL, data were converted to mmol/L by dividing the HR (SE) by 0.02586 for TC, LDL-C and HDL-C, or by 0.01129 for TG. Meta-analyses were conducted using a Bayesian hierarchical model using a non-informative uniform prior for the log hazard ratio, and a weakly informative prior for the heterogeneity parameter (half-normal with standard deviation of 0.5). As sensitivity analysis, we varied the prior for the heterogeneity parameter using either a standard deviation of 0.25 or 1.0.[19] Funnel plots were used to visually inspect publication bias and study heterogeneity (**Figure 3**). We estimated the heterogeneity between studies using the I^2 statistic and expressed this as percentage. The meta-analyses provide the pooled hazard ratio on survival across studies for each biomarker of lipid metabolism in mmol/L.

Population-based cohort

For the second part of the study, we conducted a prospective analysis of the national registry of The Netherlands ALS Center, selecting all consecutive patients diagnosed in the University Medical Center Utrecht (UMCU), Utrecht, The Netherlands, between 1 January 2012 and 31 December 2017 to ensure sufficient follow-up time for survival. All patients were diagnosed with either possible, probable laboratory supported, probable, or definite ALS.[17] The UMCU is a referral center for all patients with ALS across our country. All clinical characteristics were

collected at the time of diagnosis. The King's clinical staging system[20] was determined according to the standard operating procedures provided by the European Network to Cure ALS (ENCALS).[24] Patients with more than 30 hexanucleotide repeats in the *C9orf72* gene were considered to be *C9orf72* carriers.[22] We defined survival time as time between date of diagnosis and date of death or date last known to be alive. Survival information was updated at quarterly intervals by cross-referencing with the municipal population register. All patients were administratively censored on 9 July 2020. Data were further supplemented with the revised ALS functional rating scale (ALSFRS-R) collected at time of diagnosis.[15] For a subset of patients, longitudinal data of the ALSFRS-R were available, obtained during either clinical follow-up or previous participation in clinical research.

Blood sample collection

Blood samples were collected from patients in a non-fasting state on the day of diagnosis or within one month after diagnosis.[23] We determined: total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides with the Beckman Coulter AU5800 clinical chemistry analyzer series. Normal ranges were defined according to the central diagnostic laboratory of the University Medical Center Utrecht: TC 3.5 - 6.5 mmol/L, LDL-C < 3.5 mmol/L, HDL-C > 0.90 mmol/L for males, HDL-C > 1.1 mmol/L for females, and TG 0.0 - 2.0 mmol/L.

Statistical analysis

We performed our statistical analyses using RStudio (version 1.1.4, RStudio: Integrated Development for R, Inc., Boston, USA, http://www.rstudio.com/). Mean and standard deviation (SD) were determined and summarized for continuous variables; for categorical variables, we determined frequency and proportion. The Cox proportional hazard model was applied to assess the association between the risk of death and biomarkers of lipid metabolism at diagnosis. All models were adjusted for the eight clinical predictors – combined in a linear predictor (LP) – from the ENCALS survival model,[24] namely: age at onset, diagnostic delay, bulbar onset, definite ALS according to the revised EEC,[17] pre-diagnostic progression rate (ΔFRS),[25] percentage (%) of predicted forced vital capacity (FVC), presence of frontotemporal dementia (FTD) and carrier of the *C9orf72* repeat expansion. For each analysis, the following sensitivity analyses were conducted: (1) adding an interaction term between biomarker level and sex (i.e. is the effect of the biomarker different for males *vs.* females?) and similarly for age at diagnosis, (2) adding quadratic terms to explore potential non-linear relationships between the risk of death and the biomarker level, and (3) additional adjustment for body mass index and weight loss, factors known to be associated with both the lipid level and survival.[26] Data missing for any variable except the outcome were addressed by creating multiple imputed datasets (n = 100), using predictive mean and bootstrapping, discarding the first 100 iterations (burn-in). In total, 9.2% of all observations were missing and, therefore, imputed. All covariates were included in a stratified imputation model per diagnostic year;

survival time was included as cumulative hazard rate (Nelson-Aalen estimator).[27] Results across imputations were pooled using Rubin's rules.[28]

We further explored longitudinal trends in disease progression rate by assessing the relationship between lipid levels at diagnosis and decrease in ALSFRS-R since diagnosis using linear mixed effects models. Models contained a fixed effect for time since diagnosis (in months), lipid level and the interaction between time and lipid level; the random part contained a random slope for time and intercept per patient. We used a likelihood ratio test to assess the significance of the interaction between lipid level and time (i.e., is the rate of ALSFRS-R progression dependent on lipid level?). In addition, we assessed the cross-sectional association between lipid levels, Body Mass Index (BMI) and King's Clinical Staging[20] at diagnosis using linear regression models. Sensitivity analyses were conducted by introducing interaction terms for sex to assess potential male-female differences. All analyses of triglycerides level were performed on the natural logarithm scale due to their right-skewed distribution.

Polygenic profile score

As an exploratory analysis, we estimated polygenic profile scores (PPS) for biomarkers of lipid metabolism.[29] The PPS estimates the sum of additive genetic effects across all alleles that affect the biomarkers of lipid metabolism at the patient level. We used the PPS to explore a potential genetic link between lipid metabolism, ALS and survival time by assessing (1) how much of the variance in biomarker levels at diagnosis can be explained by genetic profile scores, and (2) whether the genetic profile score itself is associated with overall survival time. As PPS do not change over time,[30] a statistical association between the genetic profile score and survival may be evidence of abnormal lipid levels caused by genetic variation, or hold potential for therapeutic interventions.[30] Moreover, their time invariance allowed us to estimate the link between the genetic profile score and overall survival time, defined as time between symptom onset and death.

For all individuals who were enrolled in both our population-based registry and our latest genome-wide association study (GWAS),[5] we calculated the PPS. PPS were based on summary statistics from a GWAS on biomarker levels of lipid metabolism in the UK Biobank.[31] For each single nucleotide polymorphism, we calculated a weight for each biomarker using the summary-BavesR module in the Genome-wide Complex Trait Bayesian analysis toolkit (default parameters),[29] and a linkage-disequilibrium matrix originating from 50,000 unrelated individuals of inferred European ancestries included in the UK Biobank. Because the genotype data originated from several different cohorts in the ALS GWAS, we scaled the PPS per GWAS cohort to a mean of zero and a standard deviation of one. Linear regression models were used to calculate how much of the variance in biomarker level was explained by their PPS (expressed as adjusted-R^2); 95% confidence intervals were obtained by means

of bootstrapping. Simple univariable Cox models for overall survival time (i.e., from onset to death) were used to estimate hazard ratios.

Standard Protocol Approvals, Registrations and Patient Consents

The medical ethics committee and institutional review board of the University Medical Center Utrecht (METC NedMec) approved this study (study registration number: METC 19-190). Written consent was obtained from all study participants prior to the study.

Data availability statement

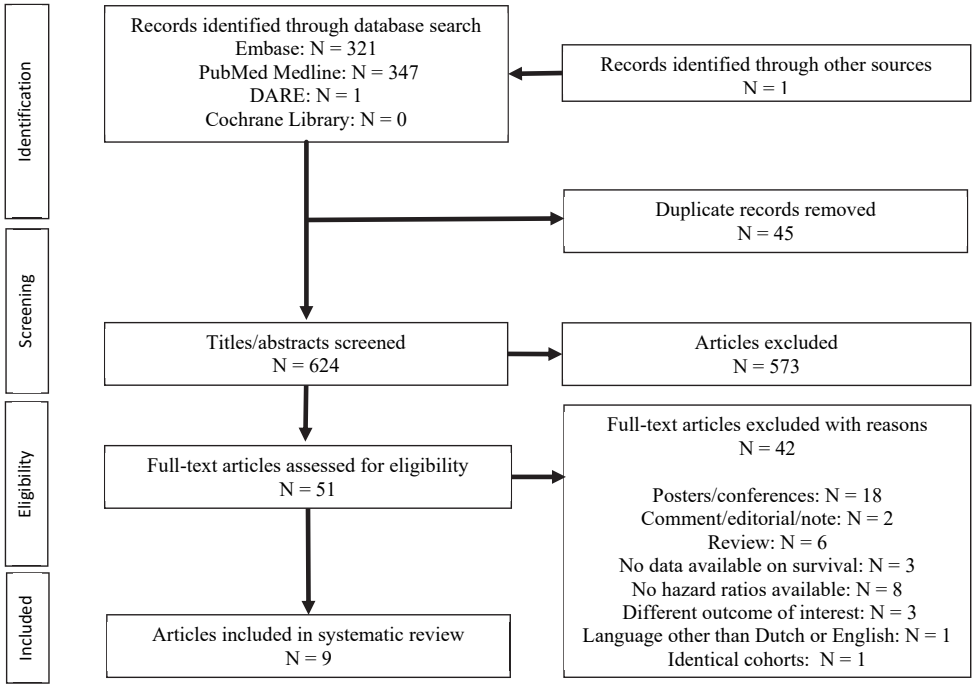
All protocol, analyses, and anonymized data will be shared on request. We take full responsibility for the data, the analyses and interpretation, and the conduct of the research.

RESULTS

Systematic review and meta-analysis

Of the 624 citations screened, nine articles were included (**Figure 1**), five of which found a significant association between survival time and serum levels of TC, LDL/HDL ratio, HDL-cholesterol or TG; their characteristics are summarized in **Table 1**.

Figure 1. Flowchart



Studies included different prognosticators in their multivariable model; none adjusted for all known prognosticators in patients with ALS.[24] Four studies reported a non-dichotomized HR and were included in the meta-analysis, resulting in a total sample size of 1,120 patients (**Figure 2**). The risk of bias assessment of the individual studies can be found in **Supplement Figure 1**. None of the biomarkers of the lipid metabolism reached statistical significance (**Figure 2**), although the 95% credible intervals included clinically relevant effect sizes. There was, however, considerable heterogeneity between study results, reflected as τ, indicating possible differences in methodology. Changing the prior assumptions resulted in similar findings (not shown). In **Figure 3** we provide the funnel plot to explore publication bias; it should be noted that, given the small number of studies, their interpretation is limited.

Table 1. Overview of included studies.

Author	Year	Survival time from	No. of patients	Lipids	Multivariable analysis	Conclusion	Risk of bias*
Nakamura et al.[32]	2022	symptom onset	78	TC, LDL-C, HDL-C, TG	Age, sex, ALSFRS-R slope, BMI slope, bulbar onset, VC	High HDL-C levels are an independent predictor of worse survival	Low
Ingre et al.[10]	2020	date of diagnosis	99	TC, LDL-C, HDL-C, TG, Ratio	Age, sex, symptom duration, site of onset, BMI, ALSFRS-R score, ΔFRS	High TC, LDL-C or ratio levels are predictors of better survival	Low **
Barone et al.[48]	2019	date of PEG placement	47	TC, Ratio	Age, BMI	No effect of cholesterol levels on survival	Moderate
Ahmed et al.[12]	2018	symptom onset	96	TC, LDL-C, HDL-C, TG, Ratio	Age, symptom duration, cognitive and/or behavioral involvement	High TC levels are a predictor of better survival	Low **
Huang et al.[13]	2015	symptom onset	413	TC, LDL-C, HDL-C, TG	Age, sex, symptom duration, ALSFRS-R score	High TG levels are a predictor of better survival	Low **
Rafiq et al.[45]	2015	study inclusion	512	TC, LDL-C, HDL-C, TG, Ratio	Age, sex, symptom duration, weight, site of onset, VC	No effect of cholesterol levels on survival	Moderate **
Sutedja et al.[49]	2011	study inclusion	303	TC, LDL, HDL, Ratio	Age, site of onset, VC	No effect of cholesterol levels on survival	Moderate
Dorst et al.[50]	2011	symptom onset	488	TC, LDL, TG, Ratio	Age, sex, ALSFRS-R score, BMI, glucose serum level	No effect of cholesterol levels on survival	Moderate
Dupuis et al.[14]	2008	symptom onset	369	TC, LDL, HDL, TG, Ratio	None	High ratio levels are a predictor of worse survival	Moderate

Table summarizes the baseline demographics of the included studies. Abbreviations: ALS = amyotrophic lateral sclerosis, FTD = frontotemporal dementia, TC = total cholesterol, LDL = low-density lipoprotein, HDL = high-density lipoprotein, TG = triglycerides, Ratio = LDL-C/HDL-C, BMI = body mass index, VC = vital capacity, ALSFRS-R = ALS functional rating scale, ΔFRS = (48 – ALSFRS-R score) / symptom duration, PEG = percutaneous endoscopic gastrostomy. *Risk of Bias based on QUIPS tool (**Supplement Figure 1**).[18] **Included in meta-analysis.

Figure 2. Forest plot of the included studies for biomarkers of lipid metabolism

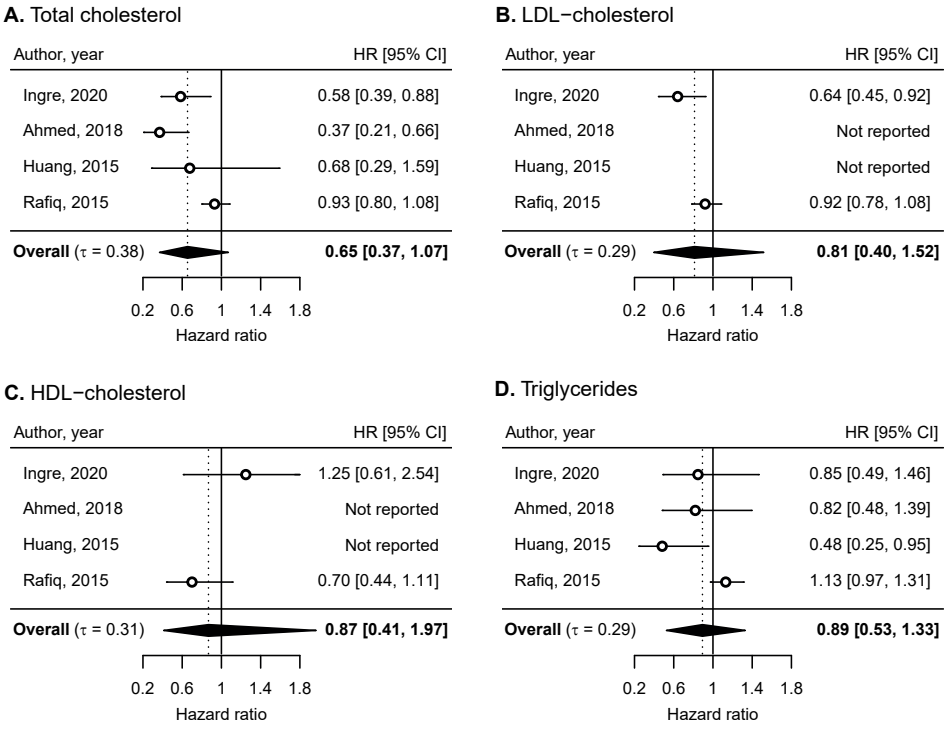


Figure 2. Meta-analysis of the reported hazard ratios in the literature. Hazard ratio of each lipid for survival, defined as the time in months from study enrollment to death from any cause or administrative censoring. The overall hazard ratio reflects the pooled hazard ratio across studies in mmol/L. Abbreviations: HR = hazard ratio; CI = credible interval.

Population-based cohort

In total, 1,324 patients with ALS were enrolled in our population-based registry. At time of administrative censoring (July 2020), 1,185 deaths (89.5% of enrolled population) had occurred during 2,370 person-years of follow-up. Median survival since diagnosis was 16.5 months (95% CI 15.7 – 17.5). Baseline characteristics of the cohort are listed in **Table 2**; 688 patients (52%) had been enrolled in our latest GWAS study and were included in the PPS analysis. Overall, 20.1% of the patients had elevated TC, 42.0% elevated LDL-C, 4.9% reduced HDL-C and 19.2% elevated TG levels on the day of diagnosis.

Figure 3. Funnel plots to explore publication bias.

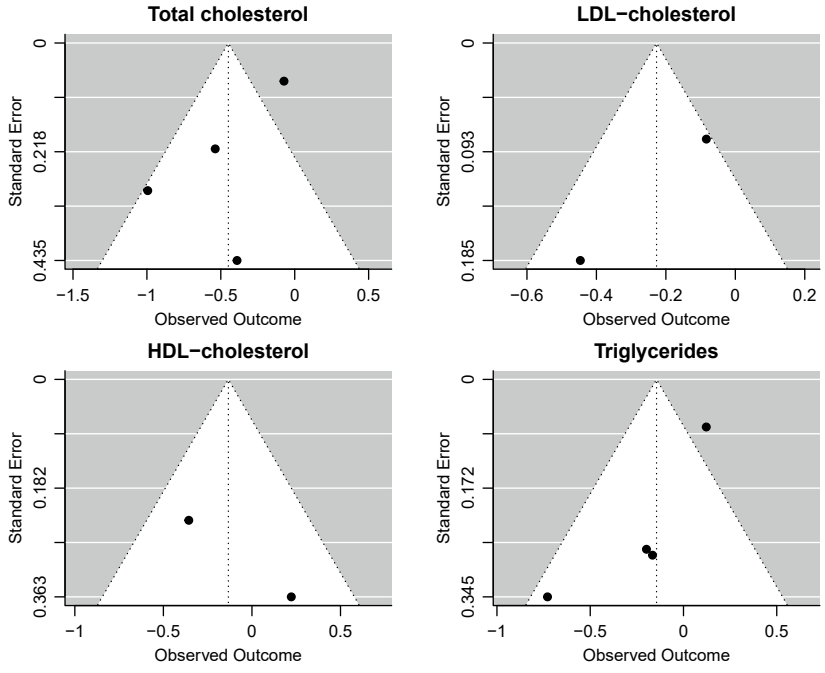


Table 2. Baseline demographics and clinical characteristics on the day of diagnosis

Characteristic	All patients (*N = 1,324*)	PPS cohort** *(N = 688)*
Age at diagnosis, years	66 (11)	66 (10)
Sex, male	748 (56%)	393 (57%)
Site of symptom onset, bulbar	449 (34%)	229 (33%)
Diagnostic delay,* months	9 (9)	9 (8)
ALSFRS-R total score	38 (6)	39 (6)
ΔFRS,* points per month	-0.83 (1.24)	-0.74 (1.07)
Forced vital capacity, %predicted	87 (22)	91 (22)
Body Mass Index, kg/m^2	25 (3)	25 (3)
Presence of frontotemporal dementia	111 (8%)	47 (7%)
Presence of *C9orf72* repeat expansion	95 (7%)	54 (8%)
Prognostic Risk Profile	-3.86 (1.67)	-3.97 (1.63)
Biomarkers of lipid metabolism		
Total cholesterol, mmol/L	5.58 (1.18)	5.54 (1.15)
LDL-C, mmol/L	3.38 (1.00)	3.36 (0.97)
HDL-C, mmol/L	1.48 (0.38)	1.48 (0.37)
Triglycerides, mmol/L	1.57 (0.88)	1.56 (0.86)

Data are expressed as mean (SD) or n (%). *Data are expressed as median (IQR). ** Patients who had GWAS data available for analysis of their polygenic profile score (PPS). Abbreviations: ALSFRS-R = revised ALS functional rating scale; ΔFRS = (48 – ALSFRS-R total score) / diagnostic delay;[25] Prognostic Risk Profile = Linear predictor of ENCALS survival model;[24] LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.

After adjustment for age, site of onset, diagnostic delay, pre-diagnostic progression rate (ΔFRS), vital capacity, presence of FTD, *C9orf72* repeated expansion and El Escorial classification,[24] a 1 mmol/L increase of HDL-C was found to be associated with a higher risk of death and shorter survival time after ALS diagnosis, HR of 1.33 (95% CI 1.14 – 1.55, $p < 0.001$, **Table 3**). This effect was larger for males than for females: HR (males) 1.48 *vs.* HR (females) 1.13, though not statistically significantly different (interaction term $p = 0.094$). The effect was similar for different ages at diagnosis (HR-interaction 1.00; 95% CI 0.98 – 1.02, $p = 0.97$). Introduction of a non-linear term did not result in a significant model improvement ($p = 0.84$). Additional adjustment for weight loss (HR of 1.37, 95% CI 1.17 - 1.61) or body-mass index (HR of 1.28, (95% CI 1.09 - 1.50) did not alter our results.

Table 3. Hazard ratios for biomarkers of lipid metabolism in population-based cohort.

Lipid	Hazard ratio	95% CI	*P*-value
Total cholesterol (mmol/L)	1.04	0.99 to 1.09	0.087
LDL-C (mmol/L)	1.03	0.98 to 1.09	0.25
HDL-C (mmol/L)	1.33	1.14 to 1.55	< 0.001
Triglyceride (log-mmol/L*)	0.91	0.80 to 1.03	0.153

Hazard ratios are determined with a Cox proportional hazards model adjusted for age, site of onset, diagnostic delay, pre-diagnostic progression rate (ΔFRS), vital capacity, presence of FTD, *C9orf72* repeat expansion and El Escorial classification.[24] A HR larger than 1 reflects a poorer survival outcome. *Analysis was performed on the natural logarithm scale due to a right-skewed distribution. Abbreviations: LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; CI = confidence interval. Hazard ratio of each lipid for survival, defined as the time in months from study enrollment to death from any cause or administrative censoring.

Longitudinal ALSFRS-R data, i.e., two or more measurements, were available for 419 of the 1,324 patients (31.6%). Average progression rate after diagnosis was 0.79 points per month (95% CI 0.73 to 0.85). With each mmol/L increase in HDL-C, the monthly ALSFRS-R progression rate increased by 0.10 points per month (95% CI -0.07 to 0.26, $p = 0.21$), indicating a similar directional effect as observed on survival, albeit not statistically significant. None of the other biomarkers of the lipid metabolism was significantly associated with the monthly progression rate (all $p > 0.15$).

Figure 4 and **Figure 5** provide the standardized distributions of the biomarkers of lipid metabolism stratified by BMI category and King's clinical stage at diagnosis, respectively. Both HDL-C (Pearson's r -0.26, 95% CI -0.32 to -0.20) and TG (Pearson's r 0.18, 95% CI 0.12 to 0.24) were associated – a negative and positive association, respectively – with BMI at diagnosis (both $p < 0.001$); these relationships were similar for males and females (both interaction terms $p > 0.40$). Similarly, TC and LDL-C depended on King's clinical staging and showed a declining trend for more advanced disease stages (both $p < 0.001$); again, these associations were similar for males and females (both interaction terms $p > 0.75$). Results were similar when categorizing the ALSFRS-R into four equal categories (*results not shown*).

Figure 4. Biomarkers of the lipid metabolism stratified by BMI category at diagnosis

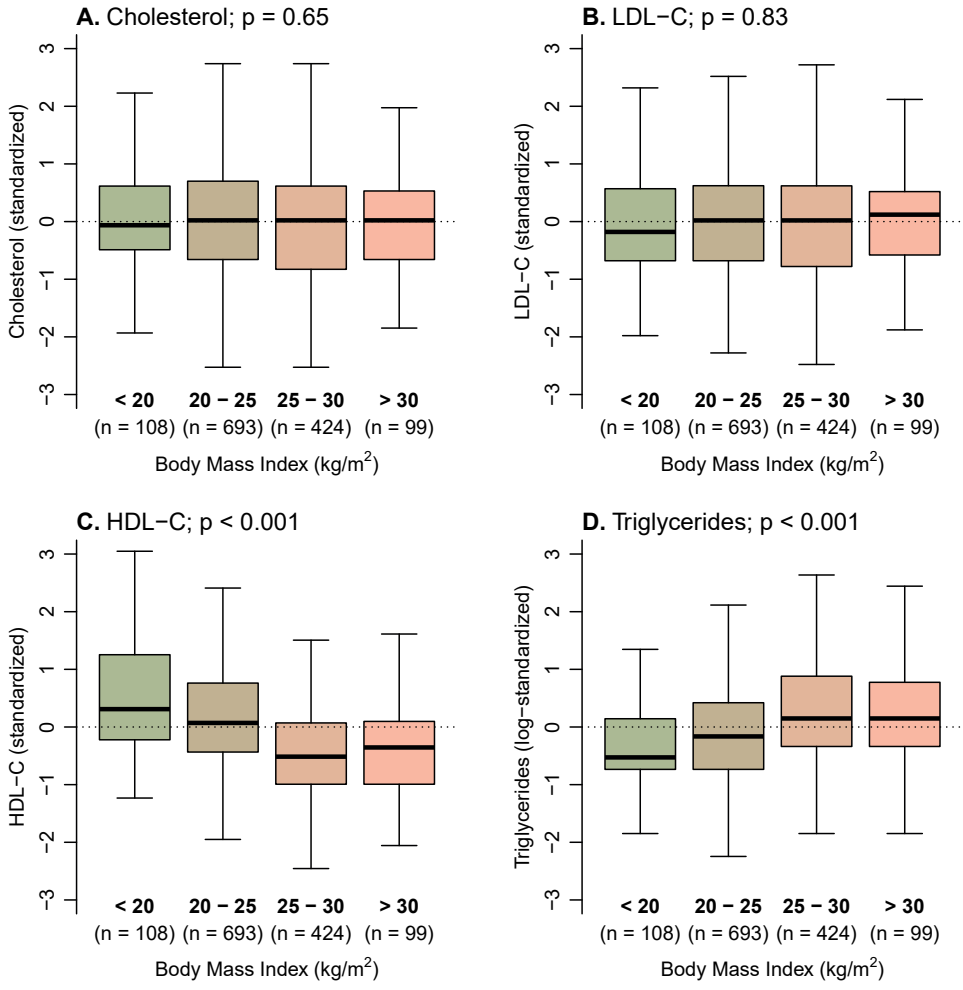


Figure 4. Boxplots summarizing the cross-sectional concentrations of the lipids linked to Body Mass Index (BMI) at diagnosis. Scales are standardized in order to provide a direct comparison between lipids; interpretation is straightforward, where the scale reflects the number of standard deviations above or below the mean lipid level as provided in **Table 2**. Abbreviations: LDL = low-density lipoprotein, HDL = high-density lipoprotein. P-values are based on the likelihood ratio test.

Figure 5. Biomarkers of the lipid metabolism stratified by King's clinical staging at diagnosis

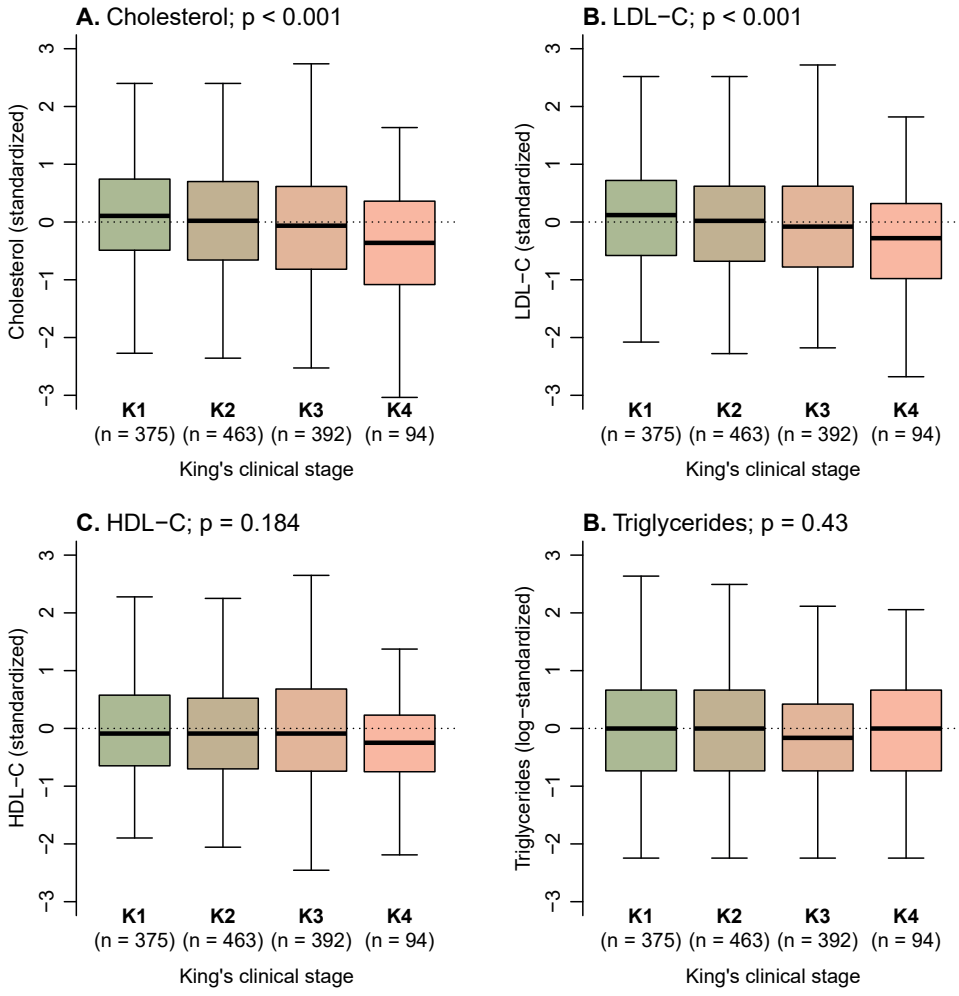


Figure 5. Boxplots summarizing the cross-sectional concentrations of the lipids linked to the four King's clinical stages. Scales are standardized in order to provide a direct comparison between lipids; interpretation is straightforward, where the scale reflects the number of standard deviations above or below the mean lipid level as provided in **Table 2**. Abbreviations: LDL = low-density lipoprotein, HDL = high-density lipoprotein. P-values are based on the likelihood ratio test.

Analysis of polygenic profile scores

Finally, in **Table 4** we summarize how much of the variance in lipid levels observed at diagnosis can be attributed to the respective PPS, expressed as adjusted-R^2, and how the PPS relate to overall survival since symptom onset. Each PPS was significantly correlated with the respective lipid level (Pearson's r_{TC} 0.11, $p = 0.002$; Pearson's $r_{LDL\text{-}C}$ 0.23, $p < 0.001$; Pearson's $r_{HDL\text{-}C}$ 0.36, $p < 0.001$; Pearson's $r_{log\text{-}TG}$ 0.33, $p < 0.001$), with the explained variance at diagnosis

ranging from 1.2% to 13.1%. None of the PPS was, however, significantly associated with overall survival time (all $p > 0.50$).

Table 4. Relationship between polygenic profile score, biomarkers level and survival.

Lipid	Explained variance at diagnosis		Relationship with survival since symptom onset		
	Adjusted-R^2	*95% CI*	*Hazard ratio*	*95% CI*	*P-value*
Total cholesterol	0.012	0.000 – 0.035	1.02	0.95 – 1.11	0.54
LDL-C	0.058	0.028 – 0.096	1.01	0.93 – 1.09	0.81
HDL-C	0.131	0.087 – 0.179	0.98	0.90 – 1.06	0.57
Triglyceride	0.107	0.066 – 0.156	1.02	0.94 – 1.10	0.64

Confidence intervals around the adjusted-R^2 were obtained by means of bootstrapping (n = 10,000) and pooled across imputations. Abbreviations: LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; CI = confidence interval. Hazard ratio of each lipid for survival, defined as the time in months from symptom onset to death from any cause or administrative censoring.

DISCUSSION

In this study, we have shown the extensive variability in the literature regarding the prognostic value of the lipid profile. The study heterogeneity is mainly driven by differences in study design, statistical models, sample size and the patient population enrolled. In the second part of our study, only HDL-cholesterol had additional prognostic value for predicting survival after diagnosis in patients with ALS in a prospective, population-based registry. Changes in components of the lipid profile were primarily related to disease severity. We found no immediate associations, however, between lipid-based polygenic scores and overall survival, yet another indication that changes in the lipid profile may be primarily a consequence of disease. Our results underscore that obtaining greater insight into lipid metabolism and longitudinal data on serum concentrations of the lipid profile could improve the monitoring of patients and potentially further disentangle ALS pathogenesis.

Firstly, our literature search into the relationship between survival and lipid profile showed that the results of these studies are mixed.[10–14,32] The included studies analyzed lipids either continuously or as binary factor (e.g., high vs. low). Binary categorization of the lipid levels into normal or abnormal may lead to spurious associations and be too limited to describe the gradual associations with prognosis. When pooling results across studies in a meta-analysis, none of the lipids were statistically significantly associated with survival, but individual study results varied considerably. The variation may be explained by (1) differences in the disease stage and the phenotype of the population enrolled, and (2) differences in study methodology (e.g., follow-up time, statistical approach, and sample size).

Secondly, our analysis of a population-based registry confirmed the non-prognostic value of most lipids; HDL-C was, however, found to be predictive of overall survival since diagnosis. This finding was recently confirmed in both Japanese[32] and Swedish[10] patients, although insignificantly in the latter. We were not able to show the association between HDL-C and disease progression determined by the ALSFRS-R, as follow-up data were limited. The prognostic value of HDL-C could be the result of a surrogate association with disease progression. Respiratory insufficiency or symptoms of dyspnea have been associated with the lipid profile,[33] while dietary changes alter lipid concentrations.[34] Weight loss is observed in up to 60% of patients with ALS,[26] and changes in BMI have a direct impact on the lipid profile.[35,36] This impact was also found in our study population: there was a strong association between HDL-C and BMI, where HDL-C increases as BMI decreases. However, adjusting for BMI or other markers of disease severity minimally impacted the association between HDL-C and survival. Albeit speculative, one could also hypothesize that the prognostic association might partially reflect a pre-manifest or prodromal sign of ALS. For example, production of oxidized derivatives of excess cholesterol might be caused by deficiencies in cholesterol metabolism,[7] which in turn may induce neuronal damage leading to muscle function loss.[7,37] Deficiencies in cholesterol metabolism may also lead to dysregulated transport of cholesterol and result in toxicity in the brain.[38]

In an attempt to disentangle this potential causality between lipids and survival, we estimated PPS for biomarkers of lipid metabolism to explore genetic links with lipid metabolism and ALS survival time. As PPS do not change over time,[30] any association between PPS and survival may be an indication that premorbid changes in lipids result in a more aggressive disease as expressed in overall survival time.[29] Our results highlight the predictive value and utility of PPS in patients with ALS as surrogate for actual lipid levels, but also underscore that over 80% of the variance in the actual lipid levels were not captured by the PPS. Taking into account the absence of a large effect between PPS and survival time, and the results from other studies in which PPS were more predictive for actual lipid levels,[39] these observations may support reverse causality, where lipid levels change as a consequence of the disease rather than vice versa.

The clinical relevance of these observations depends on the setting and the intended use of the PPS. Despite the large sample size of our cohort, we were primarily powered to detect HRs of 1.1 or greater. An HR of 1.1 would translate to a 46.4% difference in hazard when comparing a patient with -2SD (~2.5th percentile) versus a patient with +2SD (~97.5th percentile). Smaller effect sizes, therefore, could still be deemed relevant, though detecting, for example, an HR of 1.05 or greater with 90% power would require approximately 4,500 survival events. Larger GWAS studies that link overall survival time to PPS may, therefore, be needed to further investigate potential causal or etiological relationships.[30] Moreover, determining whether a change in lipid level precedes a change in clinical progression

requires longitudinal observations with repeated blood samples to provide more definite evidence.[40] In such studies, it would be key to carefully collect other parameters that influence lipids, which were not collected in our study, such as smoking,[41] diet or the use of cholesterol-lowering drugs (CLD),[42] and preferably assess serum concentration in a fasting state to minimize variability.[43,44] Finally, 42.0% of our patient population had elevated serum concentrations of LDL-C; the mean serum HDL-C was comparable to that of the general Dutch population.[43] Studies that enrolled patients with ALS have reported similar serum concentrations.[10,45] HDL-C values were more or less the same as those found in the general population; however, an elevated LDL-C can be found in about 50 – 60% of people of similar age in The Netherlands.[46,47] Patients with ALS, therefore, may have lower levels of LDL-C compared to the general population,[46] supporting our finding of decreasing levels in more advanced disease stages. Enrollment of a more geographically and culturally diverse population may improve generalizability of the exact association between lipids and overall survival in ALS, but dedicated case-control studies are needed to confirm true differences in lipids levels between patients with ALS and the general population. Moreover, though our study indicates a relationship with cross-sectional clinical stages, determining whether a change in lipid level precedes a change in clinical progression, requires longitudinal observations with repeated blood samples to provide more definite evidence.

In conclusion, lipids may contain valuable information about disease severity and prognosis, because serum concentrations seem to be dependent on disease severity. Our results underscore that gaining further insight into lipid metabolism and longitudinal data on serum concentrations of the lipid profile could improve the monitoring of patients. As our results are not in line with previous studies on a causal effect of the lipid profile on ALS disease progression, we believe this new information may contribute to ongoing efforts to disentangle ALS pathogenesis.

REFERENCES

1. Tracey TJ, Kirk SE, Steyn FJ, Ngo ST. The role of lipids in the central nervous system and their pathological implications in amyotrophic lateral sclerosis. Semin. Cell Dev. Biol. 2020;(Online ahead of print)
2. Cunha-Oliveira T, Montezinho L, Mendes C, et al. Oxidative Stress in Amyotrophic Lateral Sclerosis: Pathophysiology and Opportunities for Pharmacological Intervention. Oxid. Med. Cell. Longev. 2020;2020:1–29.
3. Al-Chalabi A, Calvo A, Chio A, et al. Analysis of amyotrophic lateral sclerosis as a multistep process: A population-based modelling study. Lancet Neurol. 2014;13(11):1108–1113.
4. Guillot SJ, Bolborea M, Dupuis L. Dysregulation of energy homeostasis in amyotrophic lateral sclerosis. Curr. Opin. Neurol. 2021;34(5):773–780.
5. van Rheenen W, van der Spek RA, Bakker MK, et al. Common and rare variant association analyses in Amyotrophic Lateral Sclerosis identify 15 risk loci with distinct genetic architectures and neuron-specific biology. Nat. Genet. 2021;53:1636–1648.
6. Thompson AG, Talbot K, Turner MR. Higher blood high density lipoprotein and apolipoprotein A1 levels are associated with reduced risk of developing amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 2021;0:1–7.
7. Abdel-Khalik J, Yutuc E, Crick PJ, et al. Defective cholesterol metabolism in amyotrophic lateral sclerosis. J. Lipid Res. 2017;58(1):267–278.
8. Cutler RG, Pedersen WA, Camandola S, Rothstein JD. Evidence that accumulation of ceramides and cholesterol esters mediates oxidative stress-induced death of motor neurons in amyotrophic lateral sclerosis. Ann. Neurol. 2002;52(4):448–457.
9. Wang Z, Bai Z, Qin X, Cheng Y. Aberrations in oxidative stress markers in amyotrophic lateral sclerosis: A systematic review and meta-analysis. Oxid. Med. Cell. Longev. 2019;2019:1–9.
10. Ingre C, Chen L, Zhan Y, Termorshuizen J, Yin L, Fang F. Lipids, apolipoproteins, and prognosis of amyotrophic lateral sclerosis. Neurology 2020;94(17):1–10.
11. Dorst J, Kühnlein P, Hendrich C, Kassubek J, Sperfeld AD, Ludolph AC. Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. J. Neurol. 2011;258(4):613–617.
12. Ahmed RM, Highton-Williamson E, Caga J, et al. Lipid Metabolism and Survival Across the Frontotemporal Dementia-Amyotrophic Lateral Sclerosis Spectrum: Relationships to Eating Behavior and Cognition. J. Alzheimer's Dis. 2018;61(2):773–783.
13. Huang R, Guo X, Chen X, et al. The serum lipid profiles of amyotrophic lateral sclerosis patients: A study from south-west China and a meta-analysis. Amyotroph. Lateral Scler. Front. Degener. 2015;16(5–6):359–365.
14. Dupuis L, Corcia P, Fergani A, et al. Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. Neurology 2008;70(13):1004–1009.
15. Ludolph AC, Dorst J, Dreyhaupt J, et al. Effect of High-Caloric Nutrition on Survival in Amyotrophic Lateral Sclerosis. Ann. Neurol. 2020;87(2):206–216.
16. Wills A-M, Hubbard J, Macklin EA, et al. Hypercaloric enteral nutrition in patients with amyotrophic lateral sclerosis: A randomised, double-blind, placebo-controlled phase 2 trial. Lancet 2014;383(9934):2065–2072.
17. Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. 2000;1(5):293–299.

18. Hayden JA, van der Windt DA, Cartwright JL, Co P. Assessing Bias in Studies of Prognostic Factors. Ann Intern Med 2013;158(4):280–286.
19. Röver C, Bender R, Dias S, et al. On weakly informative prior distributions for the heterogeneity parameter in Bayesian random-effects meta-analysis. Res. Synth. Methods 2021;12(4):448–474.
20. Roche JC, Rojas-Garcia R, Scott KM, et al. A proposed staging system for amyotrophic lateral sclerosis. Brain 2012;135(3):847–852.
21. Network to Cure ALS (ENCALS) E. King's ALS Staging SOP. 2014; Accessed 05-01-2021.
22. Cooper-Knock J, Hewitt C, Highley JR, et al. Clinico-pathological features in amyotrophic lateral sclerosis with expansions in C9ORF72. Brain 2012;135(3):751–764.
23. Craig SR, Amin R V., Russell DW, Paradise NF. Blood cholesterol screening: Influence of fasting state on cholesterol results and management decisions. J. Gen. Intern. Med. 2000;15(6):395–399.
24. Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol. 2018;17(5):423–433.
25. Kimura F, Fujimura C, Ishida S, et al. Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. Neurology 2006;66(2):265–267.
26. Janse Van Mantgem MR, Van Eijk RPA, Van Der Burgh HK, et al. Prognostic value of weight loss in patients with amyotrophic lateral sclerosis: A population-based study. J. Neurol. Neurosurg. Psychiatry 2020;91(8):867–875.
27. White I, Royston P. Imputing missing covariate values for the Coxmodel. Stat. Med. 2009;28(10):1982–1998.
28. Rubin D, Schenker N. Multiple imputation in health-care databases: an overview and some applications. Stat. Med. 1991;10(4):585–598.
29. Wray NR, Lin T, Austin J, et al. From Basic Science to Clinical Application of Polygenic Risk Scores: A Primer. JAMA Psychiatry 2021;78(1):101–109.
30. Meisner A, Kundu P, Zhang YD, et al. Combined Utility of 25 Disease and Risk Factor Polygenic Risk Scores for Stratifying Risk of All-Cause Mortality. Am. J. Hum. Genet. 2020;107(3):418–431.
31. Richardson TG, Sanderson E, Palmerid TM, et al. Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: A multivariable Mendelian randomisation analysis. PLoS Med. 2020;17(3):1–22.
32. Nakamura R, Kurihara M, Ogawa N, et al. Investigation of the prognostic predictive value of serum lipid profiles in amyotrophic lateral sclerosis: roles of sex and hypermetabolism. Sci. Rep. 2022;12(1):1–10.
33. Chio A, Calvo A, Ilardi A, et al. Lower serum lipid levels are related to respiratory impairment in patients with ALS. Neurology 2009;73(20):1681–1685.
34. Schwingshackl L, Hoffmann G. Comparison of Effects of Long-Term Low-Fat vs High-Fat Diets on Blood Lipid Levels in Overweight orObese Patients: A Systematic Review and Meta-Analysis. J. Acad. Nutr. Diet. 2013;113(12):1640–1661.
35. Poobalan A, Aucott L, Smith WCS, Avenell A, Jung R, Broom J. Effects of weight loss in overweight/obese individuals and long-term hypertension outcomes: A systematic review. Obes. Rev. 2004;5:43–50.
36. Shamai L, Lurix E, Shen M, et al. Association of body mass index and lipid profiles: Evaluation of a broad spectrum of body mass index patients including the morbidly obese. Obes. Surg. 2011;21(1):42–47.

37. Bigini P, Steffensen KR, Ferrario A, et al. Neuropathologic and biochemical changes during disease progression in liver x receptor β-/-mice, a model of adult neuron disease. J. Neuropathol. Exp. Neurol. 2010;69(6):593–605.
38. De Aguilar JLG. Lipid biomarkers for amyotrophic lateral sclerosis. Front. Neurol. 2019;10(APR):1–6.
39. Ye Y, Chen X, Han J, Jiang W, Natarajan P, Zhao H. Interactions between Enhanced Polygenic Risk Scores and Lifestyle for Cardiovascular Disease, Diabetes, and Lipid Levels. Circ. Genomic Precis. Med. 2021;14(1):1–11.
40. Holdom CJ, Janse van Mantgem MR, van Eijk RPA, et al. Venous creatinine as a biomarker for loss of fat-free mass and disease progression in patients with amyotrophic lateral sclerosis. Eur. J. Neurol. 2021;28(11):3615–3625.
41. Craig W, Palomaki G, Haddow J. Cigarette smoking and serum lipid and lipoprotein concentrations: an analysis of published data. BMJ 1989;298(6676):784–788.
42. Kakafika A, Athyros V, Tziomalos K, Karagiannis A, Mikhailidis D. High Density Lipoprotein Cholesterol and Statin Trials. Curr. Med. Chem. 2008;15(22):2265–2270.
43. Balder JW, de Vries JK, Nolte IM, Lansberg PJ, Kuivenhoven JA, Kamphuisen PW. Lipid and lipoprotein reference values from 133,450 Dutch Lifelines participants: Age- and gender-specific baseline lipid values and percentiles. J. Clin. Lipidol. 2017;11(4):1055–1064.
44. Nordestgaard BG, Langsted A, Mora S, et al. Fasting is not routinely required for determination of a lipid profile: Clinical and laboratory implications including flagging at desirable concentration cut-points - A joint consensus statement from the European Atherosclerosis Society and European Fede. Eur. Heart J. 2016;37(25):1944–1958.
45. Rafiq MK, Lee E, Bradburn M, Mcdermott CJ, Shaw PJ. Effect of lipid profile on prognosis in the patients with amyotrophic lateral sclerosis: Insights from the olesoxime clinical trial. Amyotroph. Lateral Scler. Front. Degener. 2015;16(7–8):478–484.
46. Nurmohamed NS, Collard D, Balder JW, Kuivenhoven JA, Stroes ESG, Reeskamp LF. From evidence to practice: development of web-based Dutch lipid reference values. Netherlands Hear. J. 2021;29(9):441–450.
47. Lipid Tools. 2022;
48. Barone M, Viggiani MT, Introna A, et al. Nutritional prognostic factors for survival in amyotrophic lateral sclerosis patients undergone percutaneous endoscopic gastrostomy placement. Amyotroph. Lateral Scler. Front. Degener. 2019;20(7–8):490–496.
49. Sutedja NA, van der Schouw YT, Fischer K, et al. Beneficial vascular risk profile is associated with amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 2011;82(6):638–642.
50. Dorst J, Kuhnlein P, Hendrich C, Kassubek J, Sperfeld AD, Ludolph AC. Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. J. Neurol. 2011;258(4):613–617.

Supplement Document 1: Systematic Review Protocol

What is the prognostic value of cholesterol measurements, i.e. total cholesterol, HDL-, LDL-cholesterol, ratio total cholesterol/HDL-cholesterol, and triglycerides, in blood of adult patients with amyotrophic lateral sclerosis?

A SYSTEMATIC REVIEW

PROTOCOL

Organization, City, Country:	University of Utrecht, Department of Neurology, ALS Center, UMC Utrecht Hersencentrum, Utrecht, The Netherlands
Prepared by:	Mark Janse van Mantgem, M.D.
Date:	April 2020
Senior supervisor:	Prof. dr. Leonard van den Berg
Project lead:	Prof. dr. Leonard van den Berg
Research team members:	Mark Janse van Mantgem, M.D., Ruben van Eijk, M.D., PhD., and Anemone Hackeng
Advisory group:	Viyanti Orie and Eva de Boer

Table of content

1.0 Background

Amyotrophic lateral sclerosis (ALS), also known as 'Lou Gehrig's disease,' is a neurodegenerative disorder of the upper motor neuron (UMN) and the lower motor neuron (LMN). A disease with an incidence of 2.0 to 3.0 per 100.000 person-years in Europe with a peak incidence between 50 and 70 years old (Logroscino et al., 2010). With a ratio of 1.5:1, the incidence and prevalence are greater in man than in women. Depending on the site of symptom onset, defined as spinal, bulbar, respiratory or generalized onset, first symptoms are mainly muscle weakness, fasciculations, dysphagia, weight loss (Janse van Mantgem et al., 2020), and speech/voice changes. However, ALS is a heterogeneous disease with a broad range of symptoms.

The complete pathophysiology has not been clarified. This means ALS is nowadays a fatal disease with a life expectancy of 3 to 5 years after diagnosis (Salameh et al., 2015). Researchers are focusing on finding a cure, but also on predicting survival more accurate. Recently, a prognostic tool has been validated to predict survival in individual patients with ALS (Westeneng et al., 2018). This tool is based on eight clinical predictors of survival in ALS. Besides the prognostic value of these eight clinical factors, other factors might play a crucial role in predicting survival in patients with ALS.

ALS is increasingly recognized as a systemic disease, instead of a pure neurological disease (Bäumer et al., 2014). Growing evidence has been found in an abnormal metabolic state of patients with ALS, because it seems to underpin disease prognosis and progression. Therefore, several predictors related to the metabolic and hormonal state have been described. A hypermetabolic state (Bouteloup et al., 2009; Steyn et al., 2018), lower BMI during the life course (Peter et al., 2017), weight loss at time of diagnosis (Janse van Mantgem et al., 2020, increased creatinine values (Van Eijk et al., 2018), reduced glucose intolerance, and increased values of the cholesterol/lipid spectrum (Ingre et al., 2020) are described as unfavorable predictors. The prognostic value of increased serum cholesterol remains unclear, because studies have different results and, therefore, conclusions. Because lipids are an important source of energy for muscles, the hypothesis arise that high values of serum cholesterol might indicate neuron loss. As neurons die, which might be due to oxidative stress, cholesterol is released. Elevated levels of cholesterol might be, therefore, a biomarker of neurodegeneration (Ingre et al., 2020). The opposite hypothesis also exists: a neuroprotective role of high cholesterol levels (Dorst et al., 2011; Dupuis et al., 2008).

Recently, an overview has been published in Neurology (Ingre et al., 2020) of several studies that have looked at the prognostic value of the cholesterol spectrum on survival in patients with ALS. However, this overview was not systematically performed, and no hazard ratios were presented. Therefore, an overview of all available literature on the predictive value of cholesterol measurements on survival will be helpful. We are pleased to write an updated and more convenient systematic review.

2.0 Objective

To overview available literature on the prognostic value of cholesterol measurements in blood, i.e. total cholesterol, HDL-, LDL-cholesterol, and triglycerides, in adult patients with amyotrophic lateral sclerosis.

3.0 Review Question

The PICOS for this systematic review is presented below.

Population (P)	Intervention (I) (as predictor)	Comparison (C)	Outcome (O)	Studies (S)
Adult patients with amyotrophic lateral sclerosis (ALS)	Abnormal values of cholesterol measurements	Cholesterol measurements within normal ranges	Survival rate	- Observational/longitudinal studies - Randomized controlled trials 15-04-2020

3.1. Search terms

Terms are carefully chosen and discussed with the outreach librarian at the UMC Utrecht hospital, Utrecht, The Netherlands. Relevant prognostic factors were chosen.

1. "Amyotrophic lateral sclerosis"[Mesh]
2. "Amyotrophic Lateral Sclerosis"*ti,ab
3. ALS.ti,ab
4. "Motor Neuron Disease"[Mesh]
5. MND.ti,ab
6. Neuron.ti,ab
7. Gehrig*.ti,ab
8. (1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7)
9. "Cholesterol"[Mesh]
10. "Cholesterol, LDL"[Mesh]
11. "Cholesterol, HDL"[Mesh]
12. "Triglycerides"[Mesh]
13. Cholesterol*.ti,ab
14. LDL*.ti,ab
15. HDL*.ti,ab
16. Triglyceride*.ti,ab
17. Lipid*.ti,ab
18. (9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17)
19. "Prognosis"[Mesh]
20. Prognos*.ti,ab
21. "Survival"[Mesh]
22. Survival*.ti,ab

23. "Mortality"[Mesh]
24. Mortalit*.ti,ab
25. "Kaplan-Meijer Estimate"[Mesh]
26. Kaplan*.ti,ab
27. "Proportional Hazard Models"[Mesh]
27. Cox.ti,ab
28. (19 OR 20 OR 21 OR 22 OR 23 OR 24 OR 25 OR 26 OR 27)
29. 8 AND 18 AND 28

Example Pubmed search:

("Amyotrophic Lateral Sclerosis"[Mesh] OR "Amyotrophic Lateral Sclerosis"[tiab] OR ALS[tiab] OR "Motor Neuron Disease"[Mesh] OR MND[tiab] OR Neuron[tiab] OR Gehrig*[tiab]) AND ("Cholesterol"[Mesh] OR "Cholesterol, LDL"[Mesh] OR "Cholesterol, HDL"[Mesh] OR "Triglycerides"[Mesh] OR Cholesterol*[tiab] OR LDL*[tiab] OR HDL*[tiab] OR Triglyceride*[tiab] OR Lipid*[tiab]) AND ("Prognosis"[Mesh] OR Prognos*[tiab] OR "Survival"[Mesh] OR Survival*[tiab] OR "Mortality"[Mesh] OR Mortalit*[tiab] OR "Kaplan-Meier Estimate"[Mesh] OR Kaplan*[tiab] OR "Proportional Hazards Models"[Mesh] OR Cox[tiab])

4.0 Evidence gathering and study selection

We will perform our search to the following search engines:

	Evidence based databases	Other resources
	Cochrane Library	Hand-searching
	The Database of Abstracts of Reviews of Effects (DARE)	
	PubMed	
	EMBASE	
	Medline	

4.1 Eligibility criteria

After gathering the evidence, the following eligibility criteria will be applied to the results and all identified references screened independently by two reviewers (Mark Janse van Mantgem and Ruben van Eijk).

4.1.1 Types of studies

- Longitudinal studies:
  - Prospective studies
  - Retrospective studies
  - Cohort studies
  - Case-control studies

- Randomized controlled trials where two (or more) interventions are compared with a positive effect on our research question.
- Studies without follow-up time (i.e. cross-sectional) will not be selected.

4.1.2 Types of participants

This literature review will include all studies which have recruited adult patients with ALS. The diagnosis 'amyotrophic lateral sclerosis' has to be officially diagnosed by a neurologist according to the El Escorial criteria.

4.1.3 Types of intervention and comparison

There are no interventions and comparisons involved in this literature review.

4.1.4 Type of outcome measures

The outcome of interest is the survival rate, preferably defined in months. The survival rate is defined as the percentage of ALS patients still alive after the time of diagnosis.

4.2 Inclusion criteria

Studies are included if they fulfil the following criteria:

1. Participants have been officially diagnosed with ALS according to the El Escorial criteria;
2. Studies have to be performed on human beings.
3. Determination of cholesterol values in blood of adult patients with ALS at or after their date of diagnosis;
4. At least one of the following cholesterol measurements have to be determined:
    a. Total cholesterol
    b. HDL-cholesterol
    c. LDL-cholesterol
    d. Triglycerides
    e. Ratio total cholesterol/HDL-cholesterol
5. Articles has to present original researches only
6. Written in English or Dutch;
7. Measured survival time.

4.3 Exclusion criteria

Studies are excluded if they fulfil the following criteria:

1. Grey literature, incomplete articles, such as: no full text available, posters, commentaries, hypothesis articles, not peer reviewed. If necessary we will get in touch with the authors.
2. Studies who performed a systematic review only.
3. If no information is given on the diagnostic procedure of ALS.
4. If patients <18 years old were included.

5. Studies who do not report survival time will be excluded (e.g. solely evaluated rate of ALSFRS-R decline and/or forced vital capacity (FVC)).
6. Studies with subjects other than human beings will be excluded.

5.0 Analysis

5.1 Study extraction

The search results will be initially selected based on the title and abstract by two reviewers (Mark Janse van Mantgem and Ruben van Eijk), working separately using the above mentioned in- and exclusion criteria to select articles. Any potential disagreement will be resolved by further discussion and if necessary by consulting our advisory group.

The final selected articles will be read in full. The first 5 articles will be read and data extracted by both reviewers. After the first 10 articles the remaining articles will be divided over both reviewers in randomized order.

The articles information will be imported in Mendeley and/or EndNote, where the study and data extraction will be performed. Which reference manager is used, is carefully balanced beforehand.

5.2 Data extraction

5.2.1 Design

Data will be extracted from the included articles. A standard sheet will be provided to note all data. Data of interest will be described in section 5.2.2.

All articles found with our search terms will be coded in the following format: ALS/SR/000. 'ALS' stands for Amyotrophic Lateral Sclerosis, 'SR' for Systematic Review, followed by a 3 digit number.

5.2.2 Content

We have made a list of information requirements that is necessary for our data extraction:

- **General information**. Title of study, author, type of publication, country of origin, source of funding, researcher performing data extraction, date of data extraction. Authors will be contacted if necessary.
- **Study characteristics**. Aim/objectives of the study, study design, in- and exclusion criteria, number of participants. If multiple articles from the same study team are available, we will include the latest published article, unless the quality of the latest article is less than the previous one.

- **Participants**. Age at onset and diagnosis, gender, site of symptom onset, El Escorial criterium, forced vital capacity, disease duration when blood is drawn, ALSFRS-R score when blood is drawn, presence of genetic mutations related to ALS, and presence of frontotemporal dementia (FTD).
- **Intervention**. Description of measured cholesterol values, i.e. total cholesterol, HDL-, LDL-cholesterol, ratio total cholesterol/HDL- cholesterol, and triglycerides. Cut-off scores of normal/abnormal ranges.
- **Comparison**. Description of measured cholesterol values, i.e. total cholesterol, HDL-, LDL-cholesterol, ratio total cholesterol/HDL-cholesterol, and triglycerides. Cut-off scores of normal/abnormal ranges.
- **Outcome.** Survival time, hazard ratio.

5.3 Meta-analysis

A meta-analysis will be considered. We expect a lot of heterogeneity between the studies, so we will assess whether a meta-analysis will produce a good overview.

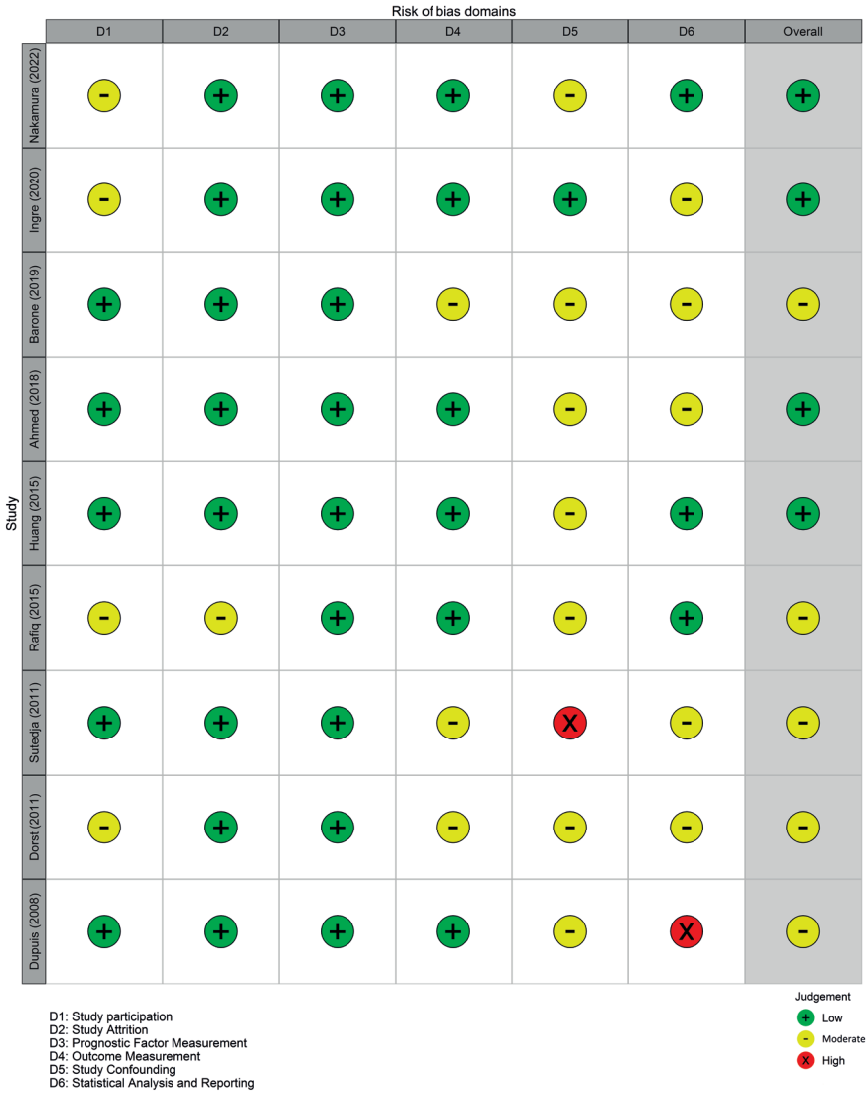
6.0 Quality assessment

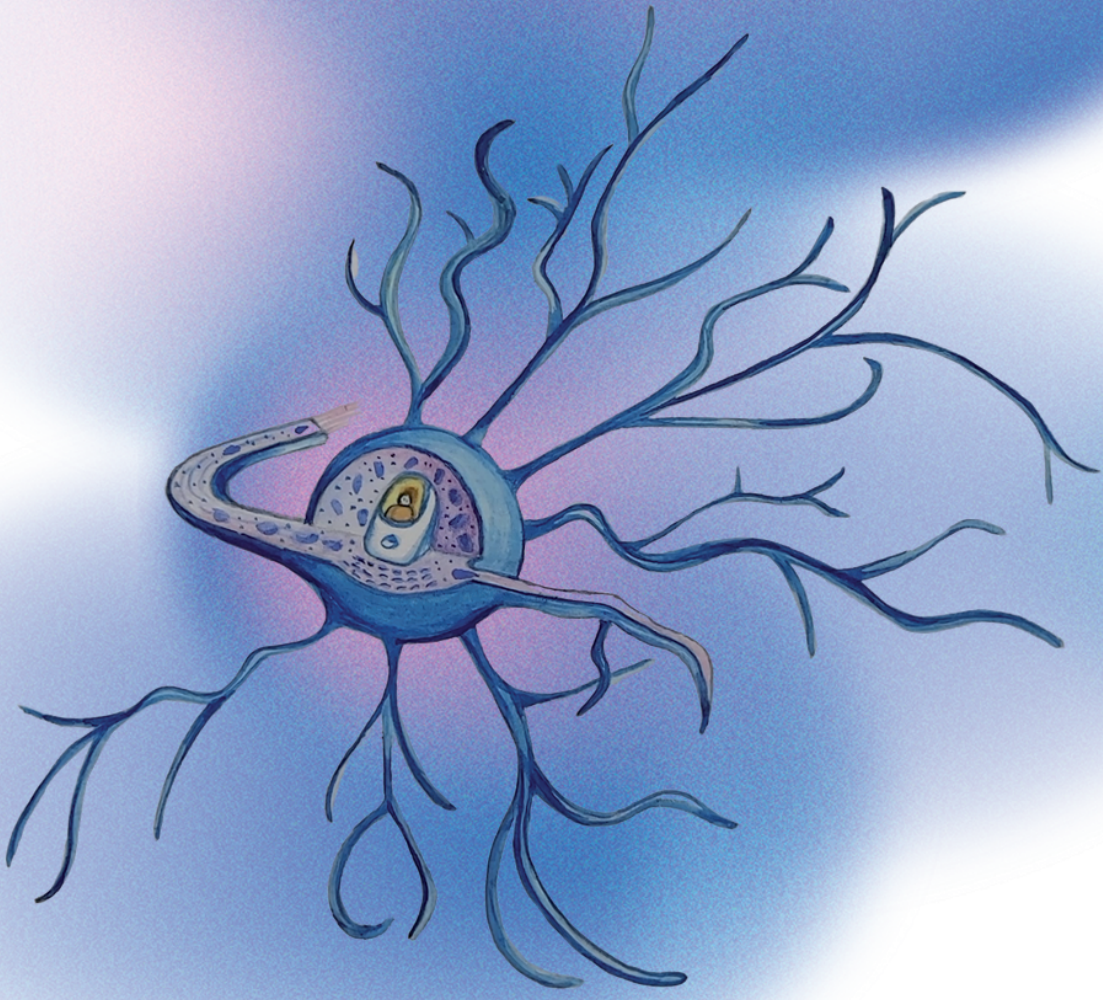
The QUIPS (Quality in Prognosis Studies tool) will be used to evaluate the quality and bias of our included articles. See Appendix A.

APPENDIX A: Quality Assessment

Domains	Prompting items for Consideration	Ratings
Study Participation	a. Adequate participation in the study by eligible persons b. Description of the source population or population of interest c. Description of the baseline study sample d. Adequate description of the sampling frame and recruitment e. Adequate description of the period and place of recruitment f. Adequate description of inclusion and exclusion criteria	**High bias:** The relationship between the PF and outcome is very likely to be different for participants and eligible nonparticipants **Moderate bias**: The relationship between the PF and outcome may be different for participants and eligible nonparticipants **Low bias**: The relationship between the PF and outcome is unlikely to be different for participants and eligible nonparticipants
Study Attrition	a. Adequate response rate for study participants b. Description of attempts to collect information on participants who dropped out c. Reasons for loss to follow-up are provided d. Adequate description of participants lost to follow-up e. There are no important differences between participants who completed the study and those who did not	**High bias**: The relationship between the PF and outcome is very likely to be different for completing and non-completing participants **Moderate bias**: The relationship between the PF and outcome may be different for completing and non-completing participants **Low bias**: The relationship between the PF and outcome is unlikely to be different for completing and non-completing participants
Prognostic Factor Measurement	a. A clear definition or description of the PF is provided b. Method of PF measurement is adequately valid and reliable c. Continuous variables are reported or appropriate cut points are used d. The method and setting of measurement of PF is the same for all study participants e. Adequate proportion of the study sample has complete data for the PF f. Appropriate methods of imputation are used	**High bias**: The measurement of the PF is very likely to be different for different levels of the outcome of interest **Moderate bias**: The measurement of the PF may be different for different levels of the outcome of interest **Low bias**: The measurement of the PF is unlikely to be different for different levels of the outcome of interest

Supplement Figure 1. Risk of bias assessment.





Chapter 4

Venous Creatinine as a Biomarker for Loss of Fat-Free Mass and Disease Progression in Patients with Amyotrophic Lateral Sclerosis

Cory J. Holdom, MSc., Mark R. Janse van Mantgem, M.D., Ruben P.A. van Eijk, M.D., PhD., Stephanie L. Howe, MSc., Leonard H. van den Berg, M.D., PhD., Pamela A. McCombe, M.D., PhD., Robert D. Henderson, M.D., PhD., Shyuan T. Ngo, PhD.*, Frederik J. Steyn, PhD.*

***Equal contribution**

Accepted at European Journal of Neurology

ABSTRACT

Background: To establish the utility of venous creatinine as a biomarker to monitor loss of fat-free mass in patients with amyotrophic lateral sclerosis (ALS).

Methods: In this multi-centre, natural history study, body composition and venous creatinine were assessed in 107 patients with ALS and 52 healthy controls. Longitudinal patterns of venous creatinine and its association with the risk of death during follow-up were determined in a cohort of patients with ALS from Australia (n=69) and the Netherlands (n=38).

Results: The mean levels of venous creatinine were 75.78±11.15 μmol/L for controls, 70.25±12.81 μmol/L for Australian patients and 59.95±14.62 μmol/L for Dutch patients with ALS. The relationship between measures of venous creatinine and fat-free mass was similar between all groups (r=0.36, p<0.001). Within patients, fat-free mass declined by 0.31 (CI: 0.22-0.40) kg/month, and venous creatinine declined by 0.52 (CI: 0.38-0.66) μmol/L/month, with a longitudinal correlation of 0.57 (CI: 0.35-0.76, *p*<0.001). Lower levels of venous creatinine were associated with increased risk for earlier death in patients with ALS (HR=0.94, CI: 0.90-0.98, p=0.007)

Conclusions: Venous creatinine is decreased in ALS and declines alongside a decline in fat-free mass over the course of disease, and may serve as practical marker to monitor the change of fat-free mass in patients with ALS. This could inform clinical care and provide an alternative endpoint for the evaluation of therapeutic interventions that focus on slowing the loss of fat-free mass and disease progression in ALS.

INTRODUCTION

Loss of fat-free mass is considered a risk factor for faster disease progression and earlier death in patients with amyotrophic lateral sclerosis (ALS).[1] However, the monitoring of loss of fat-free mass is not routinely performed, as necessary equipment and technologies to do so are not widely available.[2] Furthermore, accurate assessment of body composition in patients with ALS can be difficult and time-consuming.

Circulating creatinine has potential to be used as a biomarker for assessing fat-free mass in healthy control individuals, since measures of creatinine are proportional to skeletal muscle mass.[3] Circulating levels of creatinine have been shown to be decreased in patients with ALS.[4] This reduction in levels of creatinine in patients with ALS is associated with greater risk for faster disease progression and earlier death.[1] However, direct comparisons between circulating levels of creatinine and fat-free mass in ALS have not been made.

In this study, we determined the relationship between venous creatinine and fat-free mass in patients with ALS and healthy control individuals. We also assessed the change in measures of venous creatinine to the change in fat-free mass in patients with ALS. Through use of point-of-care measures of venous creatinine, which provides a rapid and cost-effective test for assessing creatinine, we present evidence to support the utility of venous creatinine as an accessible marker for change in fat-free mass and disease progression in patients with ALS.

METHODS

Study design and clinical assessment

This prospective case-control multicohort study was conducted between June 2016 and November 2019 at the Royal Brisbane and Women's Hospital (RBWH, Brisbane, Australia), and University Medical Centre (UMC, Utrecht, Netherlands). All data and sample collection procedures were standardized between collection sites. Patients who received a probable or definite diagnosis of ALS at the RBWH MND clinic or UMC were invited to participate (**Figure 1**).

Figure 1. Study design.

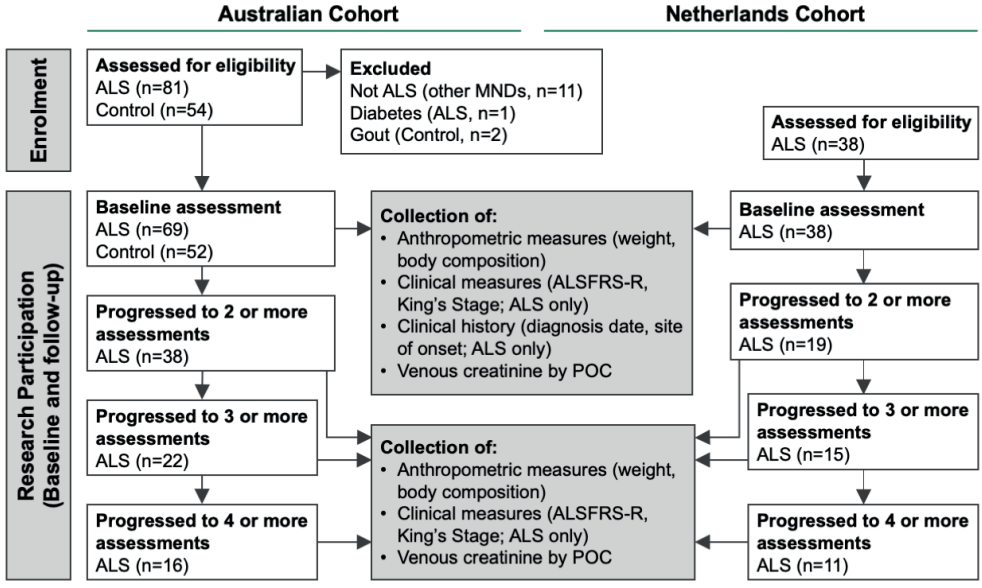


Figure 1. Schematic summarizing the number of individuals (Australian and Netherlands cohort) during enrolment, participation and follow-up.

Diagnosis was determined using the revised El Escorial criteria.[5] Eighty-one patients from the RBWH Motor Neurone Disease (MND) clinic and 38 patients from UMC with ALS were assessed for eligibility. From the Australian cohort, 11 patients were excluded for having a final diagnosis other than ALS, and 1 patient with ALS was excluded due to a history of diabetes. Information on patient demographics and disease onset was collected at enrolment. Thirty-eight patients in Australia, and 19 patients in the Netherlands completed 2 or more assessments. Assessments were completed at ~4-month intervals for up to 32.20 months (mean latency between assessments: 4.16 ± 2.18 months); total follow-up duration was 612.11 person-months with a mean duration of 10.74±7.42 months/patient. Fifty-four healthy control participants were recruited as a convenience sample of partners and friends of patients with ALS (RBWH, Brisbane, Australia only). Following enrolment, 2 control participants were excluded from analysis due to a self-reported history of gout. Other exclusion criteria were respiratory impairment, where forced vital capacity (FVC) <60% (cases) at assessment for eligibility, or a history of kidney disease (cases and controls). None of the participants who were invited to take part in the study met these exclusion criteria. Demographics for all participants at baseline are presented in **Table 1**. Demographics for patients with ALS who completed 2 or more assessments are detailed in **Table 2**. This study was approved by the University of Queensland and the RBWH human research ethics committees (Australia),

and the medical ethics committee and institutional board of UMC Utrecht (Netherlands). All participants provided written, informed consent.

Anthropometric and clinical measures

Participants were asked to fast overnight for 12 hours and to avoid strenuous physical activity prior to attending a research visit for the collection of clinical and anthropometric measures, and analysis of venous creatinine. Research visits commenced at 8am. Participant height was measured using a stadiometer. Fat-free mass was determined via air displacement plethysmography using the BOD POD Gold Standard system (Cosmed USA Inc.),[6] as we do routinely.[7] Use of predicted total gas volume of the lungs does not significantly impact predictions of body composition,[8] and so adjustments for total gas volume of the lungs was made using the Siri equation. Body mass index (BMI) was calculated as an individual's body mass divided by the square of their height.

The clinical history of each participant was noted, and for patients with ALS, the ALS Functional Rating Scale – Revised (ALSFRS-R)[9] was implemented by a clinical research nurse. The total ALSFRS-R, ALSFRS-R bulbar sub-score (questions 1-3), ALSFRS-R limb sub-scores (ALSFRS-R upper + lower limb scores; questions 4-9), ALSFRS-R respiratory sub-scores (questions 10-12) and King's Stage[10] were noted. For respiratory function scores, seated FVC as a % of predicted FVC were included, and this information was sourced from clinical records, if done within 1 month of assessment.

Assessment of venous creatinine

For the Australian cohort, venous blood samples were collected into a 10 mL BD Lithium Heparin Vacutainer® tube (BD #367880). Within this cohort, 100 µL of whole blood was used for the analysis of creatinine (within 5 min of collection) using a CHEM8+ cartridge (Abbott #09P31-25) and an i-STAT Alinity point-of-care device (Abbott). For the Dutch cohort, venous blood was collected into a 3 mL BD Lithium Heparin Vacutainer® tube (BD #367374). Within 30 minutes, room temperature samples were centrifuged at 3756 g for 5 minutes, and the plasma creatinine was quantified using an Atellica® CH Analyzer and an Atellica CH Enzymatic Creatinine_2 (ECre_2) kit (Siemens #11097533).

Statistical analysis

Measures at the first research visit (baseline) were compared using Welch-adjusted Student's t-tests. BMI, diagnostic delay and ΔFRS – defined as [(48 – ALSFRS-R score on the day of sample collection) / months since symptom onset][11] – were natural log-transformed before comparison. Proportions were compared using Fisher's exact test. Effect sizes were reported using Cohen's *d* and Cohen's *w* for continuous and proportional data, respectively. Correlations were determined using Pearson's r and Kendall's τ_B. Data are presented as mean ± standard deviation, amount (%), and 95% confidence interval (CI).

To assess changes in clinical and anthropometric measures throughout the course of disease (longitudinal assessment) relative to changes in venous creatinine, we modelled each measure (body mass, fat-free mass, creatinine, ALSFRS-R total, and ALSFRS-R sub-scores) using linear mixed effects models.[12,13] The models were constructed using Douglas Bates' *lme4* (version 1.1-25) package in *R* (version 3.6.3) and included a random intercept and slope for time per participant. For each outcome, we extracted the best linear unbiased predictions from the model, which can be interpreted as a participant-specific adjusted rate of decline. Rates of decline between outcomes were subsequently compared using Pearson's correlation coefficient, addressing whether, for example, a change in venous creatinine was associated with a change in fat-free mass. Following this, we modelled the relationship between venous creatinine with fat-free mass, body mass and ALSFRS-R using restricted cubic splines in a mixed model framework. Outcomes were standardized to obtain a longitudinal correlation coefficient, i.e., reflecting how many standard deviations venous creatinine increases with each standard deviation increase in, for example, fat-free mass. Confidence intervals were obtained by means of bootstrapping (n=1,000).

For the time-to-event endpoint (i.e., time from enrolment to death or last follow-up), we assessed the association between the hazard of an event and venous creatinine, fat-free mass, age, sex and ΔFRS using a multivariable Cox proportional hazards model. Kaplan-Meier plots were used to show the probability of survival based on venous creatinine and fat-free mass (25^{th} and 75^{th} percentiles), assuming median age and ΔFRS, and sex. Given associations between BMI and survival,[14] we also considered survival relative to BMI. For all tests, statistical significance was determined at an alpha of 0.05. Anonymized data will be shared upon reasonable request from any qualified investigator.

RESULTS

The baseline cohort consisted of 69 patients with ALS and 52 healthy controls from Australia (AUS), and 38 patients with ALS from the Netherlands (NLD; **Table 1** and **Figure 1**). Within the Australian cohort, cases were matched to controls by age, body mass and composition, however a larger proportion of females participated as controls. Fat-free mass was comparable between patients with ALS and controls (**Figure 2A**; Australian cohort), whereas levels of venous creatinine were lower in patients with ALS (**Figure 2B**; Australian cohort). Venous creatinine correlated with fat-free mass in ALS ($r=0.43$, $p<0.001$) and control participants ($r=0.57$, $p<0.001$, **Figure 2C**; Australian cohort). Similarly, in the Netherlands patient cohort, venous creatinine correlated with fat-free mass ($r=0.34$, $p=0.034$, **Figure 2D**). A correlation was also observed between venous creatinine and total body mass (Australia: $r=0.26$, $p=0.034$; Netherlands: $r=0.35$, $p=0.031$), whereas there was no significant association between venous creatinine and BMI (Australia: $r=0.15$, $p=0.38$; Netherlands: $r=0.24$, $p=0.142$). When fitting a

Table 1: Characteristics of patients with ALS (cases) and controls at the time of collection of anthropometric, clinical and venous creatinine measures.

	Case/control comparison				Within case comparison		
	Control (AUS) (n=52)	ALS (AUS) (n=69)	Effect size (95% CI)	p	ALS (NLD) (n=38)	Effect size	p
Demographics							
Age at enrolment, years	56.67 ±11.45	59.00 ±9.39	-0.23 (-0.59 – 0.14)	0.234	59.19 ±10.56	-0.02 (-0.42 – 0.38)	0.928
Sex, female	29 (55.8)	16 (23.2)	0.33 (0.16 – 0.50)	**<0.001**	13 (34.2)	0.12 (-0.08 – 0.23)	0.129
Body mass, kg	77.84 ±15.76	77.47 ±13.87	0.02 (-0.34 – 0.39)	0.894	79.17 ±10.35	-0.13 (-0.53 – 0.27)	0.477
BMI, kg/m2	26.37 ±4.84	25.82 ±4.19	0.12 (-0.24 – 0.49)	0.549	25.33 ±2.54	0.13 (-0.27 – 0.53)	0.450
Fat-free mass, kg	51.48 ±10.80	52.49 ±9.38	-0.10 (-0.47 – 0.27)	0.594	52.87 ±10.60	-0.04 (-0.44 – 0.36)	0.855
Fat-free mass, %	66.74 ±10.65	68.46 ±10.67	-0.16 (-0.53 –0.21)	0.387	66.63 ±8.72	0.18 (-0.22 – 0.59)	0.344
Clinical phenotype							
Diagnostic delay, months		12.84 ±9.18			16.19 ±21.70	-0.22 (-0.63 – 0.18)	0.947
Bulbar onset		16 (28.6)			10 (26.3)	0.02 (-0.20 – 0.04)	0.750
ALSFRS-R		37.59 ±5.49			38.71 ±4.78	-0.21 (-0.61 – 0.19)	0.373
ΔFRS		0.63 ±0.47			0.57 ±0.50	0.11 (-0.29 – 0.51)	0.079
King's Staging						0.15 (-0.02 – 0.20)	0.583
1		15 (21.7)			10 (26.3)		
2		30 (43.5)			16 (42.1)		
3		20 (29.0)			12 (31.6)		
4		4 (5.8)			0 (0)		
FVC % (of predicted)		86.22 ±19.11			95.22 ±22.27	-0.44 (-0.91 – 0.04)	0.073
Venous creatinine, umol/L	75.78 ±11.15	70.25 ±12.81			59.95 ±14.62	0.76 (0.35 – 1.18)	**<0.001**
Spinal onset, umol/L		67.70 ±12.10	0.46 (0.09 – 0.83)	**0.012**	58.36 ±15.41		
Bulbar onset, umol/L		75.69 ±13.81	0.63 (0.03 – 1.24)	**0.042**	64.40 ±11.69		

Abbreviations: ALS = amyotrophic lateral sclerosis; AUS = Australia; NLD = Netherlands; BMI = body mass index; ALSFRS-R = ALS functional rating scale-revised; ΔFRS = change in the ALSFRS-R (points/month since date of symptom onset); FVC = forced vital capacity (seated, as % of predicted).

Data are mean ± standard deviation or n (%).

Factor variables were compared using Fisher's exact test. Numerical variables were compared using Welch-adjusted two-sided t tests. BMI, ΔFRS and diagnostic delay were log-transformed before statistical comparisons.

Numerical effect sizes are standardised differences determined with Cohen's *d* (95% CI). Proportional effect sizes determined with Cohen's *w* (95% CI).

linear regression, there was no significant difference in the slope between venous creatinine and fat-free mass between cases and controls (p=0.941, **Figure 2C**) or between patients from the Australia and the Netherlands (p=0.590, **Figure 2D**); on average, an increase of 1 kg fat-free mass predicted an increase of 0.53 µmol/L venous creatinine (CI: 0.33–0.73, p<0.001). We found a lower intercept for venous creatinine in Australian patients with ALS when compared to controls (p=0.002), confirming lower venous creatinine relative to fat-free mass in patients with ALS. The intercept for venous creatinine was lower in patients with ALS from the Netherlands when compared to those enrolled in Australia (p<0.001). This suggests an overall decrease in venous creatinine levels in patients with ALS from the Netherlands. When fitting a linear regression with country, ALS status, fat-free mass, age and sex as predictors of venous creatinine, age (CI: 0.01–0.37, p=0.042) and being male (CI: 0.02–12.08, p=0.049) were found to be independently associated with an increase in venous creatinine.

Within-case comparisons

Fat-free mass was not different between patients with spinal- or bulbar-onset disease (p=0.632, **Figure 2E**). However, levels of venous creatinine were lower in patients with spinal-onset disease (p=0.041, **Figure 2F**). We found no correlation between levels of venous creatinine and total ALSFRS-R scores (p=0.447), or the respiratory domain ALSFRS-R sub-scores (p=0.374). We were unable to show a significant correlation between venous creatinine and FVC (% of predicted) (p=0.868). However, correlations were observed between venous creatinine and ALSFRS-R bulbar (τ_B=-0.13, p=0.067) and combined upper and lower limb (τ_B=0.17, p=0.013) sub-scores. Venous creatinine did not change relative to King's staging (**Figure 2G**).

Longitudinal analyses

Fifty-seven patients returned for 2 or more assessments (n=38, Australia, n=19 Netherlands, **Figure 1**). Demographics at baseline for patients with two or more research visits are presented in **Table 2**. Body mass, fat-free mass, and venous creatinine declined over time (**Figure 3**). FVC (% of predicted; Slope = -1.30 [-1.77, -0.87], p = <0.001), total ALSFRS-R scores, and ALSFRS-R bulbar and combined ALSFRS-R upper and lower limb sub-scores also declined over time (**Figure 3**, data for FVC (% of predicted) not shown).

Figure 2. Measures of fat-free mass and venous creatinine at first assessment (baseline).

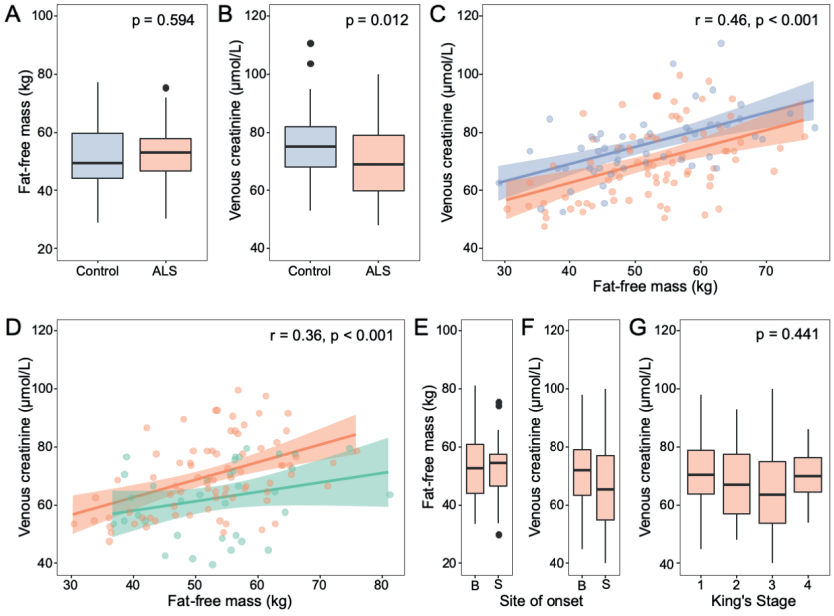


Figure 2. (A) Fat-free mass in patients with ALS from the Australian study cohort (red) was comparable to fat-free mass in control participants (blue), (B) whereas levels of venous creatinine were decreased. (C) A linear association was observed between fat-free mass and venous creatinine. This relationship was conserved between cases (red) and controls (blue; p=0.986) in the Australian cohort; reported r and p indicate correlation (combined cases and controls). (D) When comparing patient cohorts from Australia (red) and The Netherlands (green), the relationship between venous creatinine and fat-free mass was conserved (p=0.641). Reported r and p indicate correlation for all pooled cases. Comparing all cases, there was no difference in (E) fat-free mass relative to site of disease onset (p=0.665), whereas (F) venous creatinine was decreased in patients with spinal-onset disease (p=0.025). (G) Venous creatinine did not differ between patients based on King's stage (p = 0.945). ALS: amyotrophic lateral sclerosis; B: Bulbar; S: Spinal. For C and D, data presented as mean±SE.

Table 2. Characteristics of patients with ALS with two or more measures, at the time of collection of anthropometric, clinical and venous creatinine measures.

	Australia (n = 38)	Netherlands (n = 19)	Effect size (95% CI)	p
Demographics				
Age at enrolment, years	58.36 ±9.85	61.37 ±10.83	-0.30 (-0.86 – 0.27)	0.317
Sex, female	9 (23.7)	9 (47.4)	0.24 (-0.02 – 0.46)	0.129
Body mass, kg	76.22 ±13.69	75.89±9.60	0.03 (-0.54 – 0.59)	0.916
BMI, kg/m2	25.02 ±3.88	24.72±2.20	0.09 (-0.47 – 0.65)	0.879
Fat-free mass, kg	52.89 ±9.06	49.78±9.99	0.33 (-0.24 – 0.90)	0.263
Fat-free mass, %	70.01 ±9.95	65.52±9.07	0.46 (-0.11 – 1.03)	0.198
Clinical phenotype				
Diagnostic delay, months	13.16 ±8.27	17.52 ±15.79	-0.38 (-0.95 – 0.19)	0.539
Bulbar onset, %	8 (25.8)	6 (31.6)	0.06 (-0.23 – 0.12)	0.750
ALSFRS-R	38.63 ±4.32	39.53 ±3.78	-0.22 (-0.78 – 0.35)	0.513
ΔFRS, pts/month	0.63 ±0.43	0.38 ±0.32	0.63 (0.05 – 1.20)	**0.007**
King's stage, %			0.23 (-0.02 – 0.34)	0.439
1	9 (23.7)	6 (31.6)		
2	17 (44.7)	6 (31.6)		
3	9 (23.7)	7 (36.8)		
4	3 (7.9)	3 (7.9)		
FVC (% of predicted)	87.72 ±18.08	104.33 ±18.42	-0.97 (-1.62 – -0.31)	**0.004**
Venous creatinine, µmol▪L-1	68.45 ±11.57	61.47 ±15.53	0.54 (-0.04 – 1.11)	0.094
Spinal onset, µmol▪L^{-1}	67.22 ±11.66	59.69 ±17.34		
Bulbar onset, µmol▪L^{-1}	71.38 ±15.56	65.33 ±10.98		

Abbreviations: ALS = amyotrophic lateral sclerosis; BMI = body mass index; ALSFRS-R ALS functional rating scale-revised; ΔFRS = change in the ALSFRS-R (points/month since date of symptom onset); FVC = forced vital capacity (seated, % of predicted).

Data are mean±standard deviation or n (%).

Factor variables were compared using Fisher's exact test. Numerical variables were compared using Welch-adjusted two-sided t tests. BMI, ΔFRS and diagnostic delay were log-transformed before statistical comparisons. Numerical effect sizes are standardised differences determined with Cohen's d (95% CI). Proportional effect sizes determined with Cohen's w (95% CI).

Figure 3. Longitudinal outcomes in patients with ALS (Australian and Netherlands cohorts).

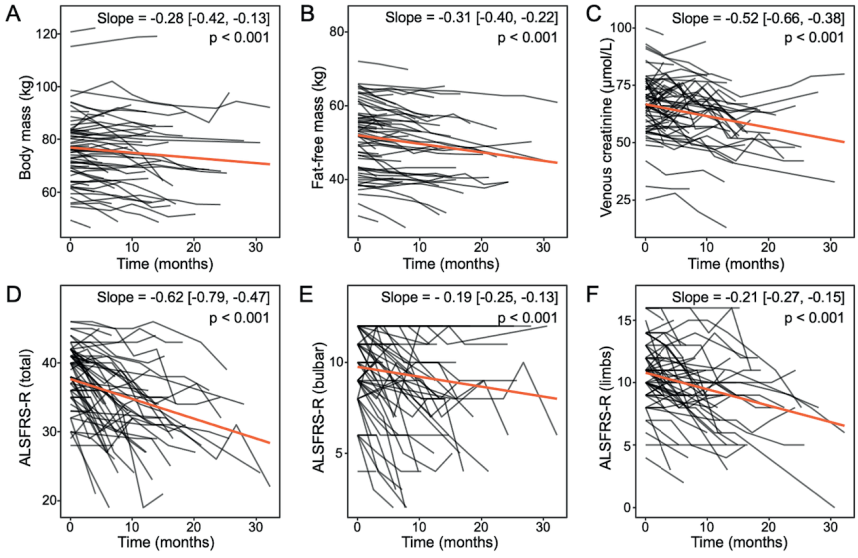


Figure 3. Significance determined with likelihood-ratio test. ALS: amyotrophic lateral sclerosis; ALSFRS-R: ALS Functional Rating Scale – Revised. Slope [95% CI].

Using mixed-effects regressions, we next compared the change in venous measures of creatinine to changes in body mass, fat-free mass, FVC, and ALSFRS-R over the course of disease. A weak correlation between the change in venous creatinine and the change in FVC (% of predicted) was observed (r=0.21, 95% CI: 0.03-0.39). A weak correlation was also observed between the change in venous creatinine and the change in ALSFRS-R (**Figure 4A**). Stronger correlations were observed between the change in venous creatinine and fat-free mass, than between the change in venous creatinine and body mass (**Figures 4B&C**). A higher baseline BMI was predictive of a slower decline in BMI. This relationship was, however, reversed when considering fat-free mass only, where a higher baseline fat-free mass predicted faster declines in BMI (**Table 3**).

Table 3. Baseline measures and their relationship with change in measures of disease progression.

	ΔFat-free mass		ΔALSFRS-R		ΔBody-mass index		ΔFVC (% of predicted)	
	***r* (95% CI)**	**p**	***r* (95% CI)**	**p**	***r* (95% CI)**	**p**	***r* (95% CI)**	**p**
Body-mass index	-0.01 (-0.29 – 0.28)	0.965	0.17 (-0.03 – 0.37)	0.103	0.39 (0.08 – 0.70)	**0.014**	0.09 (-0.19, 0.48)	0.801
Creatinine	0.31 (0.03 – 0.59)	**0.030**	-0.01 (-0.21 – 0.19)	0.921	0.15 (-0.15 – 0.45)	0.316	-0.13 (-0.48, 0.22)	0.460
Fat-free mass	-0.17 (-0.57 – 0.2)	0.413	-0.21 (-0.49 – 0.08)	0.154	-0.43 (-0.87 – 0.01)	0.054	-0.03 (-0.52, 0.46)	0.910
Age	0.01 (-0.27 – 0.28)	0.964	-0.03 (-0.22 – 0.17)	0.782	-0.05 (-0.35 – 0.26)	0.735	-0.1 (-0.45, 0.25)	0.566
Male	-0.25 (-1.07 – 0.56)	0.535	-0.31 (-0.27 – 0.88)	0.290	0.78 (-0.11 – 1.66)	0.084	-0.15 (-1.09, 0.79)	0.748
ΔFRS	-0.47 (-0.72 – -0.23)	**<0.001**	-0.83 (-1.00 – -0.65)	**<0.001**	-0.24 (-0.50 – 0.03)	0.083	-0.50 (-0.78, -0.23)	**0.001**

For patients with repeat assessments, their Δ Fat-free mass, Δ ALSFRS-R, Δ Body-mass index and Δ FVC (seated, % of predicted) were determined by fitting a linear mixed-effects regression with time as a fixed effect and subject as a random effect, and estimating a random intercept and slope. Correlations between these per-subject slopes were estimated by scaling each variable by the number of standard deviations from the group mean and fitting a linear regression to these scaled variables.

There was no significant relationship between baseline creatinine and loss of BMI. Using Cox proportional hazard regressions, we next modelled patient survival relative to measures of venous creatinine and fat-free mass. Age, sex, ΔFRS, and FVC (% of predicted) at first assessment were also considered as predictors (**Table 4**). Kaplan-Meier plots of fat-free mass and venous creatinine Cox predictors are shown in **Figures 4D&E**. Having lower levels of venous creatinine (25th percentile) was associated with a greater risk for shorter survival (**Figure 4E**).

Table 4. Risk factors at baseline and their relationship with survival

	HR	p
Creatinine	0.94 (0.90 – 0.98)	**0.007**
Fat-free mass	1.09 (1.00 – 1.18)	**0.047**
Age	1.09 (1.03 – 1.15)	**0.002**
Sex (Male)	0.4 (0.09 – 1.79)	0.228
ΔFRS	5.47 (2.37 – 12.59)	**<0.001**
FVC (% of predicted)	0.96 (0.94 – 0.99)	**0.002**

Abbreviations: HR = hazard ratio (95% CI); ΔFRS = change in the revised amyotrophic lateral sclerosis functional rating scale (points/month since date of symptom onset); FVC = forced vital capacity (seated, % of predicted).
Of the 71 patients included, 29 (40.8 %), died during follow-up. Time to death since symptom onset: mean ± SD = 25.29 ± 14.58 months.
Likelihood ratio, Wald, score tests: $p < 0.001$.

Figure 4. Correlations between venous creatinine and clinical and anthropomorphic measures, and Kaplan-Meier plots based on a Cox proportional hazards model, with venous creatinine, fat-free mass, age, sex, and ΔFRS as predictors.

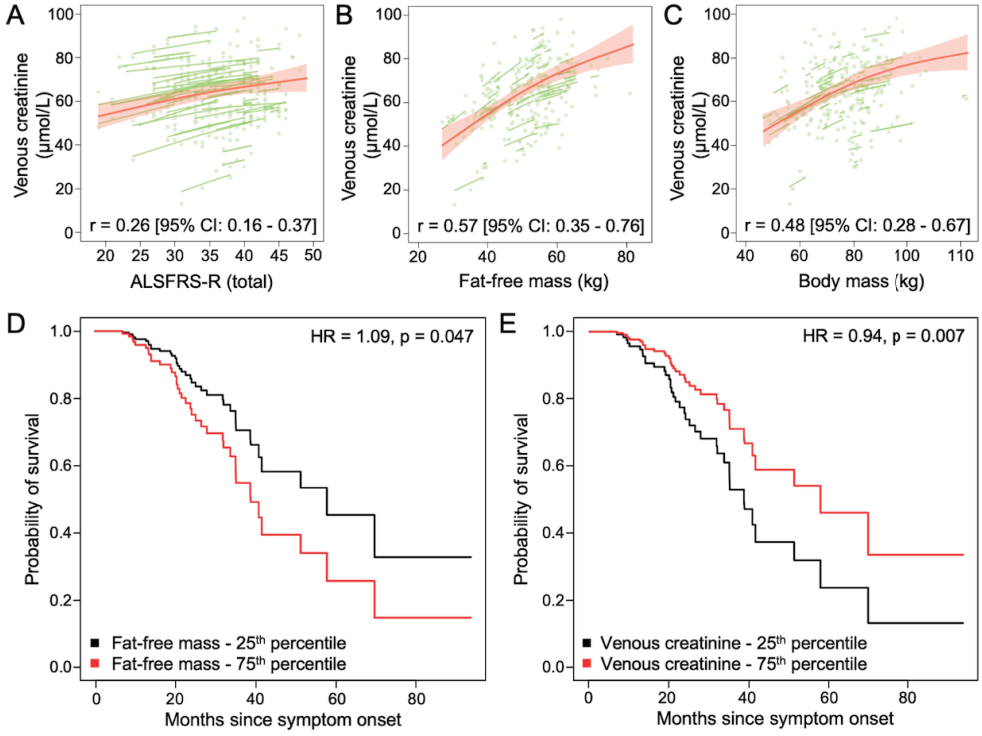


Figure 4. Within-case correlations between venous creatinine, and **(A)** ALSFRS-R, **(B)** fat-free mass, **(C)** body mass. Plots show within-case relations (green) and a global fit (red) with standard deviation. Models were fitted with restricted cubic splines, and errors were estimated using bootstrapping. Cumulative survival curves were predicted for **(D)** fat-free mass and **(E)** venous creatinine using their 25th and 75th percentiles. Kaplan-Meier plots show probability for survival during follow-up for a patient while holding other predictors constant at their mean. ALSFRS-R: Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised. HR = hazard ratio; ΔFRS = (48-ALSFRS-R)/months since symptom onset.

DISCUSSION

Venous creatinine has been shown to correlate with fat-free mass in healthy controls,[15] and it is suggested to be a biomarker for disease progression in patients with ALS.[16] In this longitudinal multicohort observational study, we assessed the reliability of measures of venous creatinine as an indirect measure of the change in fat-free mass in patients with ALS. We observed a correlation between fat-free mass and venous creatinine in cases and controls. A decline in venous creatinine was observed alongside the loss of fat-free mass over the course of disease. This is consistent with neurogenic wasting of muscle in ALS.[1] Lower levels of creatinine in patients with ALS were also associated with shorter survival. We show that

serial measures of venous creatinine can be used to monitor the change in fat-free mass in patients over the course of disease, and thus may serve as an ancillary endpoint in clinicals trials aimed at slowing the loss of fat-free mass and disease progression.

At baseline, we found venous creatinine to be lower in patients with ALS despite observing similar amounts of fat-free mass between cases and controls. The reason for this decrease in creatinine levels relative to fat-free mass remains unclear. Venous creatinine is a nonenzymatic product of intracellular ATP turnover,[17] and a reduction in creatinine in patients with ALS may be indicative of reduced ATP utilisation, as is proposed to occur in ALS.[18] Several studies have reported an impact of physical activity on venous creatinine.[15,19–22] Venous creatinine increases with increased exercise,[20] however may be decreased in people who participate in endurance exercise.[21,22] Whether a reduction in venous measures of creatinine in patients with ALS might be associated with impairments in ATP turnover or reductions in physical activity secondary to disability remains to be determined. Measures of venous creatinine were also decreased in patients with ALS from the Netherlands when compared to measures from the Australian cohort. Differences in venous creatinine between the two patient cohorts may indicate variation in underlying disease pathology. However, given that these cohorts had similar diagnostic delay and functional capacity (as inferred by similar ALSFRS-R scores), this may also be due to differences in population lifestyles, including variations in dietary intake and physical activity between cohorts; dietary intake[23] and physical activity[15,22] may impact measures of creatinine. While the reason for this difference is not clear, it is important to recognize. When conducting large multinational studies, differences between cohorts could impact study outcomes where measures are limited by single baseline comparisons.

Venous creatinine is known to be a correlate for striated muscle in healthy control research participants.[3] Previously, a relationship between plasma creatinine and measures of muscle strength in ALS has been observed.[16] Here we now show that, when using a one-off measure of creatinine, a moderate correlation can be observed between venous creatinine and fat-free mass in patients with ALS. Single measures of venous creatinine relative to fat-free mass varied considerably between participants, suggesting limited potential for one-off measures of venous creatinine to directly infer absolute measures of fat-free mass. Multiple factors could impact measures of venous creatinine. For example, venous creatinine is known to be increased in males,[24] as was confirmed in our study cohort. It has also been shown to increase with age,[24] however we did not observe a significant correlation in our participants.

Levels of venous creatinine in patients with ALS declined alongside a progressive decline in fat-free mass. This relationship between venous creatinine and fat-free mass was stronger when compared to the relationship between venous creatinine and declines in total body mass, whereas no relationship was observed for BMI. This is not unexpected; it is established that levels of creatinine are proportional to skeletal muscle mass,[3] whereas measures of BMI

include both fat-free mass and fat mass. Of interest, we found that declines in BMI were greater in patients with higher baseline fat-free mass. While BMI is generally considered as a measure of adiposity, variation in BMI is also due to changes in muscle mass.[25] Thus, a greater decline in BMI can be expected in patients with more fat-free mass and who experience significant muscle wasting. Overall, observations demonstrate that serial measures of creatinine can be used to infer the change in fat-free mass that occurs with disease progression in patients with ALS. A loss of fat-free mass due to neurogenic wasting would precede decreased ability to utilize the remaining muscle. This was reflected in our patient cohort as a decrease in ALSFRS-R over the course of disease. This decrease in ALSFRS-R shared a medium correlation with the decrease in measures of venous creatinine, which recapitulates previous findings that show that creatinine can be used as a longitudinal objective physiological biomarker for disease progression.[16]

We found that venous creatinine was independently predictive for shorter survival (correcting for covariates: sex, age, fat-free mass, ΔFRS, and FVC (% of predicted)), albeit to a lesser extent than ALSFRS-R. The greater sensitivity for ALSFRS-R to predict survival may be attributed to inclusion of measures that reflect changes in respiratory function, which itself greatly impacts prognosis.[26,27] By contrast, the relationship between venous creatinine and survival outcome is likely to correspond to more widespread muscle wasting that is associated with more progressive disease. Our outcomes suggest that venous creatinine may be an effective indictor of disease progression in ALS, and thus could inform clinical care. It would be useful however to further validate the potential for venous creatinine as a biomarker for disease progression by comparing it against neurophysiological biomarkers that have been shown to inform of changes in disease progression.[28]

Our use of point-of care methodology supports the notion that measures of venous creatinine can inform clinical outcomes during routine patient visits, and that changes in fat-free mass in patients with ALS can be inferred without the need for expensive or intensive compositional assessments. Indeed, similar approaches have been of benefit to other patient cohorts with limited access to specialist facilities that are essential for care.[29] While we argue for the clinical utility of rapid testing of venous creatinine in ALS, our approach does not discount 24-hr urine creatine excretion as a stronger correlate. 24-hr urine creatine excretion rate is an established marker for muscle mass in healthy individuals,[30] and evidence supports the use of 24-hr urine creatine excretion rate as a correlate for fat-free mass in patients at risk of sarcopenia.[31] Therefore, potential to improve the use of creatinine as a correlate for a change in muscle mass and disease progression in ALS remains. Also, our assessment of the utility of venous creatinine as a predictor for survival did not consider all factors known to modify survival, including genetics,[32] particularly C9orf72 status,[33] and extra-motor features such as cognitive impairment.[34] These factors may also contribute to changes in fat-free mass,[35] and so the impact of "disease modifiers" on the utility of venous creatinine as a marker for

change in fat-free mass remains unknown. While fat-free mass may not decline more rapidly in patients with loss of appetite[7] associations between dietary intake and creatinine[23] would suggest that alterations in food intake in patients with ALS could impact circulating levels of creatinine. Therefore, it is important that future studies also consider the interplay between nutritional status, creatinine, body composition, and survival.

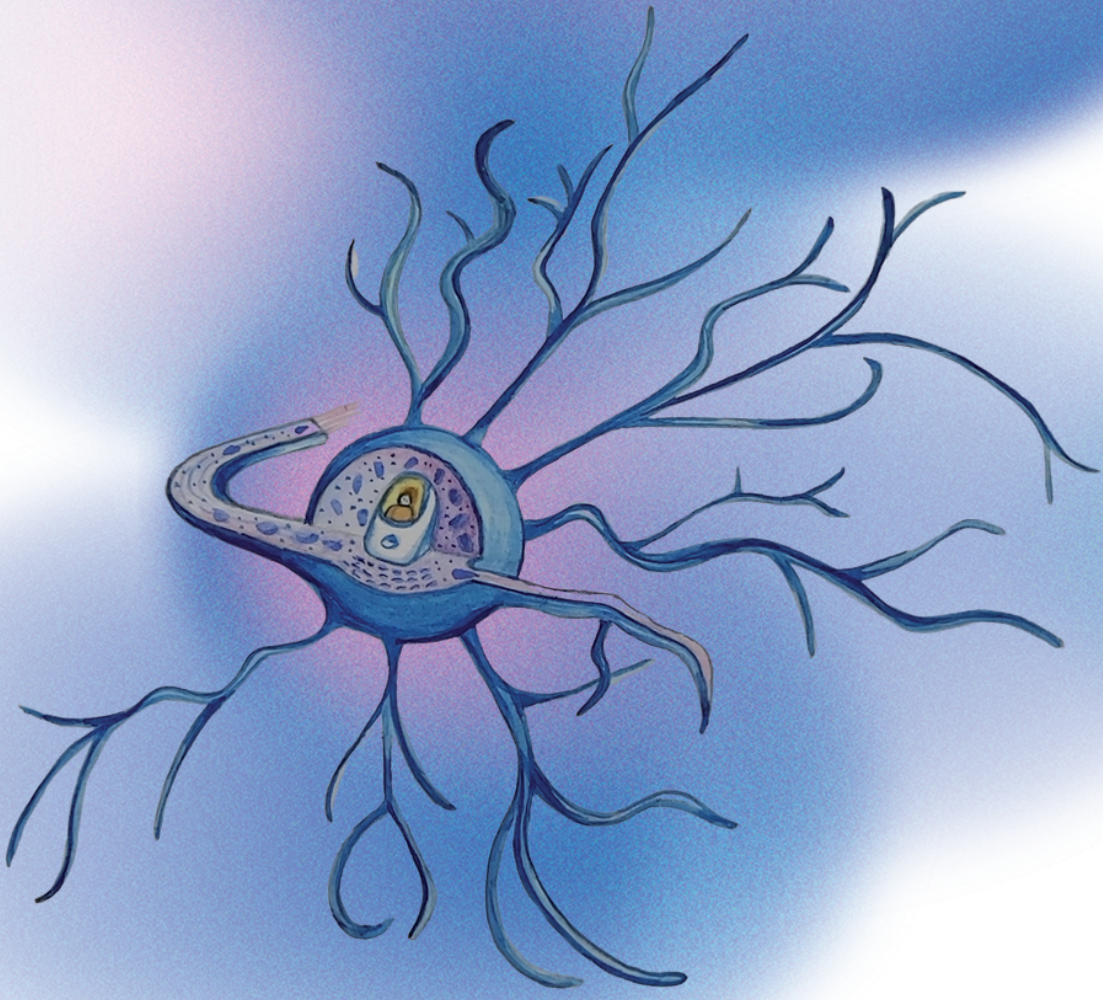
CONCLUSION

Venous creatinine has been proposed to be a measure of disease progression in ALS. Here we show that use of a point-of-care device to assess venous creatinine can be helpful for monitoring the progressive loss of fat-free mass in patients with ALS. We also show that venous creatinine is predictive of survival outcome. These observations highlight the potential for monitoring venous creatinine as part of routine clinical assessments, and as a proxy to evaluate the benefit of therapies aimed at slowing the loss of fat-free mass during disease progression.

REFERENCES

1. Brown RH, Al-Chalabi A. Amyotrophic Lateral Sclerosis. New England Journal of Medicine 2017;377(2):162–172.
2. Heymsfield SB, Adamek M, Gonzalez MC, Jia G, Thomas DM. Assessing skeletal muscle mass: Historical overview and state of the art. J Cachexia Sarcopenia Muscle 2014;5(1):9–18.
3. Schutte JE, Longhurst JC, Gaffney FA, Bastian BC, Gunnar C. Total plasma creatinine: an accurate measure of total striated muscle mass. J Appl Physiol Respir Environ Exerc Physiol 1981;51(3):762–766.
4. Chiò A, Calvo A, Bovio G, et al. Amyotrophic lateral sclerosis outcome measures and the role of albumin and creatinine: A population-based study. JAMA Neurol 2014;71(9):1134–1142.
5. Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders 2000;1(5):293–299.
6. Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. Nutrition 1993;9(5):480–491.
7. Ngo ST, van Eijk RPA, Chachay V, et al. Loss of appetite is associated with a loss of weight and fat mass in patients with amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2019;20(7–8):497–505.
8. McCrory MA, Molé PA, Gomez TD, Dewey KG, Bernauer EM. Body composition by air-displacement plethysmography by using predicted and measured thoracic gas volumes. J. Appl. Physiol 1998;84(4):1475–1479.
9. Cedarbaum JM, Stambler N, Fuller C, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. J Neurol Sci 1999;169:13–21.
10. Balendra R, Jones A, Jivraj N, et al. Estimating clinical stage of amyotrophic lateral sclerosis from the ALS Functional Rating Scale. Amyotroph Lateral Scler Frontotemporal Degener 2014;15(3–4):279–284.
11. Kimura F, Fujimura C, Ishida S, et al. Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. Neurology 2006;66(2):265–267.
12. Cai B, Dunson DB. Bayesian covariance selection in generalized linear mixed models. Biometrics 2006;62(2):446–457.
13. Ariyo O, Lesaffre E, Verbeke G, Quintero A. Model selection for Bayesian linear mixed models with longitudinal data: Sensitivity to the choice of priors. Commun Stat Simul Comput 2022;51(4):1591–1615.
14. Ngo ST, Steyn FJ, McCombe PA. Body mass index and dietary intervention: Implications for prognosis of amyotrophic lateral sclerosis. J Neurol Sci 2014;340(1–2):5–12.
15. Baxmann AC, Ahmed MS, Marques NC, et al. Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. Clinical Journal of the American Society of Nephrology 2008;3(2):348–354.
16. Van Eijk RPA, Eijkemans MJC, Ferguson TA, Nikolakopoulos S, Veldink JH, Van Den Berg LH. Monitoring disease progression with plasma creatinine in amyotrophic lateral sclerosis clinical trials. J Neurol Neurosurg Psychiatry 2018;89(2):156–161.
17. Wyss M, Kaddurah-Daouk R, Hoffmann- F, Roche L. Creatine and Creatinine Metabolism. Physiol Rev 2000;80(3):1107–1213.
18. Smith EF, Shaw PJ, De Vos KJ. The role of mitochondria in amyotrophic lateral sclerosis. Neurosci Lett 2019;710:132933.

19. Janssen GME, Degenaar CP, Menheeree PPCA, Habets HML, Geurten P. Plasma Urea, Creatinine, Uric Acid, Albumin, and Total Protein Concentrations Before and After 15-, 25-, and 42-km Contests. Int J Sports Med 1989;10:S132–S138.
20. Fragala MS, Bi C, Chaump M, Kaufman HW, Kroll MH. Associations of aerobic and strength exercise with clinical laboratory test values. PLoS One 2017;12(10):1–22.
21. Lippi G, Brocco G, Franchini M, Schena F, Guidi G. Comparison of serum creatinine, uric acid, albumin and glucose in male professional endurance athletes compared with healthy controls. Clin Chem Lab Med 2004;42(6):644–647.
22. Lippi G, Salvagno GL, Montagnana M, Schena F, Ballestrieri F, Guidi GC. Influence of physical exercise and relationship with biochemical variables of NT-pro-brain natriuretic peptide and ischemia modified albumin. Clinica Chimica Acta 2006;367(1–2):175–180.
23. Nair S, O'Brien S V., Hayden K, et al. Effect of a cooked meat meal on serum creatinine and estimated glomerular filtration rate in diabetes-related kidney disease objective. Diabetes Care 2014;37(2):483–487.
24. Hannemann A, Friedrich N, Dittmann K, et al. Age-and sex-specific reference limits for creatinine, cystatin C and the estimated glomerular filtration rate. Clin Chem Lab Med 2012;50(5):919–926.
25. Heymsfield SB, Scherzer R, Pietrobelli A, Lewis CE, Grunfeld C. Body mass index as a phenotypic expression of adiposity: Quantitative contribution of muscularity in a population-based sample. Int J Obes 2009;33(12):1363–1373.
26. Hardiman O. Hardiman - Symptomatic treatment of respiratory and nutritional failure in amyotrophic lateral sclerosis. J Neurol 2000;247(4):245–251.
27. Traynor BJ, Alexander M, Corr B. Effect of a multidisciplinary amyotrophic lateral sclerosis (ALS) clinic on ALS survival: a population based study, 1996-2000. J Neurol Neurosurg Psychiatry 2003;74:1258–1261.
28. de Carvalho M, Chio A, Dengler R, Hecht M, Weber M, Swash M. Neurophysiological measures in amyotrophic lateral sclerosis: Markers of progression in clinical trials. Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders 2005;6(1):17–28.
29. Tideman PA, Tirimacco R, Senior DP, et al. Impact of a regionalised clinical cardiac support network on mortality among rural patients with myocardial infarction. Medical Journal of Australia 2014;200(3):157–160.
30. Heymsfield SB, Arteaga C, McManus CM, Smith J, Moffitt S. Measurement of muscle mass in humans: Validity of the 24-hour urinary creatinine method. American Journal of Clinical Nutrition 1983;37(3):478–494.
31. Jassal SK, Wassel CL, Laughlin GA, Barrett-Connor E, Rifkin DE, Ix JH. Urine creatinine-based estimates of fat-free mass in community-dwelling older persons: The Rancho Bernardo study. Journal of Renal Nutrition 2015;25(2):97–102.
32. Simon NG, Turner MR, Vucic S, et al. Quantifying disease progression in amyotrophic lateral sclerosis. Ann Neurol 2014;76(5):643–657.
33. Umoh ME, Fournier C, Li Y, et al. Comparative analysis of C9orf72 and sporadic disease in an ALS clinic population. Neurology 2016;87(10):1024–1030.
34. Huynh W, Ahmed R, Mahoney CJ, et al. The impact of cognitive and behavioral impairment in amyotrophic lateral sclerosis. Expert Rev Neurother 2020;20(3):281–293.
35. Iacoangeli A, Lin T, Al Khleifat A, et al. Genome-wide Meta-analysis Finds the ACSL5-ZDHHC6 Locus Is Associated with ALS and Links Weight Loss to the Disease Genetics. Cell Rep 2020;33(4):108323.



Chapter 5

Variation in Resting Metabolic Rate Impacts Identification of Metabolic Change in Geographically Distinct Cohorts of Amyotrophic Lateral Sclerosis Patients

Cory J. Holdom, MSc., Mark R. Janse van Mantgem, M.D., Ji He, M.D., Stephanie L. Howe, MSc., Pamela A. McCombe, M.D., PhD., Dongsheng Fan, M.D., PhD., Robert D. Henderson, M.D., PhD., Leonard H. van den Berg, M.D., PhD., Ruben P.A. van Eijk, M.D., PhD., Frederik J. Steyn, PhD.*, Shyuan T. Ngo, PhD.*

***Equal contribution**

Accepted at Neurology

ABSTRACT

Background: Altered metabolism is observed in Amyotrophic Lateral Sclerosis (ALS). However, without a standardized methodology to define metabolic changes, our understanding of factors contributing to, and the clinical significance of altered metabolism in ALS is limited.

Methods: We aimed to determine how geographic variation in metabolic rates influences estimates and accuracy of predicted resting energy expenditure (REE) in ALS patients and controls, while validating the effectiveness of cohort-specific approaches in predicting altered metabolic rate in ALS. Participants from three geographically distinct sites across Australia, China, and the Netherlands underwent REE assessments, and we considered 22 unique equations for estimating REE. Analyses evaluated equation performance and the influence of demographics on metabolic status. Comparisons were made using standardized and local reference values to identify metabolic alterations.

Results: 606 participants were included from Australia (ALS: 140, Controls: 154), The Netherlands (ALS: 79, Controls: 37) and China (ALS: 67, Controls: 129). Measured REE was variable across geographic cohorts, with fat free mass contributing to this variation across all patients ($p=0.002$ to $p<0.001$). Of the 22 predication equations assessed, the Sabounchi Structure 4 (S4) equation best predicted metabolic rate across all control cohorts. Use of prediction thresholds generated using data from Australian controls generally increased the prevalence of and hypermetabolism in Chinese (55%, [43-67%]) and Dutch (44%, [33-55%]) cases when compared to Australian cases (30%, [22-38%]). Adjustment of prediction thresholds to consider geographically distinct characteristics from matched control cohorts resulted in a decrease in the proportion of hypermetabolic cases in Chinese and Dutch cohorts (25-31% vs. 55% and 20-34% vs. 43-44%, respectively), and increased prevalence of hypometabolism in Dutch cases with ALS (1% to 8-10%).

Conclusions: The identification of hypermetabolism in ALS is influenced by the formulae and demographic-specific prediction thresholds used for defining alterations in metabolic rate. A consensus approach is needed for identification of metabolic changes in ALS and will facilitate improved understanding of the cause and clinical significance of this in ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive weakness leading to death from respiratory failure. ALS is heterogeneous in clinical features; patients have variable signs of upper and lower motor neuron dysfunction, vary in age of onset, site of disease presentation, spread of disease, and survival outcome.[1] 5-10% of all cases of ALS have a family history of the disease, and some of these families have autosomal dominant inheritance of disease-linked gene mutations.[2] Around 21–33% of cases without a family history of ALS harbour potentially pathogenic gene mutations.[3-5] Non-genetic factors also contribute to the risk of developing ALS.[6-8] Although the biological processes underlying ALS are not fully understood, the identification of disease-modifying factors is crucial for gaining insights into potential pathways for treatment.

A change in resting energy expenditure (REE) is one of the many metabolic perturbations observed in ALS,[9] wherein hypometabolism is defined as a reduction and hypermetabolism as an increase in measured REE (mREE) relative to predicted REE (pREE), respectively. Both hypometabolism and hypermetabolism are associated with disease outcome, suggesting that metabolic rate may be a disease modifier.[10-16] Studies of metabolic rate in ALS have used different approaches for identifying alterations in REE.[9] Furthermore, there is no standardization of normal values across different populations. As such, it is difficult to compare studies. This is especially pertinent given the number of studies aimed at understanding the causes and consequences of metabolic perturbations in ALS,[9] and clinical trials aimed at targeting hypermetabolism in ALS (i.e., NCT04788745).

Here we explore the use of a standardized approach for defining altered metabolic rate across geographically diverse cohorts of people living with ALS. We also investigate whether cohort specific references for defining hypo- and hypermetabolism are more appropriate than values established from a single reference population.

METHODS

Study design and clinical assessment

This case-control, multicohort study was conducted between April 2015 and December 2022 with data collected across three sites: The Royal Brisbane and Women's Hospital (RBWH; Brisbane, Australia), Peking University Third Hospital (PUTH, Beijing, China) and the University Medical Centre (UMC; Utrecht, the Netherlands). Patients who received a diagnosis of probable or definite ALS using the revised El Escorial criteria[17] were invited to participate (**Figure 1**).

Figure 1. Flowchart

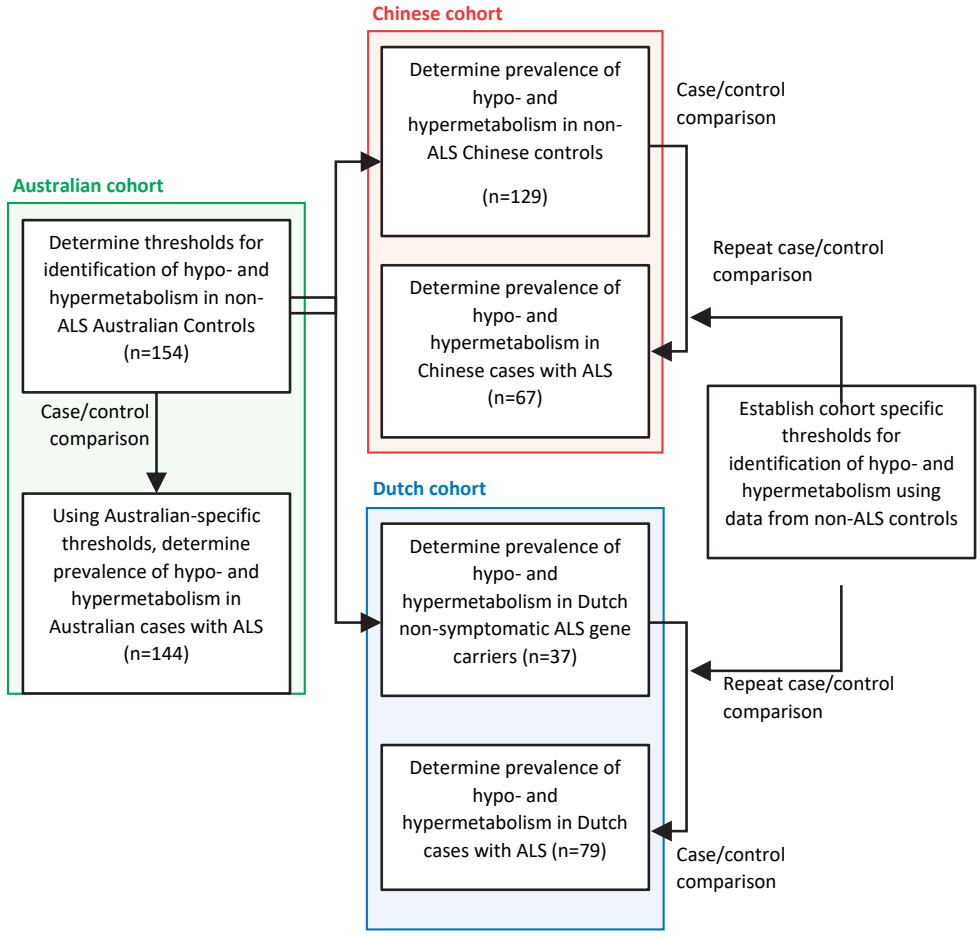


Figure 1. Schematic summarising number of participants (Cases and Controls) enrolled from each geographically distinct cohort, and the development of Australian and geographic-cohort specific criteria for the identification of hypo- and hypermetabolism in patients with ALS.

Information on patient demographics and disease onset was collected at enrolment. Each site provided data from a cohort of controls with no history of neuromuscular disease. For Australian and Chinese cohorts, non-ALS controls were family and friends of patients with ALS. Dutch controls were non-symptomatic ALS gene carriers or relatives of patients with familial ALS. Demographics for all participants at baseline are presented in **Table 1**. While the Chinese cohort was demographically well-matched, there were notable differences in age and sex distributions in the Australian and Dutch cohorts. The clinical history of participants was noted. For patients with ALS, the ALS Functional Rating Scale-Revised (ALSFRS-R)[18] was collected. This study was approved by: Australia: The University of Queensland (2015/HE000022), RBWH (HREC/14/QRBW/495), and Wesley Hospital (1622) human research ethics

committees; China: The Research Ethics Committee of Peking University Third Hospital; Netherlands: Medical ethics committee and institutional board of UMC Utrecht, The Netherlands, permit number 15-656 (MEASURE). All participants provided written, informed consent.

Body composition and metabolic assessments

Australian and Dutch participants were asked to fast overnight for at least 12h and to avoid strenuous physical activity prior to metabolic assessment. Height was measured using a stadiometer. Body composition was measured using previously reported methods;[16, 19] the BODPOD Gold Standard system (COSMED, Concord, CA, USA) was used to measure body density, from which fat and fat-free mass was inferred. Body mass index (BMI) was calculated as body mass divided by the square of height. For metabolic assessments, participants were asked to lie on an examination bed at a semi-supine (~30-45°) position. A Quark RMR or Quark Q-NRG (COSMED, Concord, CA, USA) equipped with a canopy hood was used to determine mREE. A ventilatory hood pump was used to maintain $FeCO_2$ at ~1%. Data were collected over 20 minutes, and the last 15 minutes of recording was used to determine mREE.

For the Chinese cohort, participants were asked to avoid eating, drinking, and exercising for at least 6 h prior to metabolic assessment. A multi-frequency bioelectrical impedance analysis (BIA) InBody 770 scanner (InBody 770; Biospace, Seoul, South Korea) was used to measure body composition. An ULTIMACardio2 gas exchange analysis system (Medgraphics Corp, USA) was used to determine mREE. Measures were collected from participants resting in a semi-supine position. Data were recorded for at least 16 minutes, and the last 11 minutes of recording were used to determine mREE. For all participants (Australian, Dutch, and Chinese cohorts) the Weir's equation was used to estimate REE from VO_2 consumption and VCO_2 production.[10]

Identification of metabolic status

We searched PubMed and Google Scholar for reports of methods for estimating REE. Twenty-two unique equations were identified and considered for selection. Equations were sorted into two groups: those correcting for body composition, and those not correcting for body composition. Equation performance for predicting metabolic rate was assessed using Bland-Altman analyses. The best performing equations were used to determine cohort-specific references for the determination of deviations in metabolic rates between cases and controls. The ratio of mREE to pREE was used to calculate the per-equation metabolic index (MI) for all individuals. Using data derived from non-ALS control cohorts, a MI>1 SD above the mean was considered "hypermetabolic". Controls with a MI<1 SD below the mean were considered "hypometabolic". Controls with a MI within 1 SD were considered "normometabolic". To evaluate the effect of using standardized versus local reference values for the determination of metabolic status, individuals were assigned a metabolic status relative to reference values

derived from the Australian non-ALS control cohort, as well as a metabolic status using reference values derived from within their geographically matched control cohort (**Figure 1**).

Data analysis

Summary numbers are presented as mean ±SD, or n (%). Group quantitative comparisons were conducted using Welch-adjusted t tests. Multiple groups were compared using ANOVA. Proportions were compared using Fisher exact tests. Correlations were assessed using Pearson correlation. Ordered logistic regression and likelihood ratio tests were used to evaluate the effect of demographic features on metabolic status. Significance was determined at $\alpha = 0.05$. All analyses were conducted in R (version 4.2.2; The R Foundation for Statistical Computing Platform, 2022). Logistic regression was conducted with the *ordinal* package (version 2022.11-16; Christensen, 2022). Data were presented using *ggplot2* (version 3.4.1; Wickham, 2016) and Microsoft PowerPoint (version 2004).

RESULTS

Population characteristics

The analysis incorporated data from 606 individuals: the Australian sample comprised 154 controls and 140 cases; the Chinese sample, 129 controls and 67 cases; and the Dutch sample, 37 controls and 79 cases. The Chinese cohort was age and sex-matched between cases and controls. For Australia and the Netherlands, there was greater proportion of males, and greater mean age in cases. Demographic data is presented in **Table 1**. Demographics by sex is presented in **Supplementary Table 1**.

Resting metabolic rate is highly variable within and between cohorts

Metabolic outcomes are presented in **Table 1** and population distributions of mREE are illustrated in **Figure 2**. Measured REE was highly variable between sexes, and across geographic cohorts. To explore factors that contributed to this heterogeneity we conducted Pearson correlations between mREE and population characteristics commonly used in predicting REE (sex, fat-free mass, fat mass, age, and height). For the factors most strongly correlated with mREE, there were large differences among countries (**Table 2**).

Table 1: Demographics and measures of metabolism in cases and controls in Australian, Chinese and Dutch study cohorts

	Australia			China			Netherlands		
	Control (n=154)	ALS (n=140)	p	Control (n=129)	ALS (n=67)	p	Control (n=37)	ALS (n=79)	p
Demographics									
Age, years	56.62 ±10.44	60.42 ±9.93	**0.002**	51.97 ±11.53	51.95 ±10.41	0.993	45.89 ±12.86	59.95 ±10.11	**<0.001**
Female	72 (47%)	39 (28%)	**0.001**	48 (38%)	27 (42%)	0.639	21 (57%)	26 (33%)	**0.025**
Anthropometric measures									
Height, cm	171.87 ±8.81	172.82 ±9.13	0.364	165.51 ±7.98	165.34 ±8.5	0.898	174.39 ±8.62	175.34 ±8.93	0.587
Body mass, kg	81.4 ±16.79	79.41 ±17.31	0.267	67.88 ±11.97	66.95 ±13.38	0.642	80.31 ±18.55	78.7 ±12.69	0.636
BMI, $kg.m^{-2}$	27.49 ±5.11	26.48 ±4.92	0.071	24.66 ±3.10	24.35 ±3.64	0.571	26.41 ±5.87	25.53 ±3.19	0.398
Fat, kg	28.4 ±12.25	27.47 ±13.1	0.531	19.98 ±5.71	20.67 ±6.69	0.487	27.4 ±13.63	26.39 ±8.09	0.680
Fat (%)	34.14 ±10.2	33.7 ±11.74	0.731	29.32 ±6.35	30.48 ±7.44	0.289	32.88 ±10.04	33.41 ±8.49	0.779
Fat-free mass, kg	52.92 ±10.96	52.1 ±11.21	0.528	47.74 ±8.79	46.47 ±9.66	0.381	52.91 ±9.78	52.31 ±10.35	0.764
Clinical measures									
Time since onset (months)		22.68 ±17.42						24.00 ±18.13	
Time since diagnosis (months)		7.51 ±9.58			14.69 ±9.77			10.36 ±10.39	
ALSFRS-R		37.79 ±5.40			42.61 ±3.99			38.76 ±5.07	
Metabolic measures/outcomes									
Resting metabolic rate, $kcal.day^{-1}$	1,601 ±354	1,656 ±410	0.218	1,504 ±374	1654 ±418	**0.017**	1,730 ±315	1,747 ±264	0.772
Harris-Benedict prediction, $kcal.day^{-1}$	1,626 ±269	1,612 ±280	0.652	1,472 ±221	1459 ±234	0.707	1,656 ±279	1,609 ±236	0.378
Harris-Benedict Index	0.98 ±0.13	1.02 ±0.16	**0.017**	1.02 ±0.20	1.13 ±0.23	**0.001**	1.04 ±0.08	1.09 ±0.10	**0.010**
Sabounchi S4 prediction, $kcal.day^{-1}$	1,609 ±243	1,589 ±245	0.482	1,461 ±194	1437 ±218	0.465	1,603 ±233	1,588 ±222	0.749
Sabounchi Index	0.99 ±0.13	1.04 ±0.18	**0.012**	1.03 ±0.22	1.15 ±0.22	**<0.001**	1.08 ±0.10	1.10 ±0.09	0.178

Data are presented as mean ± sd, or n (%). P-values estimated from Welch-adjusted t tests to compare means, and Fisher exact tests to compare proportions. Bold text highlights p <0.05.

Figure 2. Distributions of resting metabolic rate in Australian, Chinese and Dutch participants.

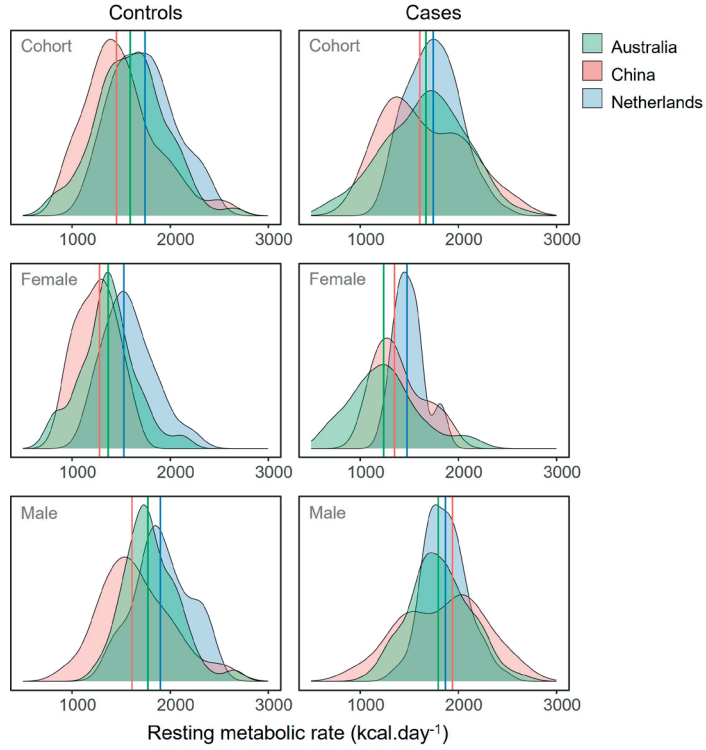


Figure 2. Distributions are colored by geographic region (Australia: green, China: red, Netherlands: blue). Colored lines indicate median of each distribution.

Table 2: Partial correlations for resting metabolic rate between cohorts

	Australia				China				Netherlands			
	Control		ALS		Control		ALS		Control		ALS	
	r	p	*r*	p	*r*	p	*r*	p	*r*	p	*r*	p
Female	0.21	**0.011**	0.38	**<0.001**	0.41	**<0.001**	0.04	0.788	0.26	0.143	0.19	0.094
Fat-free mass	0.49	**<0.001**	0.3	**0.002**	-0.08	0.409	0.36	**0.005**	0.44	**0.011**	0.64	**<0.001**
Fat mass	0.53	**<0.001**	0.5	**<0.001**	0.28	**0.002**	-0.08	0.550	0.70	**<0.001**	0.29	**0.013**
Age	-0.14	0.099	-0.29	**<0.001**	-0.27	**0.002**	-0.05	0.730	-0.46	**0.008**	-0.26	**0.024**
Height	-0.09	0.287	0.1	0.201	0.15	0.089	0.02	0.895	0.06	0.739	-0.24	**0.042**

Partial Pearson correlations shown. All predictors are shown adjusting for the other reported covariates. Bold text highlights p <0.05.

Within the Australian cohort, fat mass and fat-free mass had comparable correlations with REE for both cases and controls. Sex was also a strong correlate; being female was associated with a lower mREE. Fat-free mass was not associated with REE for the Chinese control cohort, however an association was observed for cases. In the Dutch cohort, fat-free mass had a similar correlation with REE as seen in Australian participants, however fat mass was more strongly associated with REE in Dutch controls. After adjusting for covariates, being female (r, Australia: control=0.21 p<0.011, ALS=0.38 p<0.001; China: control=0.41 p<0.001, ALS=0.04 p=0.788; Netherlands: control=0.26 p=0.143, ALS=0.19 p=0.094) and of older age (r, Australia: control=-0.14 p=0.099, ALS=-0.29 p<0.001; China: control=-0.27 p=0.002, ALS=-0.05 p=0.730; Netherlands: control=-0.46 p=0.008, ALS=-0.26 p=0.024) tended to be associated with a lower mREE.

Selection of equations for use in the study

Using Bland-Altman analyses we screened 22 equations commonly reported in the literature for their accuracy in predicting REE. Equations were ranked on bias (**Figure 3A**), with those producing the lowest bias (mREE-pREE) and highest accuracy (proportion of individuals with a pREE within ±10% of the mREE) in identifying mREE of Australian control participants considered the best-performing equations. Based on these criteria, we selected two equations for inclusion in models of MI; the Harris-Benedict (1919) equation[20], and an equation that adjusts for body composition that was first introduced by Sabounchi as part of a meta-analysis in 2014.[21] The 1919 Harris-Benedict (1919 HB) equation incorporates sex, age, height, and body mass. The Sabounchi (2014) equation incorporates sex and measures of body composition; of those validated, the Sabounchi's Structure 4 (Sabounchi S4) equation performed best.

Use of an Australian reference value identifies higher prevalence of hypermetabolism in Chinese and Dutch ALS cases

We derived an MI as the mREE/pREE for each equation (Harris-Benedict Index, HI; Sabounchi Index, SI). Using data from the Australian control cohort, we identified individuals as having a mean HI or SI within 1 SD of the mean as normometabolic; controls above 1 SD were considered hypermetabolic, controls below 1 SD were considered hypometabolic (**Figure 3B**). Within this cohort, the average HI was 0.98, with thresholds at 0.86 for hypo- and 1.11 for hypermetabolism. The average SI was 0.99, with thresholds at 0.86 and 1.12, for hypo- and hypermetabolism, respectively. As shown in **Supplemental table 2**, when applying the HI or SI, these thresholds identified 71% and 78% of Australian non-ALS controls as normometabolic, respectively. By comparison, 57% of cases (for both the HI and SI) were normometabolic, while ~30% of cases were hypermetabolic compared to 12-16% in controls. Using the same Australian reference values, we found an overall higher proportion of hypermetabolic individuals (both cases and controls) in Chinese and Dutch cohorts (**Supplemental table 2, Figure 4**). The proportion of hypometabolic patients with ALS were lower in the Chinese and Dutch cohorts. Within the Dutch cohort, only 1 individual was identified as being hypometabolic. Data specific to sex are presented in **Supplemental table 3**.

Figure 3. Selection and application of prediction equations for the determination of metabolic index in patients with ALS.

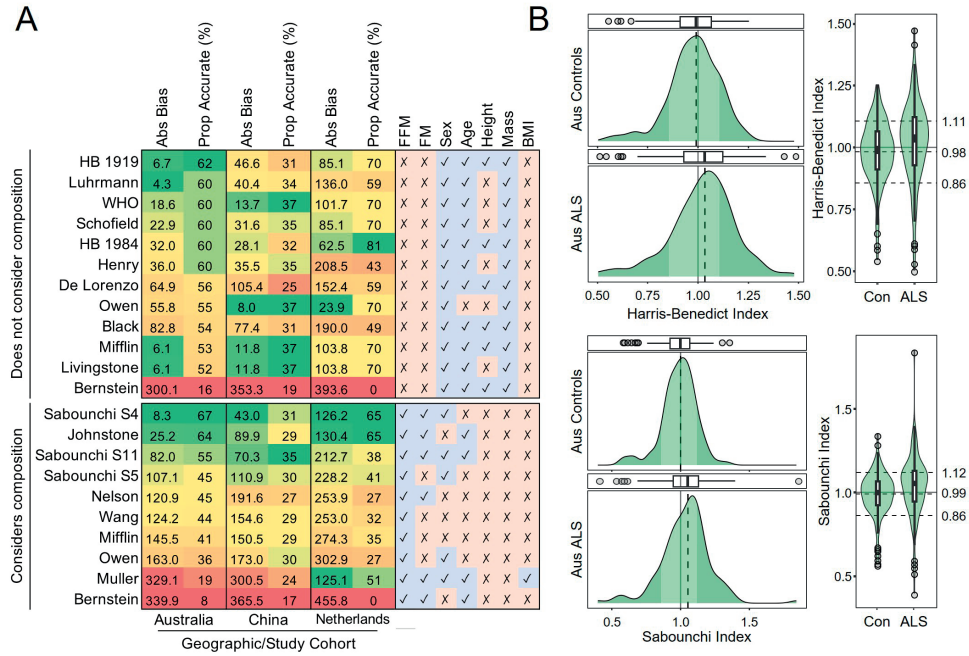


Figure 3. A. Reference equations for predicted resting metabolic rate were ranked based on inclusion of body composition, and accuracy in predicting RMR. Factors incorporated in each prediction model are indicated with a P, whereas those excluded are indicated with a O. Bland-Altman analysis was used to rank equations based on their absolute bias. Reported "Prop Accurate" identifies the proportion of Australian controls with a measured resting metabolic rate within ±10% of the equation's predicted metabolic rate. Better scores (low bias, high accuracy) are green; worse scores are red. **B.** Distribution of metabolic index calculated using the 1919 Harris-Benedict (top panels) or Sabounchi S4 (bottom panels) prediction equations; measures presented are limited to data from Australian controls (Con) and cases (ALS). Solid lines indicate mean MI, with the shaded regions highlighting the proportion of participants > 1 or < 1 standard deviation of the control mean MI. Dashed lines indicate the median metabolic index within each group.

Figure 4. Geographical variation on the classification of metabolic status

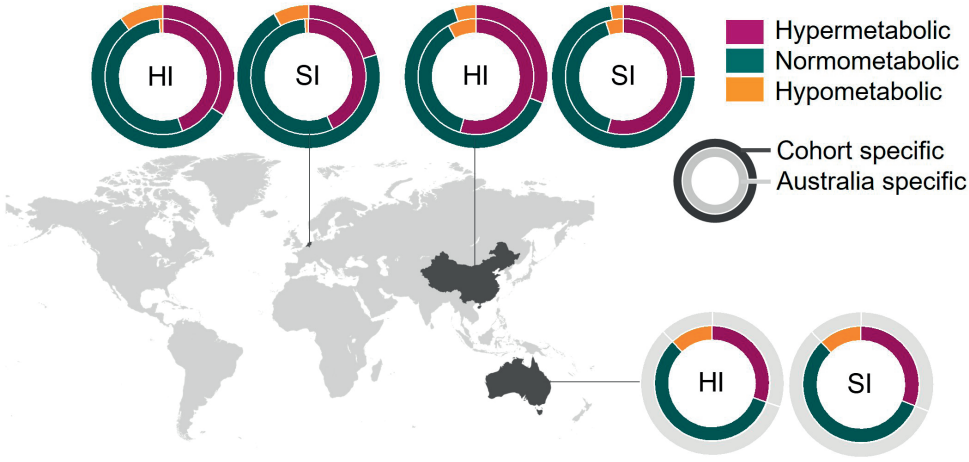


Figure 4. Comparison of Australian (inner circles) versus geographic cohort specific reference values (outer circles) on the classification of metabolic status in patients with ALS from Australia, China, and the Netherlands. For inner circles, information derived from non-ALS controls in Australia were used to generate reference values for hyper-, normo- and hypometabolism. Abbreviations: HI = 1919 Harris-Benedict Index, SI = Sabounchi S4 Index.

Reference values for the identification of hypo- and hypermetabolism in ALS should be determined in non-ALS controls matched to the ALS cohort

Next, for each country we used the local control cohort as a reference population for the development of reference values for identifying hypo- and hypermetabolic cases. For Chinese participants, reference values for the HI were 0.82 for hypometabolism, and 1.22 for hypermetabolism. For the SI, these were 0.81 and 1.25, respectively. For Dutch participants, reference values using the HI were 0.96 for hypometabolism, and 1.12 for hypermetabolism. For the SI, these were 0.98 and 1.17, respectively.

When compared to the Australian controls, the range between reference values for hypo- and hypermetabolism in Chinese controls was greater, consistent with the increased metabolic variability observed in this cohort. For Dutch controls, thresholds were generally higher, resulting in an overall upward shift in REE. Application of cohort-specific reference values resulted in an adjustment of prevalence of hypo- and hypermetabolism to fall within similar proportions to that seen in the Australian cases; the Chinese and Dutch groups had a comparable proportion of hyper-, normo- and hypometabolic controls when compared to proportions identified in the Australian group. When considering hypometabolism, this effect was greatest for the Dutch ALS cohort. Here, application of a cohort-specific reference value contributed to an 8- to 10-fold increase in the proportion of individuals identified as being hypometabolic.

Factors affecting metabolic status

We examined factors that affected the metabolic status of cases across Australian, Chinese, and Dutch cohorts. **Table 3** presents Pearson correlations for demographic features, and the HI or SI; a correlation suggests that the prediction used under- or overestimates the contribution to metabolic rate. For controls, sex and body composition were associated with MI in the Chinese cohort, suggesting that current equations may not accurately capture the contribution of these factors to MI in Chinese participants. For Australian and Dutch control cohorts, fat mass was associated with the SI, suggesting that the S4 equation may not completely account for the contribution of fat mass to these cohorts. Overall, the HI and SI performed generally well within all controls, as effect sizes were small. Of interest, when considering Australian cases, the SI was associated with sex, fat mass and age, suggesting that these factors contributed to some variation. We extended analysis to also consider disease duration on metabolic predictions in patients with ALS. We found no correlation between disease duration since onset or diagnosis and HI or SI (**Supplemental table 4**). Thus, disease duration is unlikely to impact the efficiency of the Sabounchi S4 and Harris-Benedict equations for predicting REE in ALS.

Finally, we explored the effect of formula and threshold selection on study demographics and the clinical characteristics of patients with ALS. As an exploratory exercise, we limited this comparison to data derived from the SI only (**Supplementary table 5**), as this was generally the best performing equation. Other than REE, sex was the most consistent differentiator between cases and controls when determining metabolic status. Demographics specific to sex and geographical location, and relative to categorization of metabolic status using the SI are presented in **Supplementary table 6**.

Table 3. Partial correlations for resting metabolic indices between cohorts

	Australia				China				Netherlands			
	Control		ALS		Control		ALS		Control		ALS	
	r	p	*r*	p	*r*	p	*r*	p	*r*	p	*r*	p
Harris-Benedict												
Female	0.06	0.455	0.28	**0.001**	0.38	**0.001**	-0.02	0.874	-0.05	0.768	-0.02	0.856
Fat-free mass	0.17	**0.043**	-0.03	0.701	-0.26	**0.004**	0.13	0.313	0.08	0.644	0.29	**0.013**
Fat mass	0,00	0.978	-0.07	0.390	0.07	0.444	-0.30	**0.021**	0.09	0.600	-0.42	**<0.001**
Age	0.13	0.123	-0.08	0.364	-0.05	0.614	0.08	0.538	0.06	0.739	0.09	0.435
Height	-0.13	0.114	0.01	0.899	0.06	0.490	-0.01	0.914	-0.11	0.528	-0.38	**<0.001**
Structure 4												
Female	0.22	**0.007**	0.38	**<0.001**	0.45	**<0.001**	0.03	0.810	0.24	0.172	0.17	0.139
Fat-free mass	-0.02	0.817	-0.28	**<0.001**	-0.39	**<0.001**	0.03	0.802	-0.26	0.152	0.03	0.814
Fat mass	0.32	**<0.001**	0.28	**<0.001**	0.20	**0.027**	-0.18	0.159	0.50	**0.004**	-0.03	0.831
Age	-0.11	0.167	-0.26	**0.002**	-0.28	**0.002**	-0.07	0.613	-0.46	**0.007**	-0.27	**0.019**
Height	-0.08	0.311	0.10	0.267	0.15	0.078	0.03	0.832	0.08	0.668	-0.26	**0.023**

Partial Pearson correlations shown. All predictors are shown, adjusting for the other covariates. Bold text highlights p <0.05.

DISCUSSION

We confirm altered levels of systemic metabolism in patients with ALS across three geographically diverse populations. We demonstrate that identification of hypo- or hypermetabolism is influenced by the criteria used to characterize metabolic status, and that variation across populations is shaped by factors specific to the demographics of each respective cohort. Accordingly, we conclude that the identification of cohort-specific reference values from non-ALS controls is important for developing selection criteria and defining thresholds for identifying hypo- and hypermetabolism in corresponding ALS cohorts.

Hypermetabolism is consistently reported in ALS[10-12, 14-16, 22-31] and is associated with more rapid disease progression and/or worse prognosis.[10-16] By comparison, hypometabolic patients with ALS have slower disease progression and longer survival.[30] While studies to date offer insights into the possible clinical consequence of altered systemic metabolism in ALS, considerable variation in the reported prevalence of hypermetabolism remains.[9] This heterogeneity may reflect naturally occurring differences in metabolic status across geographical regions or ancestry, or it could be linked to disease specific factors unique to each study population. Variation in disease characteristics is not uncommon in ALS.[32] For example, genetic predisposition for ALS (both sporadic and familial) is geographically distinct. *C9ORF72* hexanucleotide repeat expansions in ALS are more common in North America, Europe, and Australia, whereas these expansions are rare in patients in Asia; in Asia SOD1

variants are more common.[33] Whether similar differences in determinants of metabolic rate exist across patients from distinct genetic and/or geographical backgrounds remains unexplored. Attempts to reconcile such differences between cohorts are limited by the use of variable selection criteria across studies. As a first step towards the development of larger population-based studies investigating metabolism in ALS, it is critical that we improve understanding of the factors that contribute to variations in systemic metabolism in ALS, and then use this information to define appropriate strategies to correctly identify patients with deviations in metabolism.

Sex, age, and height are all associated with the variation in mREE. However, their contributions are different across geographic cohorts, and between cases and controls. By comparison, fat-free mass consistently contributed to variation in mREE across cases, regardless of geographic location. This is not unexpected as muscle accounts for 20-30% of resting energy requirements,[34] ALS is associated with neurogenic wasting of muscle,[35] and changes in muscle function in ALS are thought to contribute to altered metabolism,[35] in part due to a shift towards increased lipid oxidation.[28] Collectively, our findings show that a common approach for accurately predicting REE in ALS should account for differences in sex and age within and between cohorts, and must consider fat-free mass, where possible.

As a first step towards the identification of a suitable formula for predicting REE in ALS, we evaluated 22 established equations for estimating REE. When considering equations that adjust for body composition, we found that the Sabounchi S4 equation provided the most reliable estimates of REE in Australian control cohorts. While performing equally well within other cohorts, we note that the Sabounchi S11 equation performed better in Dutch participants when considering the accuracy of predictions only, whereas there was little difference between the Sabounchi S4 and Johnstone equations in the Chinese cohort. The Sabounchi S4 equation was established following a relatively recent and comprehensive review of prediction equations for REE using data from twenty geographically and ethnically distinct subpopulations. Within this study, the Sabounchi S4 equation was one of the most accurate options and was well-supported by empirical evidence across most populations.[21] For predictions of REE where measures of fat-free mass are not available, there was little agreement on the most suitable prediction equation, although the 1919 Harris-Benedict prediction equation performed well within the Australian control cohort. Variations of the Harris-Benedict equation are routinely used estimate REE in studies in ALS.[10, 13-15, 22-26, 29, 30] While our studies suggest that Harris-Benedict equations may offer a suitable alternative for predicting metabolic rate in ALS when measures of body composition are not available, recent findings show that dependence on body weight and BMI for predicting REE introduces error, and as such, may not be suitable for clinical use[36].

Variation in mREE in control cohorts is not unexpected, and identification of hypo- and hypermetabolism in ALS must account for this. Existing strategies for identifying hypermetabolism in ALS have generally identified cut-off values based on a 10-20% deviation in mREE vs pREE,[9] however specific thresholds were not calculated. Considering the Australian cohort, and assuming a 1 SD variation in MI, we found that thresholds for selecting hypo- and hypermetabolism were 86% of mREE for hypometabolism, and between 111-112% of mREE for hypermetabolism. Thresholds varied considerably between geographically distinct cohorts, suggesting that Australian reference values may not be appropriate for the identification of hypo- or hypometabolism in non-Australian patients; for Chinese and Dutch patients, the use of Australian reference values universally increased the proportion of patients identified as being hypermetabolic. Adjustment to cohort-specific thresholds reduced the prevalence of hypermetabolism in Chinese patients, whereas the proportion of hypometabolic individuals remained mostly unchanged. For patients from the Netherlands, the proportion of patients identified as hypermetabolic was reduced, whereas we observed an increase in patients identified as hypometabolic, bringing this in line with what was observed in Australian and Chinese cohorts.

In interpreting these findings, we note several study limitations. While differences in metabolic rate between groups may reflect physiological traits between cohorts, differences in methodology and variation in data collection protocols across cohorts may have influenced the results. Specifically, differences in approaches for determining body composition and discrepancies in patient settings (in-care versus out-of-hospital) may introduce inconsistencies. While the Chinese cohort employed bioelectrical impedance analysis for body composition assessments, the Dutch and Australian cohorts utilized air-displacement plethysmography. Moreover, the fasting duration in the Chinese cohort was at least 6 hours, whereas the Dutch and Australian cohorts fasted overnight. These differences in methodologies reflect the diversity of approaches in current clinical and research practices. All approaches are commonly used in metabolic research. However, the impact of variations in study approach on ALS metabolic evaluations remains undetermined. This underscores the need for future research to delineate the comparative effects and potential nuance of different methods in assessing metabolic change and highlights the value of considering standardized testing protocols for consistent metabolic evaluations in patients with ALS.

Differences in the characteristics of controls may also impact the accuracy of selection criteria and reference values for metabolic change. The Dutch control dataset included gene carriers who might have inherent metabolic alterations, potentially biasing findings towards these individuals and limiting their generalizability. For example, a recent study reported hypometabolism in presymptomatic ALS gene carriers when compared to non-carriers[37], suggesting that alterations in metabolism might emerge during the asymptomatic phase of disease. These limitations are likely amplified by the sample size of our study. Our limited

sample size and possible errors in categorization might complicate the interpretation of future research in this area, especially when considering the clinical consequences of hypo- and hypermetabolism. Additionally, the absence of repeated measures fails to account for potential variability or progression of metabolic alterations over time in patients with ALS. We did not consider disease progression and survival alongside metabolic change. Rather, we focussed on improving understanding of the variability in predicting metabolic rate in ALS patients across different methodologies and cohorts; a foundational step for future multicohort studies that will robustly explore correlations between metabolic status and disease progression and survival. We did, however, explore the impact of disease duration on metabolic predictions. We found no association between disease duration and HI or SI, suggesting that the metabolic status of participants may vary little throughout the course of disease. While consistent with prior literature showing that hypermetabolism is an early and persistent feature of ALS[24], this needs further validation. Finally, the geographic reach of this study, whilst covering three distinct populations, remains constrained and does not encapsulate all potential geographic or genetic variables. This may limit the understanding of metabolic changes across broader ALS populations. Thus, our proposed approach of categorizing patients with ALS into metabolic states, though instructive, might be overly simplistic and may not fully capture the complexity of metabolic changes in ALS. Future research should consider a more nuanced approach to better reflect the spectrum of metabolic alterations in this disease. Despite these limitations, our findings contribute to the evolving understanding and narrative of metabolic dysregulation in ALS and paves the way for more comprehensive, larger scale studies wherein a common approach for accurate modelling of REE could be considered.

CONCLUSIONS

While observing altered systemic metabolism in three distinct cohorts of patients with ALS, we show that identification of hypo- and hypermetabolism hinges upon the definitional criteria used for metabolic categorization. Interpopulation discrepancies are attributed to demographic-specific variables inherent to each respective cohort, including age, sex, and body composition. Nevertheless, we demonstrate that the Sabounchi S4 (when measures of body composition are available) and 1919 Harris-Benedict (when measures of body composition are not available) equations perform well in predicting REE across Australian, Chinese, and Dutch cohorts. Consequently, deriving cohort-specific metabolic benchmarks from non-ALS controls using these equations is beneficial for the generation of robust selection metrics and the development of thresholds for the identification of metabolic deviations in analogous ALS cohorts. Strengthening methodologies for a more rigorous and standardized approach for identifying metabolic changes will contribute to a more refined understanding of the etiological underpinnings and clinical implications of hypo- and hypermetabolism in ALS.

REFERENCES

1. Swinnen B, Robberecht W. The phenotypic variability of amyotrophic lateral sclerosis. Nat Rev Neurol 2014;10:661-670.
2. Renton AE, Chio A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. Nat Neurosci 2014;17:17-23.
3. McCann EP, Henden L, Fifita JA, et al. Evidence for polygenic and oligogenic basis of Australian sporadic amyotrophic lateral sclerosis. J Med Genet 2020.
4. Mehta PR, Iacoangeli A, Opie-Martin S, et al. The impact of age on genetic testing decisions in amyotrophic lateral sclerosis. Brain 2022;145:4440-4447.
5. Shepheard SR, Parker MD, Cooper-Knock J, et al. Value of systematic genetic screening of patients with amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2021;92:510-518.
6. Al-Chalabi A, Calvo A, Chio A, et al. Analysis of amyotrophic lateral sclerosis as a multistep process: a population-based modelling study. Lancet Neurol 2014;13:1108-1113.
7. Chio A, Mazzini L, D'Alfonso S, et al. The multistep hypothesis of ALS revisited: The role of genetic mutations. Neurology 2018;91:e635-e642.
8. Vucic S, Westeneng HJ, Al-Chalabi A, Van Den Berg LH, Talman P, Kiernan MC. Amyotrophic lateral sclerosis as a multi-step process: an Australia population study. Amyotroph Lateral Scler Frontotemporal Degener 2019;20:532-537.
9. Ludolph A, Dupuis L, Kasarskis E, Steyn F, Ngo S, McDermott C. Nurtitional and metabolic factors in amyotrophic lateral sclerosis. Nat Rev Neurol 2023:In Press.
10. He J, Fu J, Zhao W, et al. Hypermetabolism associated with worse prognosis of amyotrophic lateral sclerosis. J Neurol 2022;269:1447-1455.
11. Jesus P, Fayemendy P, Marin B, et al. Increased resting energy expenditure compared with predictive theoretical equations in amyotrophic lateral sclerosis. Nutrition 2020;77:110805.
12. Jesus P, Fayemendy P, Nicol M, et al. Hypermetabolism is a deleterious prognostic factor in patients with amyotrophic lateral sclerosis. Eur J Neurol 2018;25:97-104.
13. Kasarskis EJ, Berryman S, Vanderleest JG, Schneider AR, McClain CJ. Nutritional status of patients with amyotrophic lateral sclerosis: relation to the proximity of death. Am J Clin Nutr 1996;63:130-137.
14. Nakamura R, Kurihara M, Ogawa N, et al. Prognostic prediction by hypermetabolism varies depending on the nutritional status in early amyotrophic lateral sclerosis. Sci Rep 2021;11:17943.
15. Nakamura R, Kurihara M, Ogawa N, et al. Investigation of the prognostic predictive value of serum lipid profiles in amyotrophic lateral sclerosis: roles of sex and hypermetabolism. Sci Rep 2022;12:1826.
16. Steyn FJ, Ioannides ZA, van Eijk RPA, et al. Hypermetabolism in ALS is associated with greater functional decline and shorter survival. J Neurol Neurosurg Psychiatry 2018;89:1016-1023.
17. Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron D. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000;1:293-299.
18. Cedarbaum JM, Stambler N, Malta E, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci 1999;169:13-21.

19. Ioannides ZA, Steyn FJ, Henderson RD, McCombe PA, Ngo ST. Anthropometric measures are not accurate predictors of fat mass in ALS. Amyotroph Lateral Scler Frontotemporal Degener 2017;18:486-491.
20. Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. Proc Natl Acad Sci U S A 1918;4:370-373.
21. Sabounchi NS, Rahmandad H, Ammerman A. Best-fitting prediction equations for basal metabolic rate: informing obesity interventions in diverse populations. Int J Obes (Lond) 2013;37:1364-1370.
22. Desport JC, Preux PM, Magy L, et al. Factors correlated with hypermetabolism in patients with amyotrophic lateral sclerosis. Am J Clin Nutr 2001;74:328-334.
23. Desport JC, Torny F, Lacoste M, Preux PM, Couratier P. Hypermetabolism in ALS: correlations with clinical and paraclinical parameters. Neurodegener Dis 2005;2:202-207.
24. Bouteloup C, Desport JC, Clavelou P, et al. Hypermetabolism in ALS patients: an early and persistent phenomenon. J Neurol 2009;256:1236-1242.
25. Funalot B, Desport JC, Sturtz F, Camu W, Couratier P. High metabolic level in patients with familial amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2009;10:113-117.
26. Vaisman N, Lusaus M, Nefussy B, et al. Do patients with amyotrophic lateral sclerosis (ALS) have increased energy needs? J Neurol Sci 2009;279:26-29.
27. Ngo ST, Restuadi R, McCrae AF, et al. Progression and survival of patients with motor neuron disease relative to their fecal microbiota. Amyotroph Lateral Scler Frontotemporal Degener 2020;21:549-562.
28. Steyn FJ, Li R, Kirk SE, et al. Altered skeletal muscle glucose-fatty acid flux in amyotrophic lateral sclerosis. Brain Commun 2020;2:fcaa154.
29. Fayemendy P, Marin B, Labrunie A, et al. Hypermetabolism is a reality in amyotrophic lateral sclerosis compared to healthy subjects. J Neurol Sci 2021;420:117257.
30. Cattaneo M, Jesus P, Lizio A, et al. The hypometabolic state: a good predictor of a better prognosis in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2022;93:41-47.
31. Chang J, Shaw TB, Holdom CJ, et al. Lower hypothalamic volume with lower body mass index is associated with shorter survival in patients with amyotrophic lateral sclerosis. Eur J Neurol 2023;30:57-68.
32. McCombe PA, Garton FC, Katz M, Wray NR, Henderson RD. What do we know about the variability in survival of patients with amyotrophic lateral sclerosis? Expert Rev Neurother 2020;20:921-941.
33. Dharmadasa T, Scaber J, Edmond E, et al. Genetic testing in motor neurone disease. Pract Neurol 2022;22:107-116.
34. Zurlo F, Larson K, Bogardus C, Ravussin E. Skeletal muscle metabolism is a major determinant of resting energy expenditure. J Clin Invest 1990;86:1423-1427.
35. Shefner JM, Musaro A, Ngo ST, et al. Skeletal muscle in amyotrophic lateral sclerosis. Brain 2023.
36. Roscoe S, Skinner E, Kabucho Kibirige E, et al. A critical view of the use of predictive energy equations for the identification of hypermetabolism in motor neuron disease: A pilot study. Clin Nutr ESPEN 2023;57:739-748.
37. Dorst J, Weydt P, Brenner D, et al. Metabolic alterations precede neurofilament changes in presymptomatic ALS gene carriers. EBioMedicine 2023;90:104521.

Supplemental Table 1: Demographics and measures of metabolism in Patients with ALS or Controls in Australian, Chinese and Dutch male and female participants

	Australia						China						Netherlands					
	Female			Male			Female			Male			Female			Male		
	Control (72)	ALS (39)	p	Control (82)	ALS (101)	p	Control (48)	ALS (27)	p	Control (81)	ALS (40)	p	Control (21)	ALS (26)	p	Control (16)	ALS (53)	p
Demographics																		
Age, years	55.75±10.11	61.89±9.97	**0,003**	57.38±10.72	59.85±9.90	0,110	54.15±10.90	52.19±11.67	0,478	50.63±11.76	51.78±9.56	0,576	45.96±13.28	59.38±11.70	**<0.001**	45.81±12.72	60.23±9.34	**<0.001**
Anthropometric measures																		
Height, cm	164.85±5.76	161.99±6.58	**0,025**	178.02±5.95	177.00±5.99	0,251	159.31±5.32	157.81±5.11	0,235	169.32±6.89	170.84±5.82	0,223	168.92±6.02	167.05±5.63	0,281	181.57±5.75	179.41±7.29	0,225
Body Mass, kg	74.36±17.18	70.39±18.83	0,279	87.58±13.82	82.88±15.41	**0,032**	61.62±7.94	60.19±9.28	0,503	71.73±12.43	71.89±13.86	0,953	75.44±18.09	70.28±10.39	0,255	86.70±17.68	82.83±11.70	0,421
BMI, $kg.m^{-2}$	27.33±6.08	26.71±6.25	0,614	27.62±4.11	26.39±4.34	0,050	24.26±2.72	24.16±3.47	0,895	24.90±3.31	24.50±3.79	0,581	26.53±6.63	25.17±3.42	0,402	26.24±4.89	25.70±3.09	0,680
Fat-free mass, kg	43.37±5.55	39.64±7.98	**0,012**	61.31±6.89	56.91±8.15	**<0.001**	40.75±4.25	38.77±5.09	0,092	52.04±8.08	52.10±8.22	0,972	46.19±5.45	41.04±4.04	**<0.001**	61.73±6.60	57.84±7.62	0,056
Fat, kg	30.78±13.55	31.35±14.35	0,840	26.30±10.62	25.97±12.34	0,843	20.99±4.95	21.61±6.22	0,655	19.37±6.08	19.98±7.02	0,653	29.25±14.11	29.24±9.09	0,998	24.96±13.02	24.99±7.25	0,993
Fat (%)	39.76±9.93	43.00±9.24	0,090	29.21±7.58	30.11±10.61	0,506	33.64±4.54	35.27±6.51	0,258	26.66±5.83	26.99±6.04	0,785	37.19±8.15	40.77±7.99	0,138	27.22±9.66	29.80±6.08	0,325
Clinical measures																		
Time since onset (months)		23.94±16.59			22.20±17.79									26.98±20.62			10.62±11.17	
Time since diagnosis (months)		7.61±10.13			7.47±9.40			16.37±11.25			13.46±8.47			10.62±11.17			10.23±10.09	
ALSFRS-R		37.28±5.16			37.99±5.51			41.44±4.48			43.46±3.40			37.85±6.27			39.21±4.37	
Metabolic measures/outcomes																		
Resting metabolic rate, $kcal.day^{-1}$	1366±284	1251±352	0,085	1808±273	1813±312	0,902	1250±206	1400±252	**0,011**	1660±369	1839±421	**0,031**	1583±257	1499±156	0,198	1922±282	1869±217	0,491
Harris-Benedict prediction, kcal.day	1440±195	1362±206	0,057	1790±213	1708±244	**0,016**	1307±96	1297±117	0,724	1574±215	1577±228	0,950	1499±162	1388±129	**0,014**	1861±270	1717±199	0,062
Harris-Benedict index	0.95±0.14	0.91±0.18	0,287	1.01±0.10	1.06±0.14	**0,002**	0.96±0.15	1.08±0.18	**0,004**	1.06±0.22	1.17±0.25	**0,027**	1.05±0.09	1.08±0.09	0,264	1.03±0.07	1.09±0.10	**0,010**
Sabounchi S4 prediction, $kcal.day^{-1}$	1415±157	1339±199	**0,054**	1780±164	1686±187	**<0.001**	1314±103	1275±121	0,166	1551±182	1556±197	0,915	1467±165	1359±100	**0,012**	1783±182	1701±172	0,123
Sabounchi index	0.96±0.15	0.93±0.20	0,447	1.01±0.10	1.08±0.16	**0,001**	0.95±0.15	1.10±0.18	**<0.001**	1.08±0.24	1.18±0.25	0,040	1.08±0.11	1.10±0.09	0,364	1.08±0.08	1.10±0.09	0,302

Data are presented as mean±sd, or n (%). P-values estimated from Welch-adjusted t tests. Bold text highlights p<0.05. BMI: body-mass index; ALSFRS-R: amyotrophic lateral sclerosis functional rating scale-revised.

Supplemental table 2: Proportion of metabolic statuses relative to Australian and cohort specific reference values

	Australia			China			Netherlands		
	Control	**MND**	**p**	**Control**	**MND**	**p**	**Control**	**MND**	**p**
Australian reference									
Harris-Benedict			**0.014**			**0.004**			0.116
Hypermetabolic	25 (16%)	42 (30%)		43 (34%)	35 (55%)		10 (27%)	35 (44%)	
Normometabolic	110 (71%)	80 (57%)		52 (41%)	24 (38%)		27 (73%)	43 (54%)	
Hypometabolic	19 (12%)	18 (13%)		31 (25%)	5 (8%)		0 (0%)	1 (1%)	
Sabounchi			**<0.001**			**<0.001**			0.607
Hypermetabolic	18 (12%)	43 (31%)		42 (33%)	35 (55%)		13 (35%)	34 (43%)	
Normometabolic	120 (78%)	80 (57%)		53 (42%)	26 (41%)		24 (65%)	44 (56%)	
Hypometabolic	16 (10%)	17 (12%)		31 (25%)	3 (5%)		0 (0%)	1 (1%)	
Cohort specific reference									
Harris-Benedict			**0.014**			**0.016**			**0.040**
Hypermetabolic	25 (16%)	42 (30%)		21 (17%)	20 (31%)		5 (14%)	27 (34%)	
Normometabolic	110 (71%)	80 (57%)		86 (68%)	41 (64%)		25 (68%)	44 (56%)	
Hypometabolic	19 (12%)	18 (13%)		19 (15%)	3 (5%)		7 (19%)	8 (10%)	
Sabounchi			**<0.001**			**0.009**			**0.037**
Hypermetabolic	18 (12%)	43 (31%)		20 (16%)	16 (25%)		8 (22%)	16 (20%)	
Normometabolic	120 (78%)	80 (57%)		85 (67%)	46 (72%)		20 (54%)	57 (72%)	
Hypometabolic	16 (10%)	17 (12%)		21 (17%)	2 (3%)		9 (24%)	6 (8%)	

Metabolic status was assigned relative to metabolic index (measured metabolism divided by predicted). The distribution of control metabolic indicies was then used to identify threshold for 1 standard deviation away from the mean. These thresholds were assigned as hypo- (low metabolism) or hypermetabolism (high metabolism). Data are presented as n (%) and metabolic proportions are compared using Fisher exact tests.

Supplemental Table 3: Within-sex proportion of metabolic statuses relative to Australian and cohort specific reference values

	Australia						China						Netherlands					
	Female			Male			Female			Male			Female			Male		
	Control	AIs	p	Control	AIs	p	Control	ALS	p	Control	ALS	p	Control	ALS	p	Control	ALS	p
Australian reference																		
Harris-Benedict			0.364			**0.009**			**0.015**			0.050			0.375			0.353
Hypermetabolic	10 (14%)	3 (8%)		15 (18%)	40 (39%)		0 (0%)	6 (22%)		21 (26%)	15 (38%)		4 (19%)	7 (27%)		1 (6%)	20 (38%)	
Normometabolic	48 (67%)	23 (61%)		62 (76%)	56 (55%)		37 (77%)	19 (70%)		51 (63%)	23 (57%)		13 (62%)	17 (65%)		12 (75%)	27 (51%)	
Hypometabolic	14 (19%)	12 (32%)		5 (6%)	6 (6%)		11 (23%)	2 (7%)		9 (11%)	2 (5%)		4 (19%)	2 (8%)		3 (19%)	6 (11%)	
Sabounchi			**0.043**			**0.002**			**<0.001**			0.097			1.000			0.539
Hypermetabolic	8 (11%)	8 (21%)		10 (12%)	33 (33%)		0 (0%)	4 (15%)		20 (26%)	12 (32%)		6 (29%)	6 (23%)		2 (13%)	10 (19%)	
Normometabolic	52 (72%)	19 (50%)		68 (83%)	61 (61%)		39 (81%)	22 (81%)		46 (59%)	24 (65%)		9 (43%)	18 (69%)		11 (69%)	39 (74%)	
Hypometabolic	12 (17%)	11 (29%)		4 (5%)	6 (6%)		9 (19%)	1 (4%)		12 (15%)	1 (3%)		6 (29%)	2 (8%)		3 (19%)	4 (8%)	
Cohort specific reference																		
Harris-Benedict			0.364			**0.009**			**<0.001**			0.266			0.516			**0.034**
Hypermetabolic	10 (14%)	3 (8%)		15 (18%)	40 (39%)		8 (17%)	13 (48%)		37 (46%)	23 (57%)		6 (29%)	11 (42%)		4 (25%)	24 (45%)	
Normometabolic	48 (67%)	23 (61%)		62 (76%)	56 (55%)		27 (56%)	11 (41%)		25 (31%)	13 (33%)		15 (71%)	15 (58%)		12 (75%)	28 (53%)	
Hypometabolic	14 (19%)	12 (32%)		5 (6%)	6 (6%)		13 (27%)	3 (11%)		19 (23%)	4 (10%)		0 (0%)	0 (0%)		0 (0%)	1 (2%)	
Sabounchi			**0.043**			**0.002**			**0.005**			0.124			0.109			0.468
Hypermetabolic	8 (11%)	8 (21%)		10 (12%)	33 (33%)		8 (17%)	14 (52%)		34 (43%)	21 (57%)		8 (38%)	11 (42%)		5 (31%)	23 (43%)	
Normometabolic	52 (72%)	19 (50%)		68 (83%)	61 (61%)		25 (52%)	12 (44%)		28 (36%)	14 (38%)		13 (62%)	15 (58%)		11 (69%)	29 (55%)	
Hypometabolic	12 (17%)	11 (29%)		4 (5%)	6 (6%)		15 (31%)	1 (4%)		16 (21%)	2 (5%)		0 (0%)	0 (0%)		0 (0%)	1 (2%)	

Metabolic status was assigned relative to metabolic index (measured metabolism divided by predicted). The distribution of control metabolic indicies was then used to identify threshold for 1 standard deviations way from the mean. These thresholds were assigned as hypo- (low metabolism) or hypermetabolism (high metabolism). Data are presented as n (%) and metabolic proportions are compared using Fisher exact tests.

Supplemental Table 4: Kendall correlations between metabolic indices and disease duration

	Sabounchi S4				Harris-Benedict			
	Time since onset	p	Time since diagnosis	p	Time since onset	p	Time since diagnosis	p
Australia	-0.00 [-0.12, 0.11]	0.954	-0.05 [-0.18, 0.06]	0.345	-0.03 [-0.15, 0.09]	0.624	-0.08 [-0.19, 0.04]	0.185
China			0.07 [-0.10, 0.23]	0.454			0.05 [-0.09. 0.20]	0.565
Netherlands	-0.05 [-0.21, 0.12]	0.520	-0.02 [-0.20, 0.17]	0.819	-0.15 [-0.32, 0.02]	0.056	-0.06 [-0.24, 0.12]	0.400

Data are presented as Kendall tau B [95% CI].

Supplemental Table 5: Demographics and measures of metabolism in Patients with ALS or Controls in Australian, Chinese and Dutch participants following reassigment into metabolic categories following correction using cohort-specific predictions.

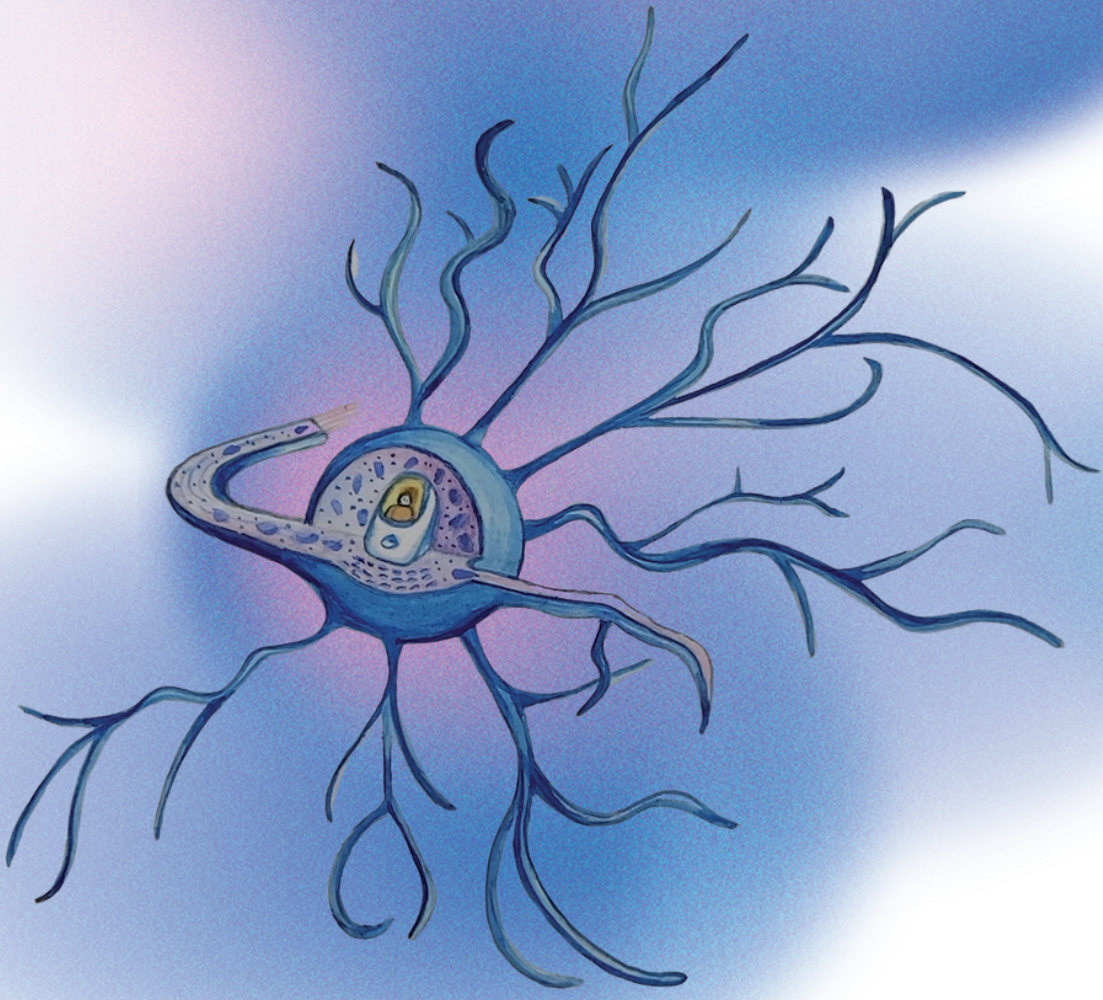
	Australia			China			Netherlands			
	Control	ALS	p[1]	Control	ALS	p[1]	Control	ALS	p[1]	p[2]
N (%)			**0.001**			**0.009**			**0.037**	0.573
Hypermetabolic	18 (12)	43 (31)		20 (16)	16 (25)		8 (22)	16 (20)		
Normometabolic	120 (78)	80 (57)		85 (67)	46 (72)		20 (54)	57 (72)		
Hypometabolic	16 (10)	17 (12)		21 (17)	2 (3)		9 (24)	6 (8)		
Metabolic rate			0.051			**0.041**			0.055	0.215
Hypermetabolic	1976±372	1998±321		2051±343	2109±247		1919±288	1872±210		
Normometabolic	1616±275	1603±278		1471±250	1524±339		1796±274	1731±270		
Hypometabolic	1065±220	1043±279		1118±201	1000±63		1413±184	1566±219		
Sabounchi index										
Hypermetabolic	1.18±0.06	1.22±0.13		1.40±0.09	1.44±0.17		1.21±0.03	1.21±0.04		
Normometabolic	1.00±0.07	1.01±0.07		1.02±0.12	1.06±0.13		1.08±0.05	1.09±0.05		
Hypometabolic	0.73±0.10	0.72±0.14		0.73±0.07	0.75±0.06		0.95±0.01	0.92±0.05		
Sex, female (%)			**0.030**			**0.018**			0.398	0.142
Hypermetabolic	8 (44)	9 (21)		0 (0)	4 (25)		6 (75)	6 (38)		
Normometabolic	52 (43)	19 (24)		39 (46)	22 (48)		9 (45)	18 (32)		
Hypometabolic	12 (75)	11 (65)		9 (43)	1 (50)		6 (67)	2 (33)		
Age, years			0.073			0.401			0.982	0.898
Hypermetabolic	53.44±12.50	57.18±9.76		45.10±13.10	51.50±7.86		39.28±12.98	57.66±10.39		
Normometabolic	56.97±9.81	61.14±9.87		53.04±11.25	51.52±11.02		47.16±11.57	60.00±9.86		
Hypometabolic	57.56±12.55	65.25±8.29		54.19±8.87	65.50±7.78		48.96±14.84	65.63±11.14		
Height, cm			0.065			0.526			0.285	**0.029**
Hypermetabolic	170.92±7.88	174.72±7.87		167.80±8.50	167.31±8.40		171.40±5.95	171.79±6.84		
Normometabolic	172.41±9.19	172.91±9.34		164.64±7.42	164.93±8.61		177.04±8.74	175.85±9.44		
Hypometabolic	168.83±6.06	167.58±9.57		166.86±9.38	159.00±1.41		171.17±9.16	179.98±6.00		
Body mass, kg			0.428			0.548			**0.024**	**0.011**
Hypermetabolic	90.40±21.50	86.92±17.11		69.21±15.26	68.30±11.00		83.57±16.75	76.93±13.31		
Normometabolic	81.72±15.39	77.11±17.05		66.84±10.51	66.63±14.25		84.43±19.98	78.34±12.58		
Hypometabolic	68.81±14.32	71.18±12.16		70.81±13.94	63.70±16.55		68.23±11.71	86.84±10.75		
Body mass index, kg.m^{-2}			0.704			0.506			0.195	**0.022**
Hypermetabolic	30.69±5.80	28.49±5.48		24.33±3.49	24.42±3.73		28.47±5.89	25.99±3.80		
Normometabolic	27.45±4.73	25.67±4.70		24.57±2.83	24.30±3.61		26.99±6.40	25.26±2.98		
Hypometabolic	24.16±5.14	25.21±2.56		25.34±3.77	25.14±6.10		23.27±3.28	26.84±3.46		
Fat-free mass, kg			0.589			0.185			0.334	0.101
Hypermetabolic	53.94±11.93	53.11±9.76		47.60±9.13	48.35±7.72		51.20±9.96	50.14±6.83		
Normometabolic	53.55±11.10	52.88±11.56		47.07±8.32	46.01±10.37		55.24±9.86	52.50±11.19		
Hypometabolic	47.07±6.70	45.91±11.62		50.59±10.12	42.20±6.79		49.25±8.99	56.34±9.67		
Fat mass, kg			0.857			0.950			0.070	0.148
Hypermetabolic	36.46±14.91	33.60±14.00		20.66±6.45	20.17±6.41		32.37±10.99	26.79±10.17		
Normometabolic	28.08±11.20	24.23±12.26		19.59±5.11	20.82±6.83		29.19±15.77	25.85±7.51		
Hypometabolic	21.73±12.55	27.14±8.97		20.95±7.27	21.25±10.11		18.99±5.27	30.50±7.52		
Fat %			0.541			0.398			**0.044**	0.336
Hypermetabolic	39.63±8.95	37.81±10.51		29.62±5.65	28.88±8.01		38.29±6.79	33.89±8.51		
Normometabolic	33.86±10.09	30.58±11.74		29.36±5.97	30.95±7.32		33.02±11.63	33.09±8.67		
Hypometabolic	30.07±10.41	37.96±10.70		28.90±8.45	32.45±7.28		27.73±5.76	35.19±7.64		
Time since diagnosis, months										
Hypermetabolic		6.97±8.45			14.25±8.12			11.74±13.00		0.397
Normometabolic		7.39±8.40			14.83±10.54			9.21±8.50		
Hypometabolic		9.41±15.88			15.00±4.24			17.40±16.78		
ALSFRS-R										0.509
Hypermetabolic		37.93±5.14			42.75±3.11			40.19±4.42		
Normometabolic		37.90±5.66			42.98±3.70			38.70±5.25		
Hypometabolic		36.94±5.04			33.00±7.07			35.50±3.78		

Data are presented as mean ± sd, or n (%). Ordinal logistic regression was used to estimate p values for the impact of each demographic, anthropometric or clinical measure on metabolic status.
p1: within-country case-control comparison. p2 between country case-control comparison.

Supplemental Table 6: Demographics and measures of metabolism in female and male Patients with ALS or Controls in Australian, Chinese and Dutch participants following reassigment into metabolic categories following correction using cohort-specific predictions.

	Australia				China				Netherlands			
	Female		Male		Female		Male		Female		Male	
	Control	ALS	Control	ALS	Control	ALS	Control	ALS	Control	ALS	Control	ALS
N (%)												
Hypermetabolic	8 (11%)	9 (23%)	10 (12%)	34 (34%)		4 (15%)	20 (26%)	12 (32%)	6 (29%)	6 (23%)	2 (13%)	10 (19%)
Normometabolic	52 (72%)	19 (49%)	68 (83%)	61 (61%)	39 (81%)	22 (81%)	46 (59%)	24 (65%)	9 (43%)	18 (69%)	11 (69%)	39 (74%)
Hypometabolic	12 (17%)	11 (28%)	4 (5%)	6 (6%)	9 (19%)	1 (4%)	12 (15%)	1 (3%)	6 (29%)	2 (8%)	3 (19%)	4 (8%)
Metabolic rate												
Hypermetabolic	1741±293	1658±322	2165±326	2088±257		1841±76	2051±343	2198±217	1811±237	1707±125	2244±135	1971±189
Normometabolic	1398±188	1271±186	1783±204	1706±214	1314±170	1336±175	1603±230	1696±364	1595±185	1447±93	1961±223	1862±218
Hypometabolic	978±178	883±161	1324±75	1337±195	972±74	1045±NA	1228±197	956±NA	1335±129	1342±166	1568±198	1678±145
Sabounchi index												
Hypermetabolic	1.18±0.05	1.15±0.02	1.18±0.07	1.24±0.13		1.39±0.08	1.40±0.09	1.45±0.19	1.21±0.03	1.21±0.05	1.21±0.02	1.21±0.04
Normometabolic	0.99±0.07	0.99±0.08	1.00±0.07	1.01±0.07	1.00±0.12	1.07±0.13	1.03±0.12	1.06±0.13	1.07±0.05	1.09±0.04	1.09±0.05	1.09±0.05
Hypometabolic	0.71±0.11	0.66±0.14	0.80±0.04	0.81±0.03	0.74±0.05	0.71±NA	0.73±0.08	0.79±NA	0.95±0.02	0.93±0.07	0.95±0.01	0.92±0.05
Age, years												
Hypermetabolic	55.25±13.56	57.33±10.08	52.00±12.11	57.13±9.83		47.75±7.54	45.10±13.10	52.75±7.86	40.88±14.55	52.63±9.22	34.49±7.77	60.67±10.28
Normometabolic	56.19±9.49	63.16±11.12	57.56±10.07	60.51±9.46	53.87±11.29	52.14±11.82	52.33±11.29	50.96±10.46	49.87±13.65	60.33±11.17	44.95±9.67	59.85±9.35
Hypometabolic	54.17±11.04	63.43±7.02	67.75±12.53	68.57±10.05	55.33±9.53	71.00±NA	53.33±8.68	60.00±NA	45.16±11.68	71.09±17.59	56.54±20.27	62.90±8.60
Height, cm												
Hypermetabolic	165.19±6.70	165.17±7.90	175.50±5.46	177.25±5.67		155.75±5.06	167.80±8.50	171.17±4.93	169.05±2.82	168.23±4.16	178.45±8.70	173.93±7.40
Normometabolic	164.47±6.02	159.92±5.49	178.49±6.01	176.95±5.98	159.62±5.17	158.09±5.25	168.89±6.31	171.21±5.84	169.26±5.36	165.87±5.71	183.41±4.78	180.45±6.92
Hypometabolic	166.29±3.78	162.95±6.47	176.45±5.28	176.07±8.68	158.00±6.06	160.00±NA	173.50±4.56	158.00±NA	168.28±9.51	174.10±4.38	176.93±5.90	182.93±4.36
Body mass, kg												
Hypermetabolic	82.08±19.71	86.17±27.72	97.06±21.45	87.12±13.64		64.62±5.73	69.21±15.26	69.53±12.23	80.22±18.09	70.88±15.27	93.63±7.61	80.56±11.26
Normometabolic	75.14±16.80	65.56±14.67	86.76±12.09	80.71±16.21	61.18±8.12	58.70±9.20	71.64±9.95	73.90±14.31	79.34±20.84	69.36±8.99	88.60±19.20	82.49±11.89
Hypometabolic	65.83±14.95	65.83±7.38	77.72±8.05	80.99±13.64	63.56±7.17	75.40±NA	76.26±15.51	52.00±NA	64.80±9.81	76.81±7.59	75.10±14.20	91.86±8.52
Body mass index, kg.m^{-2}												
Hypermetabolic	29.82±5.66	31.32±8.63	31.38±6.12	27.74±4.18		26.68±2.60	24.33±3.49	23.67±3.82	28.17±6.94	25.02±5.25	29.39±0.47	26.57±2.78
Normometabolic	27.76±6.02	25.63±5.62	27.22±3.46	25.68±4.43	23.98±2.73	23.46±3.30	25.06±2.86	25.06±3.77	27.81±7.64	25.20±2.86	26.32±5.49	25.29±3.07
Hypometabolic	23.84±5.59	24.81±2.55	25.12±4.00	25.94±2.64	25.46±2.46	29.45±NA	25.25±4.63	20.83±NA	22.96±3.59	25.43±3.78	23.87±3.17	27.55±3.63
Fat-free mass, kg												
Hypermetabolic	44.19±7.18	42.19±10.55	61.75±8.74	55.99±7.30		40.45±2.94	47.60±9.13	50.98±6.97	46.77±6.52	44.17±3.66	64.50±3.15	53.73±5.67
Normometabolic	43.16±5.80	37.66±6.09	61.49±6.75	57.62±8.28	40.85±4.43	38.09±5.15	52.34±7.14	53.27±8.44	46.28±4.82	39.62±3.59	62.57±5.91	58.44±7.99
Hypometabolic	43.73±3.03	40.98±8.44	57.08±3.53	54.97±11.74	40.31±3.59	47.00±NA	58.30±5.04	37.40±NA	45.46±6.17	44.46±2.13	56.82±9.95	62.28±3.62
Fat mass, kg												
Hypermetabolic	37.89±12.96	42.99±19.28	35.31±16.91	31.12±11.35		24.17±4.13	20.66±6.45	18.83±6.61	33.45±12.63	26.71±14.44	29.13±4.46	26.84±7.52
Normometabolic	31.69±12.74	27.90±11.91	25.31±9.01	23.09±12.25	20.47±5.09	20.84±6.43	18.84±5.05	20.79±7.31	33.05±16.59	29.74±7.40	26.03±15.10	24.05±6.93
Hypometabolic	22.10±14.24	27.78±8.45	20.64±6.44	25.98±10.61	23.24±3.73	28.40±NA	19.23±8.85	14.10±NA	19.34±5.62	32.34±5.46	18.28±5.58	29.58±9.00
Fat %												
Hypermetabolic	45.27±4.77	48.61±9.25	35.11±9.09	34.96±8.92		37.27±4.05	29.62±5.65	26.08±6.99	40.72±5.93	35.62±12.44	31.00±2.26	32.86±5.62
Normometabolic	40.85±9.12	41.24±8.69	28.52±7.12	27.26±10.57	33.01±4.75	34.80±6.99	26.26±5.12	27.43±5.75	39.92±7.91	42.36±5.96	27.38±11.36	28.81±5.95
Hypometabolic	31.37±11.46	41.45±9.13	26.18±5.78	31.57±11.13	36.40±1.95	37.60±NA	23.27±6.81	27.30±NA	29.55±5.64	41.96±2.96	24.10±4.83	31.81±6.96
Time since diagnosis, months												
Hypermetabolic		5.61±3.63		7.34±9.35		13.25±2.06		14.58±9.39		8.11±10.03		13.92±14.55
Normometabolic		9.86±13.87		6.59±5.6		17.14±12.36		12.71±8.25		10.83±11.16		8.45±6.95
Hypometabolic		5.36±3.83		16.84±25.99		12±0.00		18±0.00		16.36±19.83		17.93±18.36
ALSFRS-R												
Hypermetabolic		36.22±5.31		38.41±5.07		43.00±4.24		42.67±2.87		42.33±2.25		38.90±4.98
Normometabolic		38.00±5.42		37.86±5.78		41.77±3.62		44.08±3.48		36.83±6.77		39.56±4.22
Hypometabolic		36.91±4.87		37.00±5.83		28.00±NA		38.00±NA		33.50±0.71		36.50±4.43

Data are presented as mean ± sd, or n (%).



Chapter 6

A comparison between Bioelectrical Impedance Analysis and Air-Displacement Plethysmography in assessing Fat-Free Mass in Patients with Motor Neurone Diseases: a cross-sectional study

Mark R. Janse van Mantgem, M.D.*, Maaike L. Soors d'Ancona, MSc*, Myrte Meyjes, MSc, Leonard H. van den Berg, M.D., PhD., Elles Steenhagen, R.D., Annemieke Kok, R.D., MSc, Ruben P.A. van Eijk, M.D., PhD.
***Equal contribution**

Accepted at Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration

ABSTRACT

Objective: To determine the validity of bioelectrical impedance analysis (BIA) in quantifying fat-free mass (FFM) compared to air-displacement plethysmography (ADP) in patients with a motor neurone disease (MND).

Methods: FFM of 140 patients diagnosed with MND was determined by ADP using the Bod-Pod (i.e., the gold standard), and by BIA using the whole-body Bodystat. FFM values were translated to predicted resting energy expenditure (REE); the actual REE was measured using indirect calorimetry, resulting in a metabolic index. Validity of the BIA compared to the ADP was assessed using Bland-Altman analysis and Pearson's *r*. To assess the clinical relevance of differences, we evaluated changes in metabolic index and in individualized protein demand.

Results: Despite the high correlation between ADP and BIA ($r = 0.93$), averaged across patients, the assessed mean fat-free mass was 51.7 kg (± 0.9) using ADP and 54.2 kg (± 1.0) using BIA. Hence, BIA overestimated fat-free mass by 2.5 kg (95% CI 1.8 to 3.2, $p < 0.001$). Clinically, an increased metabolic index would be more often underdiagnosed in patients with MND using BIA (31.4% according to BIA *vs*. 44.2% according to ADP, $p = 0.048$). A clinically relevant overestimation of ≥ 15 g in protein demand was observed for 4 (2.9%) patients using BIA.

Conclusions: BIA systematically overestimates FFM in patients with MND. Although the differences are limited with ADP, underscoring the utility of BIA for research, overestimation of fat-free mass may have consequences for clinical decision-making, especially when interest lies in determining the metabolic index.

INTRODUCTION

Body composition plays a pivotal role in the clinical management of patients living with Motor Neurone Disease (MND). Tracking changes in body composition, such as quantifying the loss of fat-free mass (FFM) or fat mass (FM), offers the opportunity for timely dietary interventions to maintain adequate protein and caloric intake.[1–3] In addition, it provides an objective method for monitoring disease progression and loss of muscle mass,[4,5] which could be useful as an endpoint in clinical trials.[6]

Air-Displacement Plethysmography (ADP) has proven to be a well-validated alternative to Dual-energy X-ray Absorptiometry (DXA) for quantifying FFM in healthy individuals.[7,8] The main benefits of ADP over DXA include the lower burden, cost, easier application, and the absence of radiation. In late-stage patients with MND, however, ADP may involve increasing operational difficulties due to the need for patient transfers.[9] In addition, ADP assessments are required to be performed in an environment with as table room temperature, and, moreover, the ADP may provide – for a currently unknown reason – outlying data in a limited number of measurements.[10] Therefore, whole-body Bioelectrical Impedance Analysis (BIA) may be an interesting alternative. BIA is highly portable and requires no effort from the patient, while being non-invasive and maintaining low costs.[7]

Besides above mentioned practical arguments for using BIA *vs.* ADP *vs.* DXA, little is known about the validity of these techniques in patients with MND. DXA has shown to be capable of detecting disease progression in patients with ALS, but no comparison between techniques was made.[11] BIA, and its limitations, has been extensively studied in healthy individuals, but not in patients with MND.[12] One study described the validity of BIA over DXA in patients with ALS.[13] The authors created a specific equation to calculate FFM by BIA. In addition, a strong correlation was found between BIA and ADP in determining FM in patients with MND; there was, however, a lack of agreement existed between the two techniques.[14] ADP and DXA are indirect methods for measuring body composition, whereas BIA is a double indirect method. Hence, BIA makes multiple assumptions to translate the impedance data to a body composition, such as hydration status, and timing and contents of last ingested meal;[12] assumptions that could be affected by the presence of atrophy.[12]

Therefore, the aim of this study is to provide more clarity on anthropometric measures in patients with MND using different methods to assess body composition. The primary objective of this study is to determine the validity of the BIA in quantifying FFM compared to ADP in patients with MND. In addition, we will explore the precision of the Gallagher formula (GAL) – a formula to predict a person's body composition – based on: sex, age, ethnicity, and BMI as a 'device-free' alternative to BIA and ADP.[15] Finally, we will examine the potential effect of

the data obtained from the BIA vs. ADP on the predicted resting energy expenditure (pREE), [16] and the resulting metabolic index (MI), to explore the clinical relevance of our findings.

METHODS

Study setting

Cross-sectional data from 140 patients with Amyotrophic Lateral Sclerosis (ALS), Primary Lateral Sclerosis (PLS) or Progressive Muscular Atrophy (PMA), were prospectively collected at the University Medical Centre Utrecht, the Netherlands. Patients with ALS were diagnosed according to the El Escorial criteria.[17] A diagnosis of PLS was defined as presence of: 1) progressive upper motor neurone (UMN) symptoms for at least two years, and 2) UMN dysfunction in at least two body regions.[18] A diagnosis of PMA was defined as having lower motor neurone signs (LMN) only in two or more body regions. Exclusion criteria included the inability to lie in supine position for one hour, presence of a tracheostomy or the use of permanent assisted ventilation, or having an intellectual disability or mental illness. The study was approved by the Medical Research Ethics Committee of the UMCU (METC 15/656, NL54833.041.15). All patients provided written informed consent.

Study procedures

Following diagnosis, patients aged ≥ 18 years were invited to participate in our study. Clinical characteristics were collected, including disease duration, forced vital capacity (FVC) as percentage of predicted,[19] and the score on the revised ALS functioning rating scale (ALSFRS-R). The rate of disease progression (ΔFRS) was calculated by: (48 – ALSFRS-R score) / symptom duration.[20] The extent of atrophy was quantified as ordinal score, ranging from 0 (no involvement) to 3 (significant and severe involvement) and scored for the right arm and leg only,[21] according to the modified Ravits scale.[22] This scale is used to assess the potential effect of the extent of atrophy on the differences in body composition between BIA and ADP. To assess patient's nutritional status, we administered the Patient-Generated Subjective Global Assessment (PG-SGA).[23] Prior to the assessment, patients were requested to abstain from food, tea/coffee and smoking for at least 10 hours, and, in addition, not to perform any exceptional physical activity. Furthermore, patients were requested to abstain from water for at least one hour, and to empty their bladder. All assessments were performed on the same day. First, we determined body composition by ADP using the BodPod (Cosmed USA, Rome, ITA),[24] according to standard operating procedures. Patients were dressed in tight/form-fitting underwear or a swimsuit, were requested to remove jewellery and glasses, and had to wear a swimming cap. Thoracic gas volume was predicted using standard prediction equations.[25–28] Subsequently, the FM (%) was estimated by ADP, based on the Siri formula.[29] Secondly, we measured the REE (mREE) by indirect calorimetry, using a Quark RMR respirometer (Cosmed). A canopy hood was placed over the patient's head for a period of twenty

minutes. Adjustment of the flow rate was allowed during the first five minutes to achieve a flow rate of between 0.8 and 1.1 L/min. No adjustment of the flow rate was allowed during the remaining 15 minutes. Finally, we performed the BIA using a whole-body multifrequency device (Bodystat Quadscan 4000; Bodystat Ltd, Douglas, UK) on the right side of the body. Patients were requested to lie in supine position; then four electrodes were placed on the right side of the body. The electrodes were placed in the middle of the dorsal surface of the right hand and right foot proximal to the metacarpal-phalangeal and metatarsal-phalangeal joint, respectively, and also medially between the distal prominences of the radius and the ulna and between the medial and lateral malleoli at the ankle. Obtained data included the body resistance and reactance at a frequency of 50 kHz. Together with height, weight and sex, these parameters were used to calculate the FFM using Kyle's equation:[30]

- FFM (kg) = – 4.104 + (0.518 * height in m^2/resistance) + 0.231 * weight in kg + 0.130 * reactance + 4.229 * male

We also determined the patient's FFM using the Gallagher formula (GAL).[15,31] Significantly, GAL does not require input from either BIA or ADP and predicts a patient's FFM as follows:

- FFM (kg) = (1 – (76.0 – 1097.8 * BMI^{-1} – 20.6 * male + 0.053 * age + 95.0 * Asian * BMI^{-1} – 0.044 * Asian * age + 154 * male * BMI^{-1} + 0.034 * male * age)) * weight in kg

For both equations, FM was simply calculated as the total body weight minus FFM. Subsequently, a patient's predicted resting energy expenditure (pREE) in kcal/day could be obtained using the Sabounchi Structure 4 formula:[16,32,33]

- pREE (kcal/day) – Males = 361 + (21.1 * FFM (kg)) + (4.77 * FM (kg))
- pREE (kcal/day) – Females = 360 + (21.0 * FFM (kg)) + (4.68 * FM (kg))

Based on these obtained parameters, we were able to calculate the metabolic index (MI) by: (mREE (kcal/day) / pREE (kcal/day)) * 100%. The MI was determined using the data obtained from both the BIA and ADP, in order to compare outcomes. The GAL was not used in this analysis, as the MI has not been determined in previous studies with a 'device-free' method. An elevated metabolic index (e.g., hypermetabolism) was defined as an MI of ≥ 110%.[34]

To summarize, the pREE outcomes are dependent on the FFM and FM, and, as a result, the metabolic index (MI) might differ between data derived from BIA or ADP. For the sake of consistency, we will refer to the outcome measures of these devices, by mentioning the device that has been used (i.e., BIA or ADP).

Finally, we performed two sensitivity analyses: 1) we used the equation defined by Lukaski et al. to calculate FFM (kg) in order to assess the degree of accuracy between the equations of Kyle et al. and Lukaski et al.,[35] as the latter has previously been used as best fitting equation for patients with ALS,[36] and 2) we evaluated the hydration status of patients with MND using bioelectrical impedance vector analysis (BIVA).[37,38] The BIA assumes a fixed hydration status

of patients of 73%; For illustrative purposes, we, therefore, assessed the hydration status of our study population to provide an insight into potential effect on BIA outcomes.[31]

Statistical analysis

Statistical analyses were performed using SPSS Statistics version 28.0 and RStudio (version 1.1.4, Rstudio: Integrated Development for R, Inc., Boston, USA, http://www.rstudio.com/). The characteristics of the patients were expressed as mean ± standard deviation (SD) or as median with their 25% – 75% interquartile range (IQR), depending on their distribution, or as frequency and percentage. The mean difference between BIA and ADP, and GAL and ADP were assessed using a paired Student's t-test. Correlations were expressed as a Pearson's *r* correlation coefficient, and differences further explored using the Bland-Altman method, together with its 95% limits of agreement.[13] Additionally, we estimated the intraclass correlation coefficient (ICC) and the standard error of measurement (SEM) using a linear mixed effects model with solely a random intercept per patient.[33] A linear regression model was used to evaluate the impact of atrophy on the difference in FFM between the BIA, GAL and ADP. To translate differences in FFM between the different methods to clinical relevance – besides determining the MI – we estimated a patient's daily protein demands. According to the Dutch guideline – which is used in clinical practice – a chronically ill patient requires 1.5 g protein per kg FFM.[39] An under- or overestimation of 15 g or more in protein demand was deemed clinically relevant. A two-sided *p*-value of < 0.05 was considered statistically significant.

Data availability statement

All protocol, analyses, and anonymized data will be shared on request from any qualified investigator. We take full responsibility for the data, the analyses and interpretation, and the conduct of the research.

RESULTS

Of the 140 included patients with MND, 77 were diagnosed with ALS, 30 with PLS and 33 with PMA. The baseline characteristics of the study population are summarised in **Table 1**. The majority of the patients with MND were male (63.6%) with a mean age of 62.0 ± 10.3 years. Patients with ALS were relatively younger (59.9 ± 10.2 years), and fewer were well-nourished (64.9%) compared to patients with PMA (83.3%) or PLS (72.7%). None of the included patients was of Asian descent.

Table 1. Baseline demographics and clinical characteristics of enrolled patients

Characteristic	All patients N = 140	ALS N = 77	PMA N = 33	PLS N = 30
Sex, male	89 (63.6%)	51 (66.2%)	23 (69.7%)	15 (50.0%)
Age at enrolment, years	62.0 ± 10.3	59.9 ± 10.2	65.3 ± 9.1	63.8 ± 11.0
Symptom onset, spinal	113 (80.7%)	58 (75.3%)	32 (97.0%)	23 (76.7%)
Symptom duration, months*	26.7 (15.7 – 68.2)	17.0 (12.9 – 27.8)	43.3 (24.5 – 82.7)	115.5 (59.4 – 177.0)
ALSFRS-R score	38.2 (5.2)	38.9 (5.0)	38.1 (5.3)	36.3 (5.3)
ΔFRS*	0.23 (0.12 – 058)	0.42 (0.20 – 0.79)	0.19 (0.08 – 0.44)	0.09 (0.06 – 0.17)
FVC, %	93.3 (19.1)	94.1 (18.5)	88.5 (22.4)	96.5 (16.3)
Riluzole use	94 (67.1)	64 (83.1)	24 (72.7)	6 (20.0)
BMI, kg/m^{2}*	25.5 (4.6)	25.1 (4.8)	25.7 (5.2)	26.0 (3.8)
Waist circumference, cm	94.4 (11.9)	93.7 (11.2)	98.1 (12.0)	92.1 (13.1)
Atrophy score, right-sided*	2.0 (1.0 – 4.0)	3.0 (1.0 – 4.0)	3.5 (2.0 – 4.0)	1.0 (0.0 – 2.0)
PG-SGA category				
Well nourished	99 (70.7%)	50 (64.9%)	24 (72.7%)	25 (83.3%)
Moderately malnourished	36 (25.7%)	25 (32.5%)	7 (21.2%)	4 (13.3%)
Severely malnourished	2 (1.4%)	0 (0.0%)	2 (6.1%)	0 (0.0%)

Data are represented as mean (SD).
* = Data are median values with their 25%-75% interquartile range.
Abbreviations: ALS = amyotrophic lateral sclerosis, PMA = progressive muscular atrophy, PLS = primary lateral sclerosis, ALSFRS-R = revised amyotrophic lateral sclerosis functional rating scale, ΔFRS = progression rate determined by: (48 - ALSFRS-R total score) / disease duration), FVC = forced vital capacity, BMI = body mass index, PG-SGA = patient-generated subjective global assessment.

Validity of BIA

The relationships between FFM estimated by BIA, GAL or ADP are presented in **Figure 1**. The Pearson's *r* correlation coefficient between BIA and ADP was 0.93 (95% CI 0.91 – 0.95, $p < 0.001$), and between GAL and ADP 0.91 (95% CI 0.88 - 0.94, $p < 0.001$).

The mean differences in FFM between BIA, GAL and ADP are presented in **Table 2**. The BIA overestimated the FFM by, on average, 2.5 kg FFM (95% CI 1.8 to 3.2, $p < 0.001$), resulting in a relative overestimation of 3.0% (95% CI 2.1 to 3.8, $p < 0.001$). The GAL overestimated FFM by, on average, 4.9 kg (95% CI 4.2 to 5.7, $p < 0.001$), with a relative overestimation of 6.2% (95% CI 5.3 to 7.1, $p < 0.001$). A potential cause might be related to a patient's hydration status, as shown in **Figure 2**. This figure illustrates that our study population is overhydrated, which may result in overestimating FFM using BIA.

Figure 1. Correlation between fat-free mass obtained by ADP versus BIA and GAL

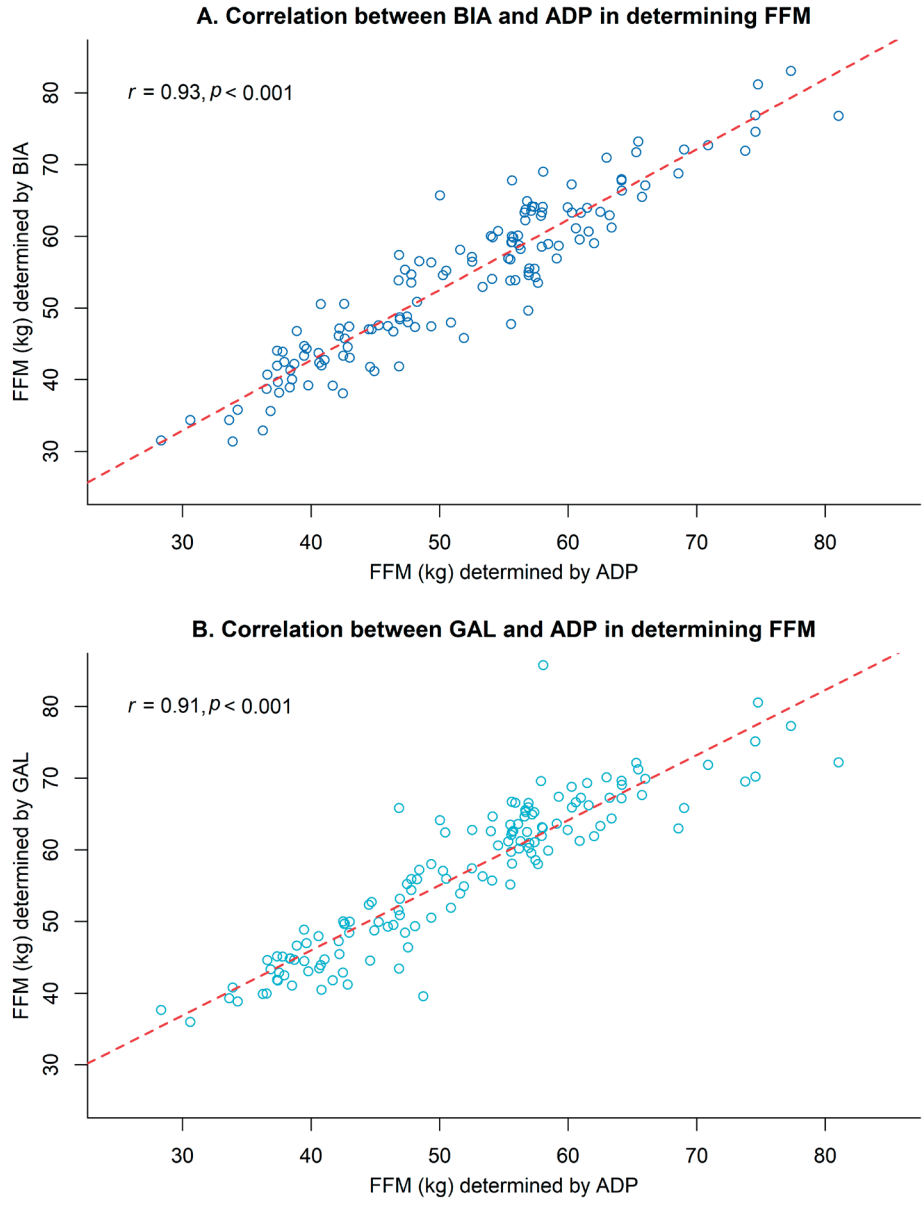


Figure 1. Scatterplot of the FFM (in kg) values obtained from the (A) BIA and (B) GAL, both compared to ADP. BIA outcomes were used in Kyle's equation.[30] Abbreviations: BIA: bioelectrical impedance analysis, ADP = air-displacement plethysmography, *r* = Pearson's *r* correlation coefficient, FFM = fat-free mass, GAL = Gallagher formula.

Table 2. Overview of the measured FFM and FM (kg and %) and the difference between the BIA, GAL and ADP in patients with MND (*n* = 140).

Method	FFM (kg)	FFM (%)	FM (kg)	FM (%)
ADP	51.7 ± 0.9	65.8 ± 0.8	27.2 ± 0.8	34.2 ± 0.8
BIA	54.2 ± 1.0	68.6 ± 0.7	24.8 ± 0.7	31.4 ± 0.7
Difference BIA *vs.* ADP (95% CI)	2.5 (1.8 – 3.2)	3.0 (2.1 – 3.8)	-2.4 (-3.2 – -1.8)	-2.8 (-3.8 – -2.1)
p-value	< 0.001	< 0.001	< 0.001	< 0.001
GAL	56.6 ± 0.9	72.0 ± 0.7	22.3 ± 0.7	28.0 ± 0.7
Difference GAL *vs.* ADP (95% CI)	4.9 (4.2 – 5.7)	6.2 (5.3 – 7.1)	-4.9 (-5.7 – -4.2)	-6.2 (-7.1 – -5.3)
p-value	< 0.001	< 0.001	< 0.001	< 0.001

Data are represented as mean ± SE. BIA outcomes were used in Kyle's equation[30] to determine FFM. Abbreviations: FFM = fat-free mass, FM = fat mass, ADP = air-displacement plethysmography, BIA = bioelectrical impedance, CI = confidence interval, GAL = Gallagher formula.

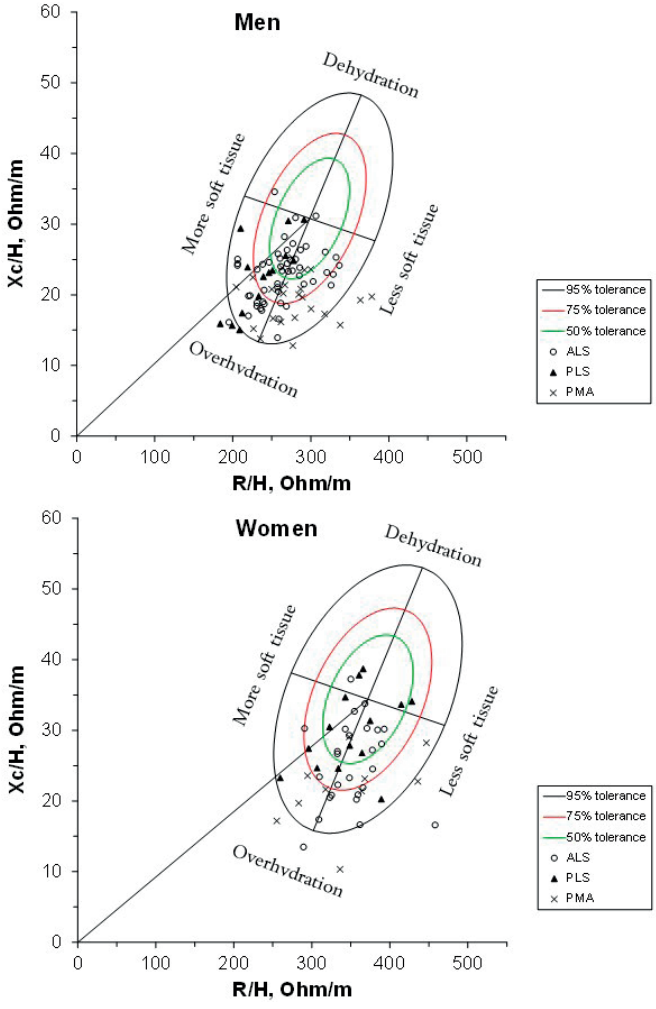


Figure 2. BIVA nomogram of patients with MND

BIVA yielding RXc point graph in men (top panel) and women (bottom panel) with MND according to diagnosis.[57] Individual impedance vectors are plotted on the 50%, 75% and 95% tolerance ellipses of the corresponding reference population (38). Abbreviations: BIVA = bioelectrical impedance vector analysis, Xc/H = height-adjusted reactance, R/H = height-adjusted resistance, ALS = amyotrophic lateral sclerosis, PLS = primary lateral sclerosis, PMA = progressive muscle atrophy.

Figure 3 presents the individual differences in FFM between BIA *vs.* ADP and GAL *vs.* ADP, with 95% limits of agreement ranging from -5.3 to 10.3 kg, and from -3.8 to 13.6 kg, respectively.

Figure 3. Differences in FFM when determined by BIA, GAL, and ADP

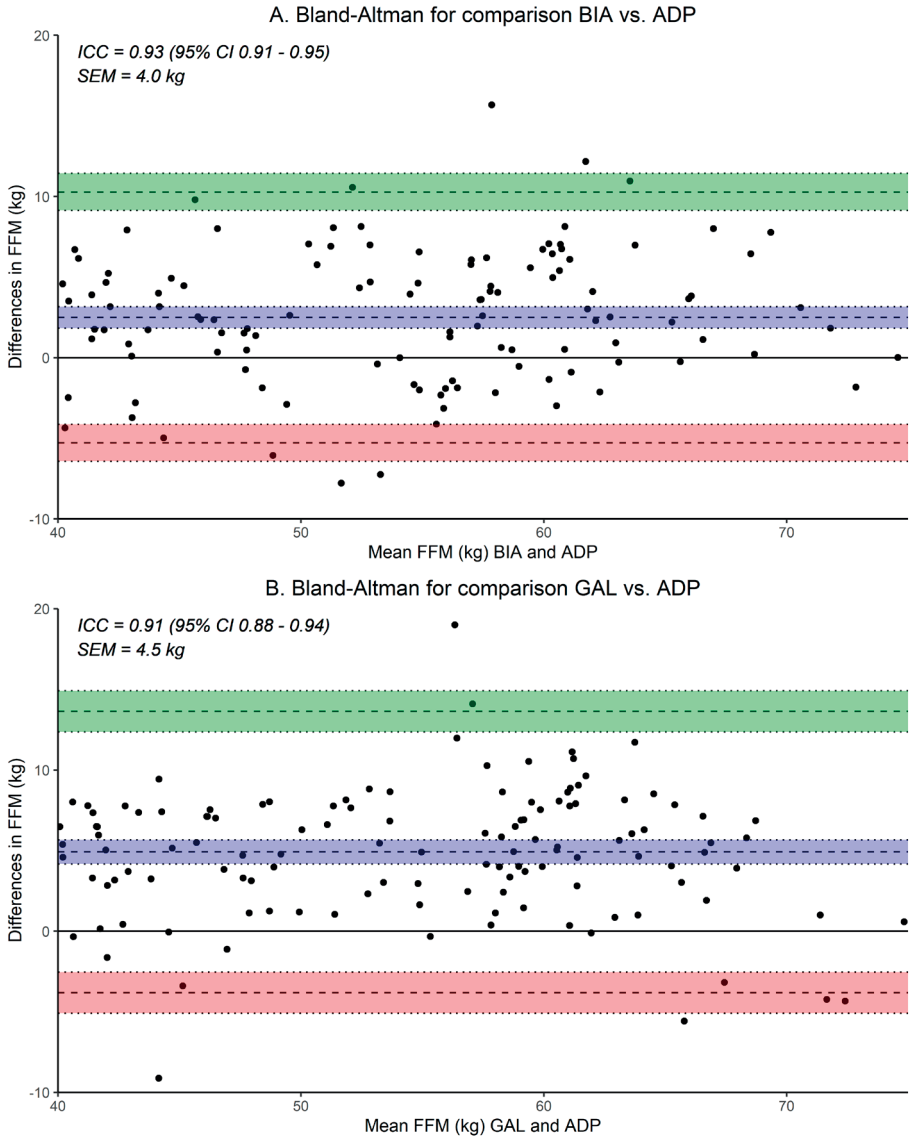


Figure 3. Bland-Altman plots visualizing the differences in FFM (in kg) of the BIA (panel A) and GAL (panel B) compared to ADP in MND patients (n = 140). Blue area indicates the 95% confidence intervals around the mean difference. Dotted lines indicate the 95% limits of agreement. BIA outcomes were used in Kyle's equation.[30] Abbreviations: BIA = bioelectrical impedance analysis, ADP = air-displacement plethysmography, ICC = intraclass correlation coefficient, CI = confidence interval, SEM = standard error of measurement, FFM = fat-free mass, GAL = Gallagher formula.

Table 3. Overview of the measured FFM (kg and %) and the difference between the BIA, GAL and ADP in patients with MND (N = 140)

		According to Lukaski's equation et al.				*According to Kyle's equation et al.*				*According to the Gallagher formula*			
Variable	ADP	BIA	Difference BIA - ADP	95% CI	p-value	BIA	Difference BIA - ADP	95% CI	p-value	GAL	Difference GAL – ADP	95% CI	p-value
FFM (kg)	51.7 ± 0.9	61.9 ± 1.2	10.2	9.1 – 11.3	<0.001	54.2 ± 1.0	2.5	1.8 – 3.2	<0.001	56.6 ± 0.9	4.9	4.2 – 5.7	<0.001
FFM (%)	65.8 ± 0.8	78.5 ± 1.1	12.8	11.5 – 14.1	<0.001	68.6 ± 0.7	3.0	2.1 – 3.8	<0.001	72.0 ± 0.7	6.2	5.3 – 7.1	<0.001
FM (kg)	27.2 ± 0.8	22.9 ± 0.8	-4.3	-5.2 – -3.5	<0.001	24.8 ± 0.7	-2.4	-3.2 – -1.8	<0.001	22.3 ± 0.7	-4.9	-5.7 – -4.2	<0.001
FM (%)	34.2 ± 0.8	28.8 ± 0.9	-5.4	-6.6 – -4.4	<0.001	31.4 ± 0.7	-2.8	-3.8 – -2.1	<0.001	28.0 ± 0.7	-6.2	-7.1 – -5.3	<0.001

Data are represented as mean ± SE. Abbreviations: ADP = air-displacement plethysmography, BIA = bioelectrical impedance analysis, CI = confidence interval, GAL = Gallagher formula, FFM = fat-free mass, FM = fat mass, SE = standard error.

The ICC for BIA was 0.93 (95% CI 0.91 to 0.95), and for GAL 0.91 (95% CI 0.88 to 0.94). Results were similar within MND subtypes (*not shown*). Lukaski et al. overestimated FFM by 10.2 kg (95% CI 9.1 to 11.3, $p < 0.001$), as shown in **table 3** and **Figure 4**.

Figure 4. Differences in FFM when determined by different formulae

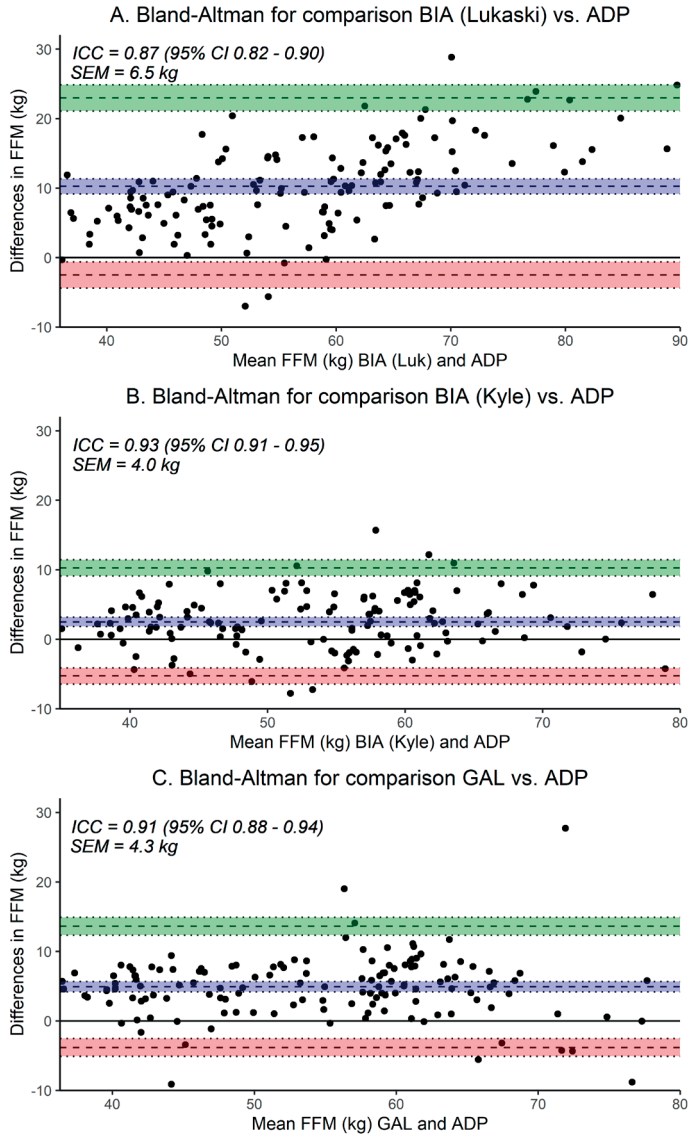


Figure 4. Bland-Altman plots visualising the differences in FFM (in kg) when determined using Lukaski's equation (panel A), Kyle's equation (panel B), and the Gallagher formula (panel C) in patients with MND ($n = 140$). Blue area indicates the 95% confidence intervals around the mean difference. Dotted lines indicate the 95% limits of agreement. Abbreviations: BIA = bioelectrical impedance analysis, ADP = air-displacement plethysmography, FFM = fat-free mass, ICC = intraclass correlation coefficient, CI = confidence interval, SEM = standard error of measurement, Luk = according to Lukaski's equation, GAL = Gallagher formula.

The difference between the BIA and ADP in FFM declined as the atrophy score increased: for each point increase in atrophy score, the mean difference in FFM between BIA and ADP declined by 0.78 kg (95% CI -1.2 to -0.4, $p < 0.001$). This relationship was less evident for GAL ($p = 0.08$).

Clinical relevance

The mean pREE was found to be 1619.9 kcal/day (SD 241.0 kcal/day) when using BIA, and 1577.9 kcal/day (SD 227.5 kcal/day) when using ADP, resulting in a mean difference of 42.0 kcal/day (95% CI -13.5 – 97.4, $p = 0.123$). We found a mean metabolic index of 105.7% (SD 10.4%) when using BIA, and 108.2% (SD 9.7%) when using ADP, resulting in a mean difference of 2.5% (95% CI 0.1 – 4.9, $p = 0.048$). A hypermetabolic state of ≥ 110% was found in 44 (31.4%) patients using BIA, and in 62 (44.2%) patients using ADP. When we used ≥ 120% as cut-off value, 9 (6.4%) patients were hypermetabolic using BIA, compared to 11 (7.9%) patients using ADP (**Figure 5**). In addition, mean daily protein demand was 77.5 g according to the ADP, which would increase to 81.3 g and 84.9 g when using the BIA and GAL, respectively. A clinically relevant underestimation of 15 g or more in protein demand was observed for 4 (2.9%) patients using BIA, and 9 (6.4%) patients using GAL. Differences in pREE were found between men (ADP: 1705.8 kcal/day (SD 169.1 kcal/day), BIA: 1751.6 kcal/day (SD 180.0 kcal/day)) and women (ADP: 1354.7 kcal/day (SD 116.8 kcal/day), BIA: 1387.8 kcal/day (SD 137.2 kcal/day)), all p-values < 0.001.

Figure 5. Differences in calculating predicted REE and MI using data provided by BIA and ADP.

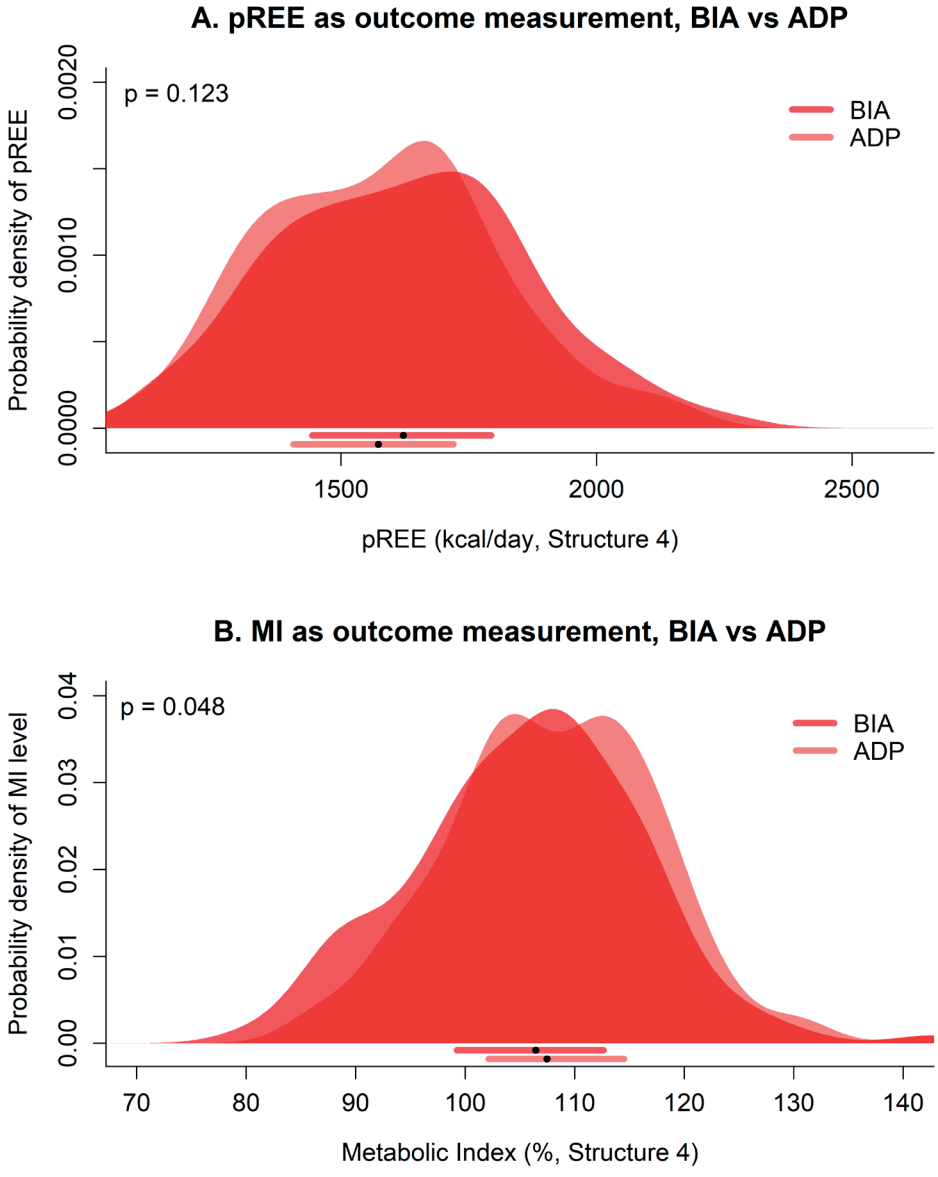


Figure 5. Figure shows probability densities of (A) the predicted REE based on data provided by BIA vs. ADP, and (B) having a certain metabolic index based on data provided by BIA vs. ADP, in patients with MND. BIA outcomes were used in Kyle's equation.[30] The horizontal bars below every panel provide the median (black dots) with their 25%-75% interquartile range. Abbreviations: pREE = predicted resting energy expenditure, BIA = bioelectrical impedance, ADP = air-displacement plethysmography, MI = metabolic index. P-values are Wilcoxon signed-rank test.

DISCUSSION

In this study, we have shown that – despite high correlations – the determination of fat-free mass using bioelectrical impedance or the Gallagher formula will result in a systematic overestimation, compared to air-displacement plethysmography, in patients with MND. As a result, protein demand was overestimated when using BIA and GAL, but this is unlikely to have significant disadvantages for a patient's health management. Nevertheless, overestimation of fat-free mass may have consequences when interest lies in determining hypermetabolism, which would be underestimated when using BIA data to predict REE. This could be of importance when counselling patients for dietary interventions or in research settings when stratifying patients according to their metabolic index.

A comparison between ADP and BIA has not previously been performed in patients with MND. However, in non-neurologically affected individuals, an overestimation of FFM was found when using BIA compared to ADP,[40–45] with differences ranging from 0.5% to 5.3%. Our results seem to be in line with these studies, as we found an overestimation of FFM when using BIA – and GAL – compared to ADP. Nevertheless, both methods were highly correlated with ADP. This suggests that using BIA is a valid and easy device to use in patients with MND, as previously suggested.[13] Moreover, repeated measures of BIA and ADP have shown both to have high reliability.[46] On the other hand, we have highlighted the clinical relevance and differences in our findings which may be of relevance when assessing REE and, subsequently, determining the MI.

Predicting a patient's REE is mainly dependent on FFM and FM,[32] in addition to their age and sex. A previous study showed that anthropometric measurements – such as body mass index (BMI) and body adiposity index (BAI) – do not seem to be sufficient to determine fat mass in ALS,[47] thus questioning the use of the Gallagher formula – which includes BMI as parameter – in patients with ALS/MND. However, as a decline in BMI,[48] and weight loss[3] have proved to indicate a less favourable prognosis, it would seem to be important to prevent anthropometric changes by starting dietary interventions in time.[49,50] Changes in outcomes of the Gallagher formula, might, therefore, be indicative of a patient's needs. However, we have shown that the BIA is able to timely identify those patients who are in need of a dietary intervention, and, moreover, this method is low-burden for patients. Besides, BIA determines FFM more accurately than GAL, and the overestimation of FFM by BIA would merely lead to an advice of hypercaloric nutrition. An essential point to emphasize is that the degree of this overestimation might also be dependent on the prediction equation used.[32,34] Clinicians should be aware of the potential consequences of the method used, as GAL and BIA overestimate FFM – and, therefore, underestimate FM – which might result in an earlier indication for dietary intervention. Besides, the proportion of patients in our study with a

clinically relevant deficient protein intake was low, which might suggest augmentation of energy intake only, as previously described.[51]

In the same way, an increased metabolic state (e.g. hypermetabolic state) has proven to be prognostically less favourable in patients with ALS.[9] It is, therefore, very important that a patient's body composition – consisting of FM and FFM – is determined accurately. We have shown differences between BIA and ADP in determining a patient's metabolic state, which may result in excluding actual hypermetabolic patients with MND from, for example, observational studies and clinical trials.[52] This might lead to unjustified exclusion of these patients. Similar to this, GAL overestimates FFM, and, therefore, pREE, which may lead to a lower estimate of the proportion of patients with a hypermetabolic state, and subsequently unjustified exclusion of patients. Furthermore, the use of these different methods to calculate FFM may affect the estimation of patient's caloric needs.

The main strength of this study is the comparison between BIA *vs.* ADP and GAL *vs.* ADP in patients with MND, and the effect on daily patient care. In addition, all assessments were performed by a trained, consistent study team, which prevented observer bias. As we have not assessed the triceps skinfold thickness (TSF), we were unable to assess the ALS-specific BIA formula.[13] The validity of TSF in ALS is arguable, however, due to abnormal fat distribution and asymmetrical muscle atrophy, as well as a rater-dependent variation.[13] Moreover, we used the Sabounchi Structure 4 equation to determine the pREE, and, subsequently, the metabolic index, which has recently been shown to be the best fitting equation in patients with MND.[33]

A limitation of our study might be the assessment of the BIA on the right side of the body only. This may have generated some deviation in outcomes due to asymmetrical muscle atrophy in this study population. Performing BIA on both sides and subsequently determining the mean, may provide additional information.[53] Future studies should compare the validity of one-sided *vs.* double-sided BIA in patients with MND. Furthermore, future studies should collect longitudinal data in this study population, as it might provide greater insight in the validity of these techniques. Moreover, a comparison between the three disease subtypes (ALS *vs.* PMA *vs.* PLS), when using BIA and ADP, might be of value, despite their shared pathological features.[54] We do, however, provide unique data on body composition and metabolic rate in these patients. A comparison of pREE and MI between disease subtypes has been described elsewhere.[55] Other limitations might be the fact that we did not include non-neurological controls in our study, and that we have not assessed the inter- and intra-rater reliability. We did, however, include a significant number of patients with PMA and PLS, conditions which have not been extensively studied in comparison with ALS. Moreover, all assessments were performed according to our study protocol, to which the same, experienced study team adhered. Finally, future studies should be aware of the impact of hydration

status when obtaining data regarding body composition.[12,31,56] Measuring body composition in MND patients is very important for creating an international consensus on the available techniques, and, subsequently, the derived data, to advise patients, their caregivers, and health care professionals more appropriately on e.g. nutritional intervention.

In conclusion, BIA and GAL systematically overestimate FFM compared to ADP in patients with MND. Differences are limited, and may have limited clinical impact for dietary interventions. However, overestimation of FFM may have consequences when determining patient's metabolic index, as the BIA underestimates the metabolic index compared to the ADP.

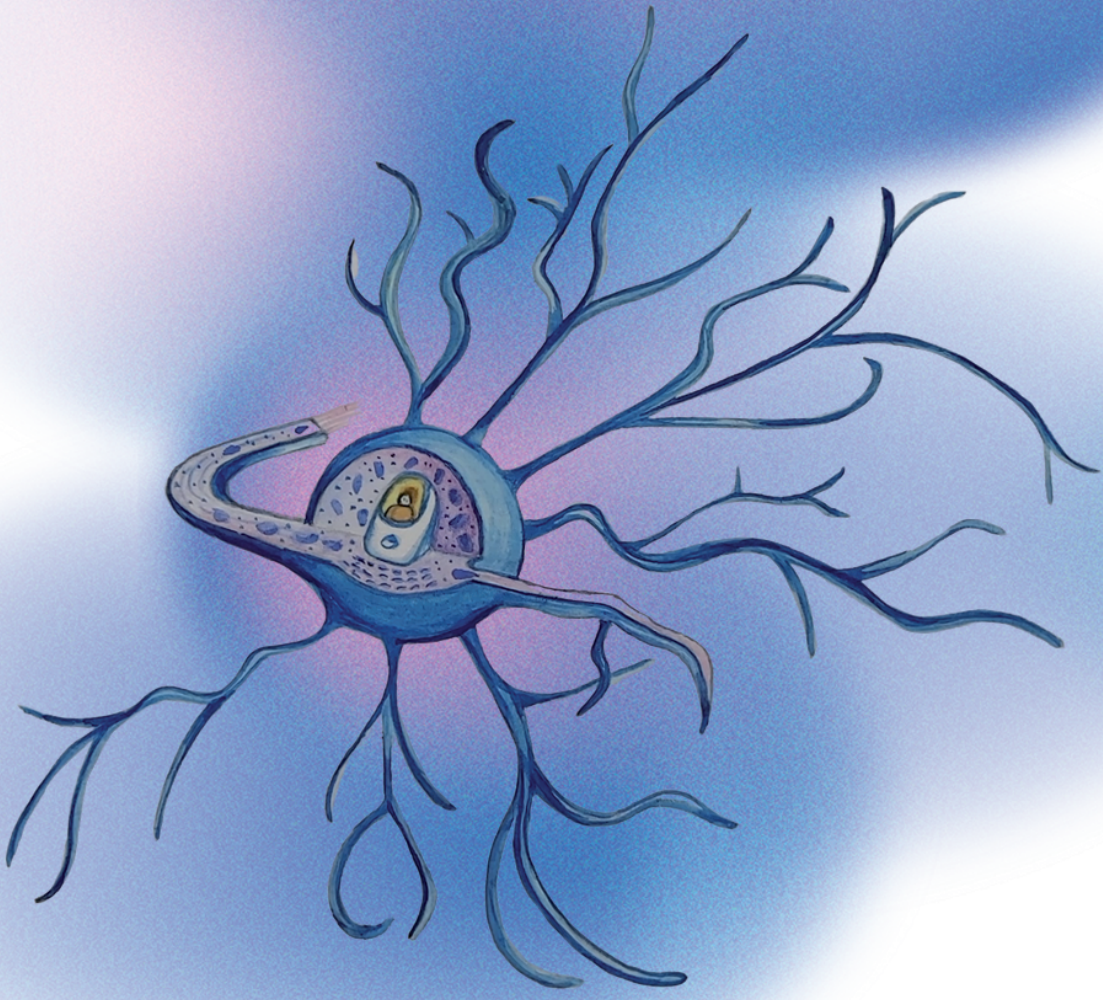
REFERENCES

1. Kruizenga H, Beijer S, Huisman-de Waal G, Jonkers-Schuitema C, Klos M, Remijnse-Meester W, et al. Richtlijn ondervoeding - Herkenning, diagnosestelling en behandleing van ondervoeding bij volwassenen. The Netherlands; 2019 p. 1–39.
2. 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. 2015 Apr 1;51(4):479–84.
3. Janse Van Mantgem MR, Van Eijk RPA, Van Der Burgh HK, Tan HHG, Westeneng HJ, Van Es MA, et al. Prognostic value of weight loss in patients with amyotrophic lateral sclerosis: A population-based study. J Neurol Neurosurg Psychiatry. 2020;91(8):867–75.
4. Holdom CJ, Janse van Mantgem MR, van Eijk RPA, Howe SL, van den Berg LH, McCombe PA, et al. Venous creatinine as a biomarker for loss of fat-free mass and disease progression in patients with amyotrophic lateral sclerosis. Eur J Neurol. 2021 Nov 1;28(11):3615–25.
5. Ngo ST, van Eijk RPA, Chachay V, van den Berg LH, McCombe PA, Henderson RD, et al. Loss of appetite is associated with a loss of weight and fat mass in patients with amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener. 2019 Oct 2;20(7–8):497–505.
6. Van Eijk RPA, Eijkemans MJC, Ferguson TA, Nikolakopoulos S, Veldink JH, Van Den Berg LH. Monitoring disease progression with plasma creatinine in amyotrophic lateral sclerosis clinical trials. J Neurol Neurosurg Psychiatry. 2018 Feb 1;89(2):156–61.
7. Fields DA, Goran MI, McCrory MA. Body-composition assessment via air-displacement plethysmography in adults and children: a review. Am J Clin Nutr. 2002;75:453–67.
8. Lowry DW, Tomiyama AJ. Air displacement plethysmography versus dual-energy X-ray absorptiometry in underweight, normal-weight, and overweight/obese individuals. PLoS One. 2015 Jan 1;10(1).
9. Steyn FJ, Ioannides ZA, Van Eijk RPA, Heggie S, Thorpe KA, Ceslis A, et al. Hypermetabolism in ALS is associated with greater functional decline and shorter survival. J Neurol Neurosurg Psychiatry. 2018;89(10):1016–23.
10. Muntean P, Micloș-Balica M, Popa A, Neagu A, Neagu M. Reliability of repeated trials protocols for body composition assessment by air displacement plethysmography. Int J Environ Res Public Health. 2021 Oct 1;18(20).
11. Lee I, Kazamel M, McPherson T, McAdam J, Bamman M, Amara A, et al. Fat mass loss correlates with faster disease progression in amyotrophic lateral sclerosis patients: Exploring the utility of dual-energy xray absorptiometry in a prospective study. PLoS One. 2021 May 1;16(5 May).
12. Hills AP, Byrne NM. Bioelectrical impedance and body composition assessment. Mal J Nutr [Internet]. 1998;4:107–12. Available from: https://www.researchgate.net/publication/225300934
13. Desport JC, Preux PM, Bouteloup-Demange C, Clavelou P, Beaufrère B, Bonnet C, et al. Validation of bioelectrical impedance analysis in patients with amyotrophic lateral sclerosis. American Journal of Clinical Nutrition. 2003;77(5):1179–85.
14. Ioannides ZA, Steyn FJ, Henderson RD, Mccombe PA, Ngo ST. Anthropometric measures are not accurate predictors of fat mass in ALS. Amyotroph Lateral Scler Frontotemporal Degener. 2017 Oct 2;18(7–8):486–91.

15. Gallagher D, Heymsfield SB, Heo M, Jebb SA, Murgatroyd PR, Sakamoto Y. Healthy percentage body fat ranges: An approach for developing guidelines based on body mass index. American Journal of Clinical Nutrition. 2000;72(3):694–701.
16. Sabounchi NS, Rahmandad H, Ammerman A. Best-fitting prediction equations for basal metabolic rate: Informing obesity interventions in diverse populations. Vol. 37, International Journal of Obesity. 2013. p. 1364–70.
17. Brooks BR, Miller RG, Swash M, Munsat TL. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis. 2000;1(5):293–9.
18. Turner MR, Barohn RJ, Corcia P, Fink JK, Harms MB, Kiernan MC, et al. Primary lateral sclerosis: Consensus diagnostic criteria. J Neurol Neurosurg Psychiatry. 2020;91(4):373–7.
19. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: The global lung function 2012 equations. European Respiratory Journal. 2012 Dec 1;40(6):1324–43.
20. Kimura F, Fujimura C, Ishida S, Nakajima H, Furutama D, Uehara H, et al. Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. Neurology. 2006;66(2):265–7.
21. Devine MS, Ballard E, Orourke P, Kiernan MC, McCombe PA, Henderson RD. Targeted assessment of lower motor neuron burden is associated with survival in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener. 2016 May 18;17(3–4):184–90.
22. Ravits J, Paul P, Jorg C. Focality of upper and lower motor neuron degeneration at the clinical onset of ALS. Neurology. 2007 Nov 30;68:1571–5.
23. Ottery F. https://pt-global.org/. 2014. PG-SGA/Pt-Global Platform.
24. COSMED - The Metabolic Company. Estimation of Resting Metabolic Rate based on Lean Body Mass with Bod Pod. 2012;(June):1.
25. Crapo RO, Morris AH, Clayton PD, Nixon CR. Lung volumes in healthy nonsmoking adults. Bull Eur Physiopathol Respir. 1982;18(3):419–25.
26. Fields DA, Hull HR, Cheline AJ, Yao M, Higgins PB. Child-specific thoracic gas volume prediction equations for air-displacement plethysmography. Obes Res. 2004;12(11):1797–804.
27. Hollander F, Hulshof P, Koster E. Measuring fat mass and fat free mas using plethysmography (BOD POD®). Standard Operating Procedure 2020 p. 1–11.
28. Stocks J, Marchal F, Kraemer R, Gutkowski P, Bar Yishay E, Godfrey S. Plethysmographic assessment of functional residual capacity and airway resistance. In: Infant Respiratory Function Testing. 1996. p. 191–238.
29. Siri WE. Body composition from fluid spaces and density: analysis of methods. 1961. Nutrition. 1993;9(5):480–91.
30. Kyle UG, Genton L, Karsegard L, Slosman DO, Pichard C. Single Prediction Equation for Bioelectrical Impedance Analysis in Adults Aged 20-94 Years. Nutrition. 2001;17:248–53.
31. Lemos T, Gallagher D. Current body composition measurement techniques. Curr Opin Endocrnol Diabetes Obes. 2017;24(5):310–4.
32. Flack KD, Siders WA, Johnson LA, Roemmich JN. Cross-Validation of Resting Metabolic Rate Prediction Equations. J Acad Nutr Diet [Internet]. 2016;116(9):1413–22. Available from: http://dx.doi.org/10.1016/j.jand.2016.03.018
33. Holdom CJ, Janse van Mantgem MR, He J, Howe S, McCombe PA, Fan D, et al. Variation in Resting Metabolic Rate Impacts Identification of Metabolic Change in Geographically Distinct Cohorts of Amyotrophic Lateral Sclerosis Patients. Accepted at Neurology. 2023;Unpublished.
34. Weijs PJM. Hypermetabolism, is it real? The example of amyotrophic lateral sclerosis. J Am Diet Assoc. 2011;111(11):1670–3.

35. Lukaski HC, Bolonchuk WW, Hall CB, Siders WA. Validation of tetrapolar bioelectrical impedance method to assess human body composition. J Appl Physiol. 1986;60(4):1327–32.
36. Nau KL, Dick AR, Peters K, Schloerb PR. Relative validity of clinical techniques for measuring the body composition of persons with amyotrophic lateral sclerosis. Vol. 1, Journal of Neurological Sciences 152 Suppl. 1997.
37. Piccoli A, Pastori G. BIVA software 2002. Padova; 2002.
38. Piccoli A, Nigrelli S, Caberlotto A, Bottazzo S, Rossi B, Pillon L, et al. Bivariate normal values of the bioelectrical impedance vector in adult and elderly populations. Am J Clin Nutr. 1995;61(2):269–70.
39. Kruizenga H, Beijer S, Huisman-de-Waal G, Jonkers-Schuitema C, Klos M, Remijnse-Meester W, et al. Richtlijn Ondervoeding - Herkenning, diagnosestelling en behandeling van ondervoeding bij volwassenen. Stuurgroep Ondervoeding. 2009. p. 1–39.
40. Azcona C, Köek N, Frühbeck G. Fat mass by air-displacement plethysmography and impedance in obese/non-obese children and adolescents. International Journal of Pediatric Obesity. 2006;1(3):176–82.
41. Flakoll PJ, Kent P, Neyra R, Levenhagen D, Chen KY, Ikizler TA. Bioelectrical Impedance vs Air Displacement Plethysmography and Dual-Energy X-ray Absorptiometry to Determine Body Composition in Patients with End-Stage Renal Disease. Journal of Parenteral and Enteral Nutrition. 2004;28(1):13–21.
42. Foucart L, De Decker A, Sioen I, De Henauw S, Michels N. Hand-to-foot bioelectrical impedance analysis to measure fat mass in healthy children: A comparison with air-displacement plethysmography. Nutrition and Dietetics. 2017 Nov 1;74(5):516–20.
43. Rodrigues NCL, Sala PC, Horie LM, Dias MCG, Torrinhas RSM de M, Romão JE, et al. Bioelectrical Impedance Analysis and Skinfold Thickness Sum in Assessing Body Fat Mass of Renal Dialysis Patients. Journal of Renal Nutrition. 2012;22(4).
44. Benton MJ, Swan PD, Schlairet MC, Sanderson S. Comparison of Body Composition Measurement With Whole Body Multifrequency Bioelectrical Impedance and Air Displacement Plethysmography in Healthy Middle-Aged Women. Health Care Women Int. 2011;32(12):1068–78.
45. Ferri-Morales A, Nascimento-Ferreira MV, Ubago-Guisado E, De Moraes ACF, Vlachopoulos D, Torres-Costoso A, et al. Agreement between standard body composition methods to estimate percentage of body fat in young male athletes. Pediatr Exerc Sci. 2018 Aug 1;30(3):402–10.
46. Von Hurst PR, Walsh DCI, Conlon CA, Ingram M, Kruger R, Stonehouse W. Validity and reliability of bioelectrical impedance analysis to estimate body fat percentage against air displacement plethysmography and dual-energy X-ray absorptiometry. Nutrition and Dietetics. 2016 Apr 1;73(2):197–204.
47. Ioannides ZA, Steyn FJ, Henderson RD, Mccombe PA, Ngo ST. Anthropometric measures are not accurate predictors of fat mass in ALS. Amyotroph Lateral Scler Frontotemporal Degener. 2017 Oct 2;18(7–8):486–91.
48. Jawaid A, Murthy SB, Wilson AM, Qureshi SU, Amro MJ, Wheaton M, et al. A decrease in body mass index is associated with faster progression of motor symptoms and shorter survival in ALS. Amyotrophic Lateral Sclerosis. 2010;11(6):542–8.
49. Altinkaynak M, Gok GK, Ozmen B, Buyukdemir S, Akpinar TS, Erten SN, et al. Prognostic Value of Regular Nutritional Treatment in Patients With Amyotrophic Lateral Sclerosis. Neurologist. 2023 May 1;28(3):166–72.

50. Burkhardt C, Neuwirth C, Sommacal A, Andersen PM, Weber M. Is survival improved by the use of NIV and PEG in amyotrophic lateral sclerosis (ALS)? A post-mortem study of 80 ALS patients. PLoS One. 2017;12(5):1–12.
51. Kasarskis EJ, Berrv S, Vanderleest JG, Schneider AR, McClain CJ. Nutritional status of patients with amyotrophic lateral sclerosis: relation to the proximity of death. American Journal of Clinical Nutrition. 1996;63(1):130–7.
52. Scaricamazza S, Salvatori I, Amadio S, Nesci V, Torcinaro A, Giacovazzo G, et al. Repurposing of Trimetazidine for amyotrophic lateral sclerosis: A study in SOD1G93A mice. Br J Pharmacol. 2022 Apr 1;179(8):1732–52.
53. Jensen B, Braun W, Both M, Gallagher D, Clark P, González DL, et al. Configuration of bioelectrical impedance measurements affects results for phase angle. Med Eng Phys. 2020 Oct 1;84:10–5.
54. Saberi S, Stauffer JE, Schulte DJ, Ravits J. Neuropathology of Amyotrophic Lateral Sclerosis and Its Variants. Neurol Clin. 2015 Nov 1;33(4):855–76.
55. Janse van Mantgem MR, van Eijk RPA, Holdom CJ, Meyjes M, Ngo ST, Steyn FJ, et al. Assessing the Metabolic Rate in Patients with Amyotrophic Lateral Sclerosis, Progressive Muscular Atrophy, and Primary Lateral Sclerosis. Submitted at Journal of Neurology. 2023;
56. Barazzoni R, Jensen GL, Correia MITD, Gonzalez MC, Higashiguchi T, Shi HP, et al. Guidance for assessment of the muscle mass phenotypic criterion for the Global Leadership Initiative on Malnutrition (GLIM) diagnosis of malnutrition. Clinical Nutrition. 2022 Apr;
57. Piccoli A, Pastori G. BIVA software. Department of Medical and Surgical Sciences, University of Padova, Padova, Italy. 2002;



Chapter 7

Assessing the Metabolic Rate in Patients with Amyotrophic Lateral Sclerosis, Progressive Muscular Atrophy, and Primary Lateral Sclerosis

Mark R. Janse van Mantgem, M.D., Ruben P.A. van Eijk, M.D., PhD, Cory J. Holdom, MSc, Myrte Meyjes, MSc, Shyuan T. Ngo, PhD, Frederik J. Steyn, PhD, Leonard H. van den Berg, M.D., PhD

Submitted at Journal of Neurology

ABSTRACT

Objective: To determine the difference in metabolic rate between patients with amyotrophic lateral sclerosis (ALS), progressive muscle atrophy (PMA), and primary lateral sclerosis (PLS).

Methods: We enrolled patients with ALS, PMA, and PLS between 2017 and 2021 in a prospective study. Indirect calorimetry was performed in a fasted state to obtain resting energy expenditure (mREE). We determined the patient's body composition with air displacement plethysmography to calculate the predicted REE (pREE). The metabolic index (MI) was defined as the ratio between mREE and pREE, and expressed as a percentage deviation from mREE. Differences between disease categories were determined using a multivariable regression model adjusting for sex, age, fat-free mass, fat mass, height, and smoking status.

Results: In total, 79 patients with ALS, 33 patients with PMA, and 30 patients with PLS were included. We observed a MI of ≥ 110% in 55.7% of patients with ALS, 27.3% of patients with PMA, and 26.7% of patients with PLS. After adjustment for key confounders, MI were found to differ significantly between disease subtypes (p = 0.017), with higher metabolic rates observed in patients with ALS (mean 109.6%, 95% CI 107.7 – 111.5), followed by PMA (mean 107.1%, 95% CI 104.1 – 110.2) and PLS (mean 104.3%, CI 101.2 –107.4). There were no correlations between metabolic measures and disease-specific characteristics.

Conclusions: Increased metabolic rates were found across MND subtypes. The altered metabolic rate was most commonly observed in patients with ALS, but was also present in patients with PMA and PLS.

INTRODUCTION

An increased metabolic rate – or hypermetabolism – is observed in patients with amyotrophic lateral sclerosis (ALS).[1,2] It has been associated with a faster rate of functional decline and shorter survival.[1,3] The pathophysiology behind these changes has yet to be unravelled. When embarking on the unravelling process, it is worth investigating whether changes in metabolic rate are unique to patients with ALS or if this is a phenomenon observed across other subtypes of motor neuron diseases (MND), including progressive muscle atrophy (PMA) and primary lateral sclerosis (PLS).

The metabolic index (MI) is defined by the measured resting energy expenditure (mREE) relative to the predicted REE (pREE).[1] Studies in MND have focused specifically on the prevalence and impact of hypermetabolism in patients with ALS,[1,4–8] while it is not known if this phenomenon is solely seen in these patients or could also be present in patients with PMA or PLS. Identification of metabolic changes in other forms of MND would improve understanding of possible factors that contribute to an altered metabolic state. Moreover, it might improve tailored care, as hypermetabolism may cause, for example, weight loss – an unfavourable prognostic symptom in patients with ALS – due to the imbalance in energy requirement and energy intake.[9] Patients with ALS tend to have an increase in mREE compared to healthy individuals.[5] However, reports on the prevalence of hypermetabolism in ALS vary considerably.[2] While factors that contribute to this heterogeneity are likely multifactorial, some variance might be due to features inherent to disease subtypes. The prevalence of hypermetabolism is increased in patients with familial ALS,[10] relative to sporadic only.[1] Heterogeneity is a hallmark of disease in ALS, and while some variability is addressed through the development of improved diagnostic criteria that consider lower (LMN) and upper motor neuron (UMN) involvement,[11] clinical presentation and progression remains variable. In this instance, the variable pathophysiological background of ALS is likely to contribute.[12] This variability could be amplified when considering metabolic changes in disease, and across disease subtypes. Besides, REE has shown to be correlated with fat-free mass (FFM)[7,13] and thus varying degrees of muscle wasting and/or involvement may uniquely impact metabolism in patients with MND.

Therefore, we aimed to determine whether an increased metabolic index is a specific disease characteristic in patients with ALS, or might also be present in patients with a final diagnosis of PMA and PLS. In addition, we aimed to relate specific clinical features of MND to the MI and REE in these subtypes.

METHODS

We conducted a prospective study, including patients with ALS, PMA, and PLS. Data were collected between March 2017 and November 2021 at the University Medical Centre Utrecht (UMCU, Utrecht, The Netherlands). Patients with ALS were diagnosed according to the El Escorial criteria (EEC) of having possible, probable laboratory supported, probable, or definite ALS.[14] Patients were categorized as PLS when 1) progressive upper motor neuron (UMN) symptoms were present for at least two years, and 2) UMN dysfunction is present in at least two body regions.[15] A diagnosis of PMA was defined as having only lower motor neuron signs (LMN) in two or more body regions. This study conforms with the World Medical Association Declaration of Helsinki and was approved by the medical ethics committee and institutional board of UMC Utrecht, The Netherlands, permit number 15-656 (MEASURE). All patients provided written informed consent prior to the first assessment.

Study procedures

Patients with a diagnosis of MND who were 18 years of age and older were invited to participate. Participants were asked to fast (except for drinking water) overnight and instructed to not perform any vigorous physical activity for at least 10 hours prior to assessment. Patients who were not able to lie down for at least one hour, had had a tracheostomy or other assisted ventilation in the preceding three months, and/or were diagnosed with a mental health condition were excluded from the study. During the visit, we performed metabolic assessments, determined forced vital capacity (FVC) as percentage of predicted, performed a neurological examination, and administered the ALSFRS-R.[16] The rate of disease progression (ΔFRS) was calculated by: (48 – ALSFRS-R score) / symptom duration in months.[17] The King's clinical stage[18] of each included patient with ALS was derived from the obtained ALSFRS-R score at study visit.[19,20] The severity of UMN and LMN signs was graded using a modified Ravits scale, where each limb was scored on a scale of 0 (no involvement) to 3 (significant and severe involvement) to indicate the involvement of UMN and/or LMN for each patient.[21,22]

Metabolic assessments and parameters

We performed all assessments for each patient under standardized circumstances (i.e. all assessments were competed in the morning, under a stable room temperature of approximately 24 degrees Celsius, and patients were asked not to talk or fall asleep during assessment). First, we determined a subject's height and weight. Second, fat-free mass (FFM) and fat mass (FM) were determined with whole body air displacement plethysmography using the BodPod system (Cosmed USA, Rome, ITA).[23] Patients were requested to wear underwear only during this assessment. Moreover, a tight shower cap was placed over the patient's head to cover all their hair. Subsequently, we calculated the pREE according to the Harris-Benedict and Structure 4 equation. Both equations were included, as the Harris-Benedict equation

is most commonly used in previous studies on metabolism in ALS, while the Sabounchi Structure 4 equation seems to fit best in patients with MND:[24]

1) Harris-Benedict equation:[25]
   - Male: 66.473 + (13.7516 * weight (kg)) + (5.0033 * height (cm)) - (6.7550 * age (years))
   - Female: 655.0955 + (9.5634 * weight (kg)) + (1.8496 * height (cm)) - (4.6756 * age (years))
2) Sabounchi Structure 4 equation:[26,27]
   - Male: 361 + (21.1 * FFM (kg)) + (4.77 * FM (kg))
   - Female: 360 + (21.0 * FFM (kg)) + (4.68 * FM (kg))

Third, we determined the mREE by indirect calorimetry using a Quark RMR respirometer (Cosmed). A canopy hood was placed over the patient's head for a period of twenty minutes. Adjustment of the flow rate was allowed during the first five minutes to achieve a flow rate between 0.8 and 1.1 L/min. No adjustment of the flow rate was allowed in the remaining 15 minutes. The collected REE was determined in kcal/day using the Weir equation: 1440 * ((3.94 * VO_2) + (1.11 * VCO_2)).[28] Subsequently, we determined the % of predicted REE (i.e., the MI) by: (mREE (kcal/day) / pREE (kcal/day)) * 100%.

Statistical analysis

We performed our statistical analyses using RStudio (version 1.1.4, RStudio: Integrated Development for R, Inc., Boston, USA, http://www.rstudio.com/). We summarized patient characteristics as frequencies and percentages, or as means and standard deviations (SD), or as medians and their interquartile range (IQR). Potential differences were determined between the three patient groups by using the chi-squared test for categorical variables and an analysis of variance (ANOVA) for continuous variables. Correlations were assessed using Pearson's correlation coefficient.

To assess differences in MI between patients with ALS, PMA, and PLS, we created a multivariable model, which included: diagnosis (ALS, PMA or PLS), sex, age, fat-free mass, fat mass, BMI, height, and smoking status. These variables were used in a linear regression model with MI as outcome. The estimated mean with their 95% CI were reported with their corresponding p-values. A two-sided p-value < 0.05 was considered statistically significant. A similar model was used to assess between-group differences in mREE and pREE.

Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

We take full responsibility for the data, the analyses and interpretation, and the conduct of the research.

RESULTS

In total, 79 patients with ALS, 33 patients with PMA, and 30 patients with PLS were enrolled in this study. Baseline characteristics are listed in **Table 1**. We observed a MI – based on the Structure 4 equation – of ≥ 110% in 55.7% of patients with ALS, 27.3% of patients with PMA, and 26.7% of patients with PLS.

Table 1. Baseline demographics and clinical characteristics

Characteristics	ALS (N = 79)	PMA (N = 33)	PLS (N = 30)	p-value
Males, no. (%)	53 (67.1%)	23 (69.7%)	16 (53.3%)	0.32
Age, years	59.9 (10.1)	65.3 (9.1)	64.3 (9.3)	0.01
Diagnostic delay, months	11.1 (6.6 – 16.0)	26.2 (10.0 – 51.1)	38.4 (26.0 – 70.9)	< 0.001
Disease duration, months	17.0 (13.0 – 28.1)	43.2 (24.4 – 82.6)	115.3 (63.3 – 176.8)	< 0.001
Site of symptom onset, bulbar (%)	19 (24.1%)	0 (0.0%)	7 (23.3%)	0.008
El Escorial				-
Definite (%)	14 (17.7%)	-	-	-
Probable (%)	28 (35.4%)	-	-	-
Lab-supported (%)	23 (29.1%)	-	-	-
Possible (%)	14 (17.7%)	-	-	-
FVC, %	94.0 (18.3)	88.5 (22.4)	94.9 (17.0)	0.32
ALSFRS-R score	38.8 (5.1)	37.9 (5.1)	36.0 (5.8)	0.06
ΔFRS*	0.42 (0.20 – 0.79)	0.19 (0.08 – 0.44)	0.09 (0.06 – 0.17)	< 0.001
C9orf72 repeat expansion (%)	8 (10.1%)	1 (3.0%)	0 (0.0%)	0.12
Riluzole use	66 (83.5%)	22 (66.7%)	3 (10.0%)	< 0.001
Smoking, current (%)	8 (10.1%)	7 (21.2%)	5 (16.7%)	0.58
BMI, kg/m^2	25.5 (3.2)	26.2 (4.5)	26.1 (4.0)	0.58
Weight, kg	78.7 (12.7)	81.1 (13.9)	78.3 (16.8)	0.65
Fat-free mass, kg	52.3 (10.3)	51.8 (9.0)	51.0 (13.0)	0.84
Fat mass, kg	26.4 (8.1)	29.4 (12.7)	27.3 (8.9)	0.32
Height, cm	175.3 (8.9)	176.1 (9.7)	172.4 (10.7)	0.26

Data are given in mean (SD) or frequency (%). = Data are median values with 25%-75% inter-quartile range. Abbreviations: ALS = amyotrophic lateral sclerosis, PMA = progressive muscular atrophy, PLS = primary lateral sclerosis, FVC = forced vital capacity, ALSFRS-R = amyotrophic lateral sclerosis functional rating scale revised, ΔFRS = progression rate determined by: (48 - ALSFRS-R total score) / disease duration), BMI = body mass index. P values are ANOVA for continuous variables and Chi-square tests for categorical variables.

On average, patients with ALS had a mREE of 1,747 kcal/day (range: 1,225 to 2,468 kcal/day), patients with PMA 1,668 kcal/day (range: 1,191 to 2,211 kcal/day), and patients with PLS 1,613 kcal/day (range: 1,161 to 2,239 kcal/day). **Figure 1** shows that increased metabolic indices, over 110%, were observed in some patients across all diagnostic categories, but more often in patients with ALS than those with PMA or PLS ($p < 0.001$). A similar trend was found when other prediction equations were applied (**Figure 2**).[26,29]

Figure 1. Metabolic parameters in motor neuron diseases

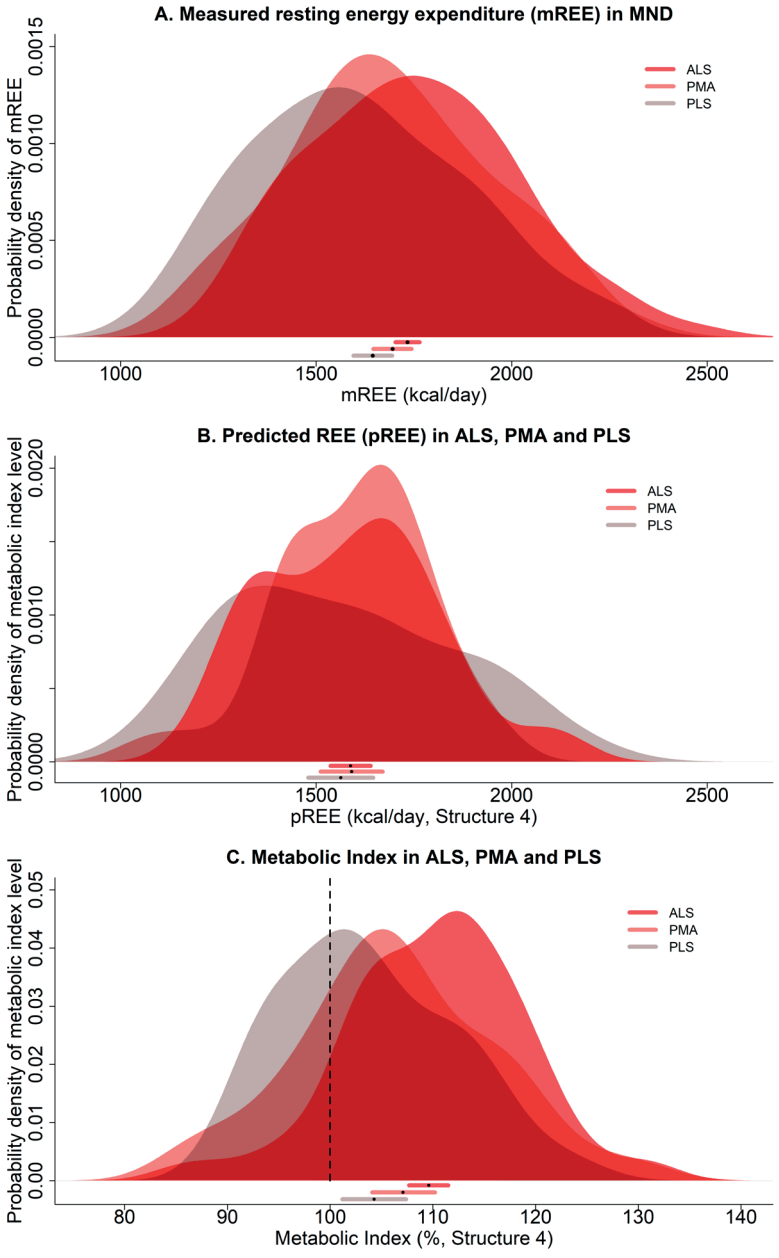


Figure 1. Figure shows probability densities of (A) having a certain measured resting energy expenditure (mREE), (B) the predicted REE according to the Structure 4 equation [26,27], or (C) having a certain metabolic index determined by the Structure 4 equation, in patients with ALS, PLS, and PMA. The horizontal bars below every panel provide the mean (black dots) with their 95% confidence intervals for each patient subgroup. Abbreviations: mREE = measured resting energy expenditure, pREE = predicted resting energy expenditure, ALS = amyotrophic lateral sclerosis, PMA = progressive muscular atrophy, PLS = primary lateral sclerosis.

Figure 2. Metabolic indices according to several prediction equations

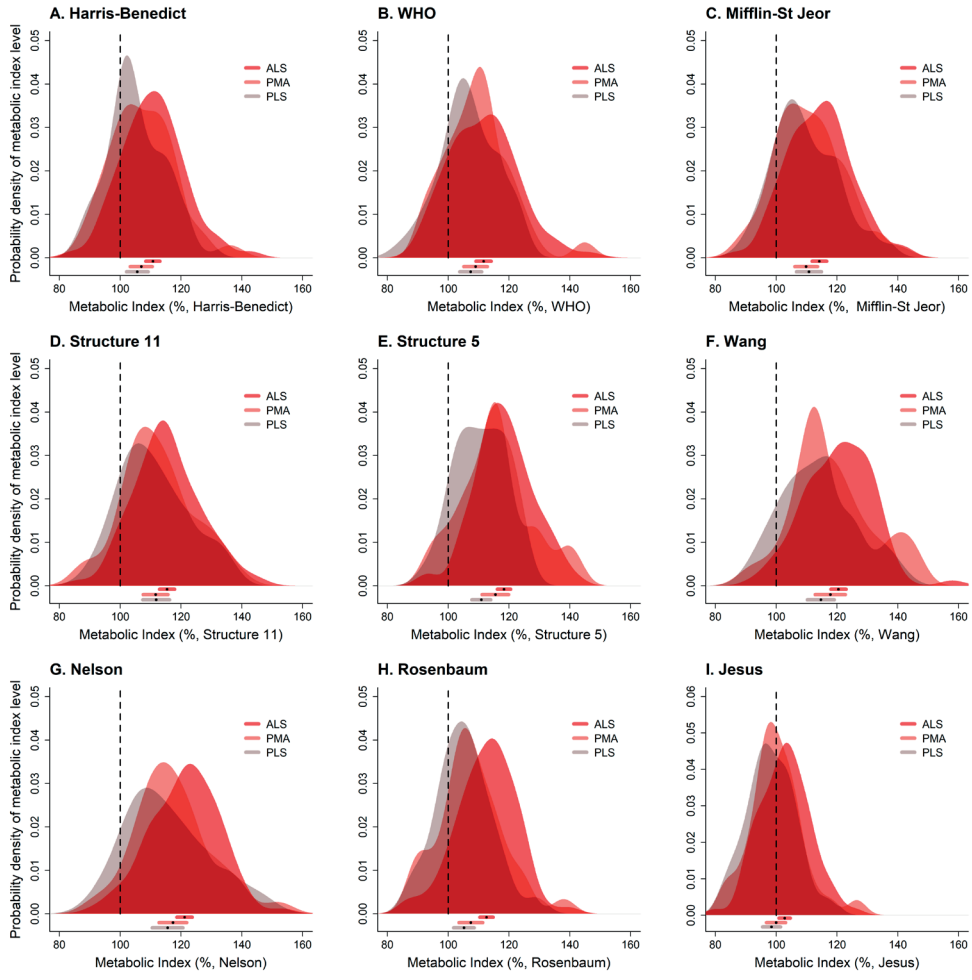


Figure 2. Figure shows probability densities of having a certain metabolic index according to nine different prediction equations[26,29] in patients with ALS, PLS, and PMA. The horizontal bars below every panel provide the mean (black dots) with their 95% confidence intervals for each patient subgroup. Abbreviations: ALS = amyotrophic lateral sclerosis, PMA = progressive muscular atrophy, PLS = primary lateral sclerosis.

Subsequently, mREE was found to differ between MND subtypes (p = 0.013), with a mean difference between ALS vs. PMA of 38.2 kcal/day (95% CI -20.5 – 97.0) and between ALS vs. PLS of 88.7 kcal/day (95% CI 85.0 – 92.4) (**Table 2**). Moreover, higher metabolic indices are seen in patients with ALS, compared to patients with PMA (mean difference of 2.5% (95% CI -1.2 – 6.2)) and PLS (mean difference of 5.3% (95% CI 1.6 – 9.0)), overall p = 0.017.

Table 2. Metabolic differences between MND subtypes

Metabolic parameter	Estimated mean	95% CI	p-value
Measured REE (fasted)			0.013*
ALS	1733.5 kcal/day	1703.0 – 1764.0	
PMA	1695.3 kcal/day	1646.5 – 1744.0	0.20**
PLS	1644.8 kcal/day	1595.3 – 1694.3	0.003**
Metabolic index, Structure 4 [26,27]			0.017*
ALS	109.6 %	107.7 – 111.5	
PMA	107.1 %	104.1 – 110.2	0.19**
PLS	104.3 %	101.2 – 107.4	0.0053**
Metabolic index, Harris-Benedict [25]			0.006*
ALS	110.7 %	108.7 – 112.7	
PMA	108.2 %	105.0 – 111.4	0.20**
PLS	104.4 %	101.1 – 107.6	0.0015**

Data represent the estimated mean in patients with ALS, PMA, and PLS with their 95% confidence interval, adjusted for: sex, age, fat-free mass, fat mass, height, and smoking status. Abbreviations: CI= confidence interval, ALS = amyotrophic lateral sclerosis, PMA = progressive muscular atrophy, PLS = primary lateral sclerosis, REE = resting energy expenditure in kcal/day. # P-values indicate if patients differ across all three disease categories. ** P-values indicate if patients with ALS differ statistically from the disease control.

Clinical features, body composition and metabolic parameters

Metabolic and anthropometric parameters (i.e. pREE, mREE, MI, FFM and FM) were not correlated significantly with %predicted FVC, total ALSFRS-R score or ALSFRS-R slope in patients with ALS, PMA or PLS (all r between -0.29 – 0.30). However, the total ALSFRS-R score and mREE tended to be correlated in patients with ALS and PMA (ALS: $r = 0.21$ (95% CI -0.02 – 0.41), $p = 0.068$, PMA: $r = 0.34$ (95% CI -0.03 – 0.62), $p = 0.070$). We found a strong correlation, in all disease categories, between weight and mREE (ALS: $r = 0.74$ (95% CI 0.62 – 0.83), $p < 0.001$, PMA: $r = 0.63$ (95% CI 0.36 – 0.80), $p < 0.001$, PLS: $r = 0.84$ (95% CI 0.68 – 0.92), $p < 0.001$), and between FFM and mREE (ALS: $r = 0.85$ (95% CI 0.77 – 0.90), $p < 0.001$, PMA: $r = 0.73$ (95% CI 0.52 – 0.86), $p < 0.001$, PLS: $r = 0.90$ (95% CI 0.80 – 0.95), $p < 0.001$). We did not find significant correlations between FM and mREE in any disease subtype (all r between 0.08 and 0.27).

When considering patients diagnosed with ALS only, we found no differences in FFM or FM in those with either bulbar or spinal symptom onset (all $p > 0.50$). Furthermore, the MI was not significantly different between the severity of UMN or LMN involvement, across diagnostic categories, as defined by the EEC, nor relative to disease duration (**Figure 3**).

Figure 3. Upper and lower motor neuron scores related to the metabolic index

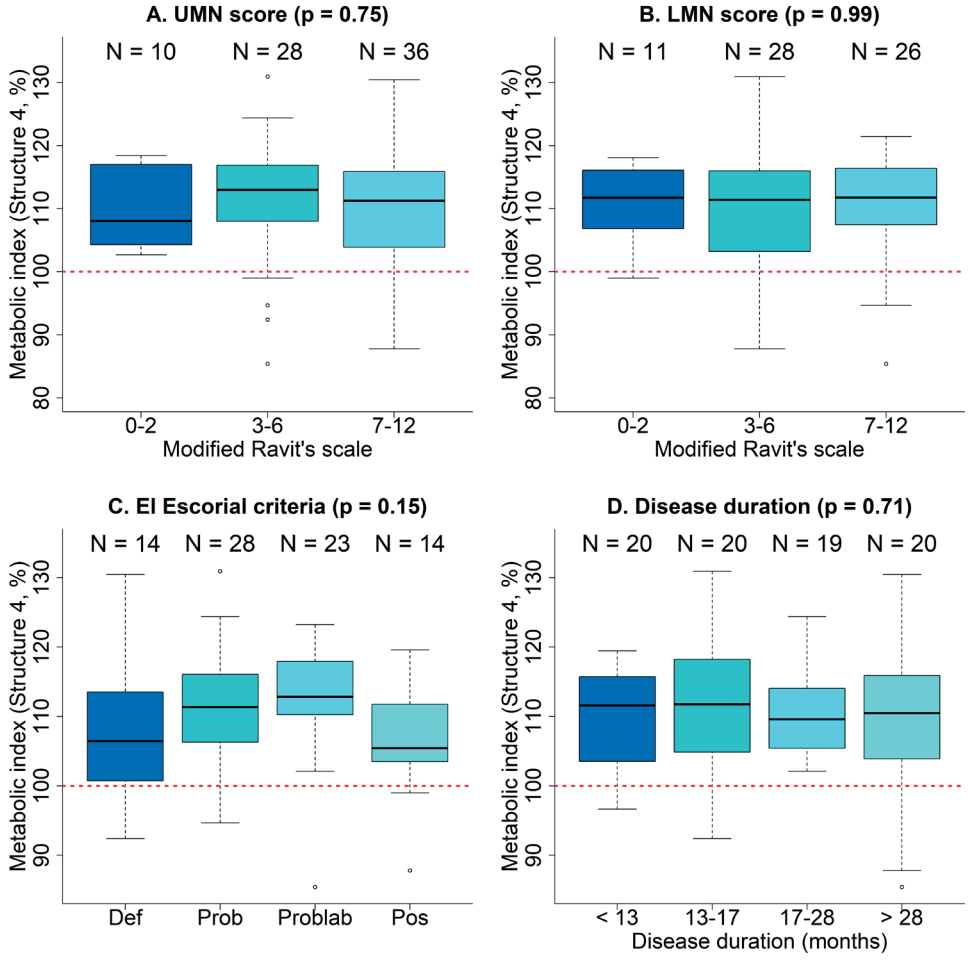


Figure 3. Boxplots show disease characteristics of patients with ALS relative to the metabolic index, according to the Structure 4 equation (Men: 361 + ((21.1 * fat-free mass in kg) + (4.77 * fat mass in kg)); Women: 360 + ((21.0 * fat-free mass in kg) + (4.68 * fat mass in kg)))[26,27]. Panels (A) and (B) illustrate the metabolic index relative to the upper (UMN) and lower motor neuron (LMN) sum score, respectively, according to the modified Ravits scale. Panel C shows the metabolic index across four subgroups according to the revised El Escorial criteria. Finally, panel D shows the metabolic index relative to disease duration in months in patients with ALS. Abbreviations: UMN = upper motor neuron, LMN = lower motor neuron, ALS = amyotrophic lateral sclerosis, Def = definite ALS, Prob = probable ALS, Problab = probable laboratory supported ALS, Pos = possible ALS.

DISCUSSION

In this prospective study, we show that an elevated metabolic state is not solely a phenomenon that occurs in patients with ALS, but, to a lesser extent, also in patients with PMA and PLS. We found that patients with PLS had a lower measured resting energy expenditure and metabolic index compared to patients with ALS, with patients with PMA falling between the prevalence of those with PLS and ALS. Significantly, the MI was not evidently dependent on an MND-specific clinical characteristic, nor was this related to the extent of lower or upper motor neuron involvement when considering ALS alone. These results are important, because they may suggest that increases in metabolic rate might occur due to a specific (shared) pathophysiological process, or that the predictive formulae used to determine the metabolic state might not be suitable in patients with MND due to the loss of FFM. The latter suggests that an altered metabolic state is secondary to the disease subtype.

In line with previous studies,[1,2,4,5] we observed a prevalence of hypermetabolism – defined as a metabolic state of ≥ 110%[27] – in 55% of the patients with ALS. We have not found a previous study that investigated the metabolic state in PMA and PLS. We show that patients with PLS differ significantly in metabolic state compared to patients with ALS. This result seems to support the approach of handling ALS, PMA, and PLS as different diseases,[30,31] despite the shared pathological features.[32] When considering patients with ALS only, we found no effect of LMN- or UMN-burden on metabolic rate. This result is in contrast with earlier observations.[1] Differences in study outcomes may be due to the use of different prediction equations to determine pREE, or the inclusion of patients with other forms of MND, instead of purely ALS.

The increased metabolic rate seems to develop due to an imbalance in energy homeostasis.[33] On a cellular level, energy homeostasis might be disturbed in motor neurons due to the presence of astrocytes and/or glutamate excitotoxicity,[34] and/or due to dysfunctions in glucose metabolism in the central nervous system.[35,36] Moreover, TDP-43 aggregation in motor neurons – which is present in approximately 95% of the patients with ALS,[37] is widely seen in patients with PMA[38] and also seems to affect UMN in PLS[39] – has been shown to alter energy homeostasis.[40] Besides, oxidative stress due to mutated mitochondrial DNA and/or altered mitochondrial morphology, might affect metabolic pathways; this has mainly been explored in motor neurons of patients with ALS.[35] Hence, multiple hypotheses are proposed to contribute to metabolic imbalance, and all factors might play a role to varying degrees in individual patients due to the heterogeneity of the disease subtypes.[12]

Independent of a possible shared pathophysiological background, FFM seems to be the main factor for predicting REE in patients with ALS,[13] and could explain 53% to 88% of the variance in predicted REE.[26,41] Indeed, we did find a strong correlation between FFM and pREE, but

also in mREE, in all three MND subtypes. Additionally, the mREE tended to be correlated with ALSFRS-R score in patients with ALS and PMA, which might be due to the sensitivity of this questionnaire in measuring LMN signs, and by proxy, loss of FFM.[16] Collectively, these results might suggest that an altered metabolic state could be due to FFM loss. However, we did not find significant differences in our multivariable model – in which we corrected for FFM – nor between patients with different UMN or LMN total scores. Next, PLS is an UMN predominant progressive disease, and, while the prevalence of an increased metabolic rate in patients with PLS were relatively lower when compared to ALS and PMA, increases in MI in patients with PLS suggest that hypermetabolism could occur independent of factors specific to neurogenic wasting of FFM.[42] Therefore, the MI does not seem to be influenced by FFM (alone).[15,43] In short, the pathophysiological drivers of altered metabolic rate is unclear, yet the increased metabolic rates (i.e. hypermetabolism) seems to be underpinned by a trait that is independent of other clinical variables. Therefore, the altered metabolic state should be further investigated, as a patient's metabolic status might help to differentiate between the three MND subtypes. This could inform better counselling of patients and their relatives regarding disease course and survival. Moreover, it might affect individual tailored care, as patients with elevated metabolic indices might be more urgently referred to a dietitian for intervention.

The main strength of this prospective study is that we included a significant number of patients with ALS, PMA, and PLS, and, therefore, collected a large amount of data on body composition and metabolic parameters across these disease subtypes. Moreover, direct comparisons are enabled through the adoption of standardized procedures; all assessments were performed in a standardized manner by a trained, consistent study team, which prevented observer bias. A limitation of this approach, however, is the requirement that all study participants were required to undergo all assessments, including measurement of height, body weight, and body composition through air displacement plethysmography. This might have resulted in selection bias, as patients with severe disability (e.g. patients to stand, walk, or step into the BodPod system) could not participate. Moreover, we did not contrast measures against a population of non-MND controls, and as such we cannot determine whether increases in MI or the prevalence of hypermetabolism in patients with ALS, PMA or PLS differ compared to the general population in the Netherlands. Future studies should incorporate this, as the metabolic rate seems to be influenced by demographic-specific factors.[24] In addition, due to the prevalence of these rare diseases, results may have been affected due to the lack of power. Despite this, we were able to include a significant number of patients with ALS, PMA, and PLS.

In conclusion, elevated metabolic indices are most commonly seen in patients with ALS; however, they are also seen in patients with PMA and PLS, albeit to a lesser extent. This finding is independent of important covariables that might affect the MI and REE. Our findings

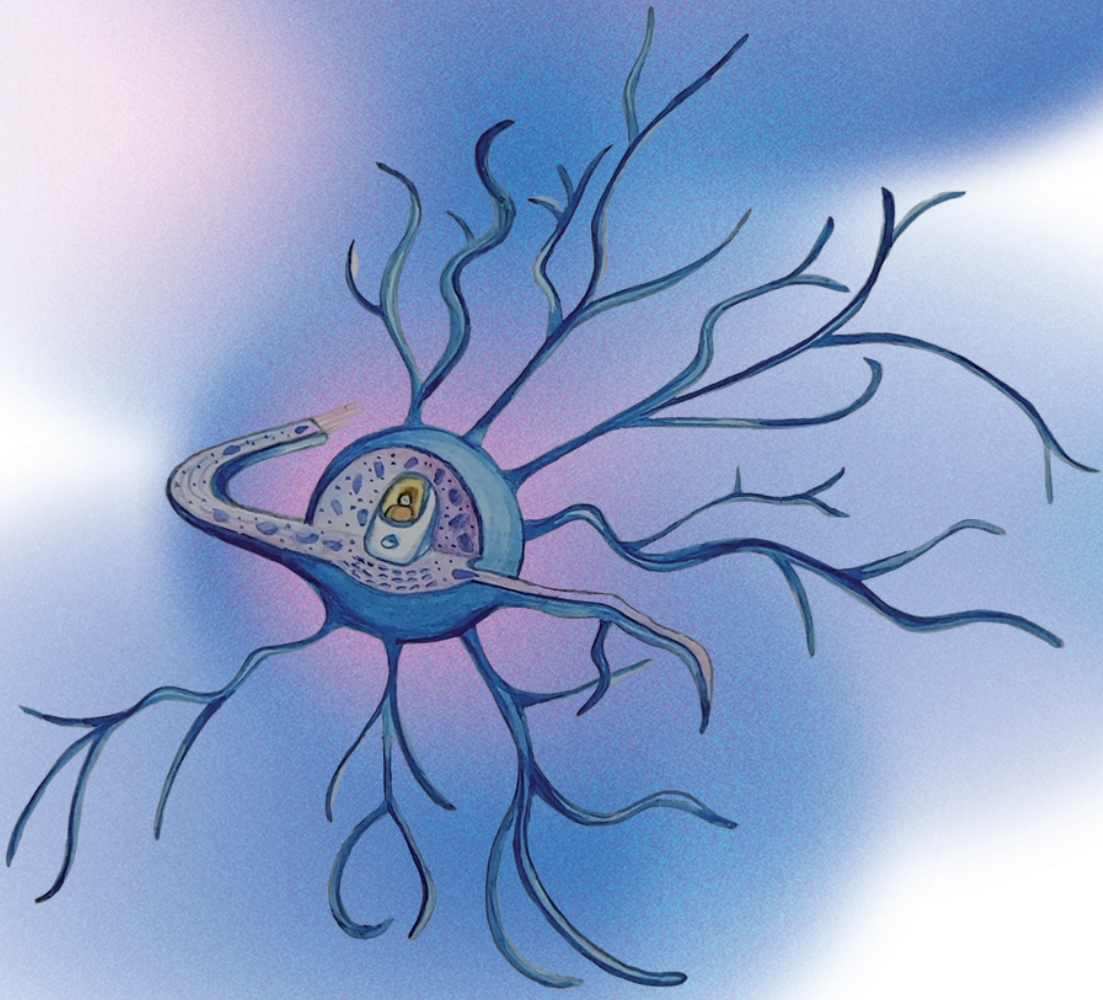
provide insights into factors that could contribute to altered metabolic state across the spectrum of MND, and suggest that factors outside of conventional clinical characteristics, and possibly shared pathophysiological background, contributes to altered metabolism in ALS, PMA, and PLS.

REFERENCES

1. Steyn FJ, Ioannides ZA, Van Eijk RPA, et al. Hypermetabolism in ALS is associated with greater functional decline and shorter survival. J Neurol Neurosurg Psychiatry 2018;89(10):1016–1023.
2. Ludolph A, Dupuis L, Kasarskis E, Steyn F, Ngo S, McDermott C. Nutritional and metabolic factors in amyotrophic lateral sclerosis. Nat Rev Neurol 2023;(Online ahead of print)
3. Jésus P, Fayemendy P, Nicol M, et al. Hypermetabolism is a deleterious prognostic factor in patients with amyotrophic lateral sclerosis. Eur J Neurol 2018;25(1):97–104.
4. He J, Fu J, Zhao W, et al. Hypermetabolism associated with worse prognosis of amyotrophic lateral sclerosis. J Neurol 2022;269(3):1447–1455.
5. Fayemendy P, Marin B, Labrunie A, et al. Hypermetabolism is a reality in amyotrophic lateral sclerosis compared to healthy subjects. J Neurol Sci 2021;420:1–5.
6. Jésus P, Fayemendy P, Nicol M, et al. Hypermetabolism is a deleterious prognostic factor in patients with amyotrophic lateral sclerosis. Eur J Neurol 2018;25(1):97–104.
7. Desport J, Lacoste M, Preux P, Couratier P. Hypermetabolism in ALS : Correlations with Clinical and Paraclinical Parameters. Neurodegener Dis 2005;2(3–4):202–207.
8. Vaisman N, Lusaus M, Nefussy B, et al. Do patients with amyotrophic lateral sclerosis (ALS) have increased energy needs? J Neurol Sci 2009;279(1–2):26–29.
9. Janse Van Mantgem MR, Van Eijk RPA, Van Der Burgh HK, et al. Prognostic value of weight loss in patients with amyotrophic lateral sclerosis: A population-based study. J Neurol Neurosurg Psychiatry 2020;91(8):867–875.
10. Funalot B, Desport J, Sturtz F, et al. High metabolic level in patients with familial amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis 2009;10:113–117.
11. Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis 2000;1(5):293–299.
12. Masrori P, Van Damme P. Amyotrophic lateral sclerosis: a clinical review. Eur J Neurol 2020;27(10):1918–1929.
13. Ioannides ZA, Steyn FJ, Mi JD, Henderson RD, McCombe PA, Ngo ST. Predictions of resting energy expenditure in amyotrophic lateral sclerosis are greatly impacted by reductions in fat free mass. Cogent Med 2017;4:1–10.
14. Brooks B, Miller R, Munsat T, Diseases WF of NRG on MN. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000;1(5):293–299.
15. Turner MR, Barohn RJ, Corcia P, et al. Primary lateral sclerosis: Consensus diagnostic criteria. J Neurol Neurosurg Psychiatry 2020;91(4):373–377.
16. Cedarbaum JM, Stambler N, Fuller C, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. J Neurol Sci 1999;169:13–21.
17. Kimura F, Fujimura C, Ishida S, et al. Progression rate of ALSFRS-R at time of diagnosis predicts survival time in ALS. Neurology 2006;66(2):265–267.
18. Roche JC, Rojas-Garcia R, Scott KM, et al. A proposed staging system for amyotrophic lateral sclerosis. Brain 2012;135(3):847–852.
19. Balendra R, Jones A, Jivraj N, et al. Estimating clinical stage of amyotrophic lateral sclerosis from the ALS Functional Rating Scale. Amyotroph Lateral Scler Frontotemporal Degener 2014;15(3–4):279–284.
20. Network to Cure ALS (ENCALS) E. King's ALS Staging SOP. 2014;Accessed 05-01-2023.

21. Ravits J, Paul P, Jorg C. Focality of upper and lower motor neuron degeneration at the clinical onset of ALS. Neurology 2007;68:1571–1575.
22. Devine MS, Ballard E, Orourke P, Kiernan MC, McCombe PA, Henderson RD. Targeted assessment of lower motor neuron burden is associated with survival in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2016;17(3–4):184–190.
23. COSMED - The Metabolic Company. Estimation of Resting Metabolic Rate based on Lean Body Mass with Bod Pod. 2012;(June):1.
24. Holdom CJ, Janse van Mantgem MR, He J, et al. Defining hypermetabolism in amyotrophic lateral sclerosis (ALS): towards a standardized approach for identifying metabolic change. Unpublished 2023;
25. Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. Proceedings of the National Academy of Sciences 1918;4(12):370–373.
26. Flack KD, Siders WA, Johnson LA, Roemmich JN. Cross-Validation of Resting Metabolic Rate Prediction Equations. J Acad Nutr Diet 2016;116(9):1413–1422.
27. Sabounchi NS, Rahmandad H, Ammerman A. Best-fitting prediction equations for basal metabolic rate: Informing obesity interventions in diverse populations. Int J Obes 2013;37(10):1364–1370.
28. Slingerland-Boot H, Adhikari S, Mensink MR, van Zanten ARH. Comparison of the Beacon and Quark indirect calorimetry devices to measure resting energy expenditure in ventilated ICU patients. Clin Nutr ESPEN 2022;48:370–377.
29. Kasarskis EJ, Mendiondo MS, Matthews DE, et al. Estimating daily energy expenditure in individuals with amyotrophic lateral sclerosis. American Journal of Clinical Nutrition 2014;99(4):792–803.
30. De Vries BS, Rustemeijer LMM, Bakker LA, et al. Cognitive and behavioural changes in PLS and PMA: challenging the concept of restricted phenotypes. J Neurol Neurosurg Psychiatry 2019;90(2):141–147.
31. McCluskey G, Morrison KE, Donaghy C, et al. Serum Neurofilaments in Motor Neuron Disease and Their Utility in Differentiating ALS, PMA and PLS. Life 2023;13(6):1301.
32. Saberi S, Stauffer JE, Schulte DJ, Ravits J. Neuropathology of Amyotrophic Lateral Sclerosis and Its Variants. Neurol Clin 2015;33(4):855–876.
33. Dupuis L, Pradat PF, Ludolph AC, Loeffler JP. Energy metabolism in amyotrophic lateral sclerosis. Lancet Neurol 2011;10(1):75–82.
34. Madji Hounoum B, Blasco H, Coque E, et al. The Metabolic Disturbances of Motoneurons Exposed to Glutamate. Mol Neurobiol 2018;55(10):7669–7676.
35. Barone C, Qi X. Altered Metabolism in Motor Neuron Diseases: Mechanism and Potential Therapeutic Target. Cells 2023;12(11):1536.
36. Tefera TW, Steyn FJ, Ngo ST, Borges K. CNS glucose metabolism in Amyotrophic Lateral Sclerosis: a therapeutic target? Cell Biosci 2021;11(1):1–17.
37. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science (1979) 2006;314(5796):130–133.
38. Geser F, Stein B, Partain M, et al. Motor neuron disease clinically limited to the lower motor neuron is a diffuse TDP-43 proteinopathy. Acta Neuropathol 2011;121(4):509–517.
39. Mackenzie IRA. Neuropathology of primary lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2020;21(S1):47–51.
40. Chiang PM, Ling J, Jeong YH, Price DL, Aja SM, Wong PC. Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism. Proc Natl Acad Sci U S A 2010;107(37):16320–16324.

41. Nelson K, Weinsier R, Long C, Schutz Y. Prediction of resting energy expenditure from fat-free mass and fat mass. American Journal of Clinical Nutrition 1992;5(56):848–856.
42. Shefner JM, Musaro A, Ngo ST, et al. Skeletal muscle in amyotrophic lateral sclerosis. Brain 2023;(Online ahead of print)
43. Sorarù G, D'Ascenzo C, Nicolao P, et al. Muscle histopathology in upper motor neuron-dominant amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis 2008;9(5):287–293.



Chapter 8

General Discussion and Future Perspectives

GENERAL DISCUSSION

This thesis provides an overview of the determination, assessment, and prognostic value of anthropometric measurements, metabolic biomarkers, and the metabolic state in patients with ALS. Due to the increase in knowledge about metabolic disturbances, the metabolic hypothesis[1] – which suggests metabolic disturbances contribute to the disease process – is of interest in these patients. The findings in this thesis support the view that metabolic alterations occur in patients with ALS and, moreover, provide novel insights related to the metabolic hypothesis. In this chapter, we discuss the main findings and conclusions of the studies performed and offer suggestions for the remaining challenges.

Metabolic biomarkers

Metabolic disturbances in patients with ALS have been found in the pre-symptomatic[2] and symptomatic stages.[3] This has led to an increase in the number of studies performed at different timepoints (e.g. during life-course, date of onset, date of diagnosis, etcetera). Therefore, drawing conclusions remains challenging. In **chapter 2** and **chapter 3** we discuss the variation in methodology in previous research when assessing weight loss and serum cholesterol, respectively. With regard to the amount of weight loss: In **chapter 2**, in a large cohort of patients with ALS, we show that the amount of weight loss between date of disease onset and date of diagnosis (e.g. the symptomatic stage) is prognostically unfavorable for survival in patients with ALS, even when corrected for important confounders in ALS.[4] Moreover, we show that weight loss also occurs in patients without bulbar symptoms or dysphagia. This supports the metabolic hypothesis in ALS.[1,5–7] Weight loss has been shown to be predictive for disease severity in the preclinical stage.[8] Hence, weight loss seems to be an important anthropometric measurement. Future studies should focus on the cause of weight loss, which might even be multifactorial, as discussed in **chapter 2**. As we show in **chapter 4**, serum creatinine is related to the loss of fat-free mass (FFM) and, subsequently, correlated with changes in the ALSFRS-R. This might suggest that the loss of FFM is the main component of total weight loss, and, therefore, should be a biomarker of interest throughout the disease course. Furthermore, in **chapter 6** we show that differences in metabolic index are due to the differences in assessing FFM, which seems to support the role of FFM as the main component of weight loss; hence, FFM is an important biomarker. There is also an increasing interest in the effect of nutritional treatment on the disease course and survival in patients with ALS, though findings are inconclusive.[9–11] Our results may offer an explanation: it would seem that it is more important to prevent the loss of FFM rather than maintain total body weight. As well as focusing on the timing of nutritional treatment, future studies should also look at its composition (for instance: high-caloric vs. protein-rich), in relation to the disease course and, moreover, survival. Equally important are longitudinal studies on changes in FFM and FM, related to the effect of nutritional management. Previous studies focused on maintaining a stable total weight rather than a stable FFM. This may be one of the

factors contributing to the various results regarding the effect of PEG placements on survival in patients with ALS.[12] In addition, longitudinal studies on body composition and the effect of nutritional treatment in pre-symptomatic *C9orf72* carriers might be of value, as there is limited evidence that genetic predisposition in pre-symptomatic individuals plays a role in body composition.[8]

These future perspectives might be combined with longitudinal follow-up of serum cholesterol variables. As shown in **chapter 3**, in a large group of patients with ALS, high HDL-cholesterol (HDL-C) levels are prognostically unfavorable for survival in patients with ALS. However, as we hypothesize, there seems to be a relationship between serum HDL-C levels and weight loss and/or BMI. It is known that there is a high correlation between HDL-C and BMI: higher BMI levels correspond to lower HDL-C levels.[13] Our study introduces the idea of altered cholesterol levels as a consequence of disease rather than a causal relationship. On the other hand, ALS models have provided evidence of defective cholesterol metabolism in motor neuron cells.[14–16] Moreover, changes in lipid levels have been found in the cerebrospinal fluid (CSF) of patients with ALS.[17] Whether these CSF alterations correspond with serum cholesterol levels should be further investigated. Moreover, the effect of BMI and weight loss on serum cholesterol levels should be further explored in longitudinal studies in these patients.

Whereas it might be challenging to determine body weight of severely affected patients, we have shown in **chapter 4** that creatinine seems to be an adequate marker to monitor the change in fat-free mass (FFM). FFM together with fat-mass (FM) results in the total body weight. Changes in creatinine might, therefore, be indicative of disease progression.[18] Determining creatinine is a non-invasive, inexpensive, and low burden method for assessing changes in FFM. However, whether the creatinine level indicates a patient's need for (dietary) intervention or reflects a potential therapeutic effect, should be further examined.

Assessing the metabolic state

Besides the alterations in anthropometric measurements, there has been an increase in evidence and knowledge regarding the metabolic state in patients with ALS.[3,19–25] The phenomenon of 'hypermetabolism' has been found consistently in a subgroup of patients – not in all patients - and has been shown to result in a poorer prognosis.[3,19–25] So far, the etiology of 'hypermetabolism' is rather hypothetical. However, a variety of metabolic disturbances has been found in the central nervous system,[7] and also in the skeletal muscles,[26] which might result in a systemic 'hypermetabolic' state. The metabolic state is determined by the metabolic index (MI): the measured resting energy expenditure (mREE) relative to the predicted REE (pREE). The pREE can be determined by several predication equations. In **chapter 5** we discuss the variety of prediction equations,[27] none of which has been validated in patients with ALS. We conclude that demographic-specific factors might have an effect on the pREE

in the general, normal population. Therefore, it is worthwhile validating specific equation(s) in this general population (e.g. non-neurological controls) to predict REE in future research, before applying this in patients with ALS. As shown in **chapter 5**, the Sabounchi Structure 4 equation seems to fit best for the study population, however, is least appropriate in the Dutch population. This suggests a country-specific equation might be necessary, as is similarly seen in the ENCALS survival model.[4] We hypothesize that differences in the metabolic index are a consequence of the disease, rather than a cause, due to the use of FM and FFM as parameters in the prediction equations, which should be assessed in future studies. There are different measurement methods for determining FM and FFM. In **chapter 6** we show that the bioelectrical impedance analysis (BIA) overestimates FFM by 2.5 kg compared to air-displacement plethysmography (ADP) using the BodPod system (the gold standard) in patients with ALS. This does not seem to affect current nutritional therapeutic strategies. However, we found an underestimation of hypermetabolic patients when using BIA. This is important when studies are focused on the MI in patients with ALS, and, moreover, when clinical trials use in- and exclusion criteria related to the MI. This might lead to patients being excluded from future clinical trials, even though they might respond to future drugs to cure ALS. Therefore, future studies should be consistent and persistent in their methods when determining FFM and FM, and, in addition, use an appropriate equation for predicting REE in their population.

As discussed in **chapter 6,** the Sabounchi Structure 4 equation seems to fit best in patients with ALS., We, therefore, applied this formula in our patients with ALS and performed a comparison with patients with progressive muscular atrophy (PMA) and primary laterals sclerosis (PLS). While ALS, PMA, and PLS share pathological features,[28] an increasing amount of evidence suggests these three motor neuron disease (MND) subtypes should be treated as different diseases.[29,30] Until the report of our findings, as described in **chapter 7**, potential metabolic disturbances had not been studied in MND-subtypes other than ALS. In **chapter 7**, we show that hypermetabolism is a commonly found phenomenon in ALS, but not solely in ALS. Moreover, our findings hold when the pREE is determined using the Harris-Benedict equation, the prediction equation applied most commonly in previous studies to determine REE. This might imply the following:

a) Determining the pREE, and subsequently the MI, might help to distinguish between MND-subtypes (i.e. ALS, PMA, and PLS). As a result, it might mean that patients and their relatives can be given more accurate information about disease course and prognosis. However, as already described, future studies should be consistent in determining FFM, FFM, and, moreover, be aware of the method used (i.e. ADP or BIA). Further studies need to assess, and, in addition, confirm, if pREE might be a useful marker for clinical practice.
b) 'Hypermetabolism' might be a phenomenon of the shared pathophysiological background in the MND-subtypes; we have shown that 'hypermetabolism' occurs in all MND-subtypes. In ALS, it has proved to be consistent throughout the disease course.[3,20]

On the other hand, there is limited evidence that the MI might change as the disease progresses.[22] Therefore, longitudinal studies assessing the pREE and MI in MND-subtypes are necessary to determine the consistency in parallel with disease characteristics.

In brief, our results are valuable in the process of unraveling the etiology of the metabolic differences.

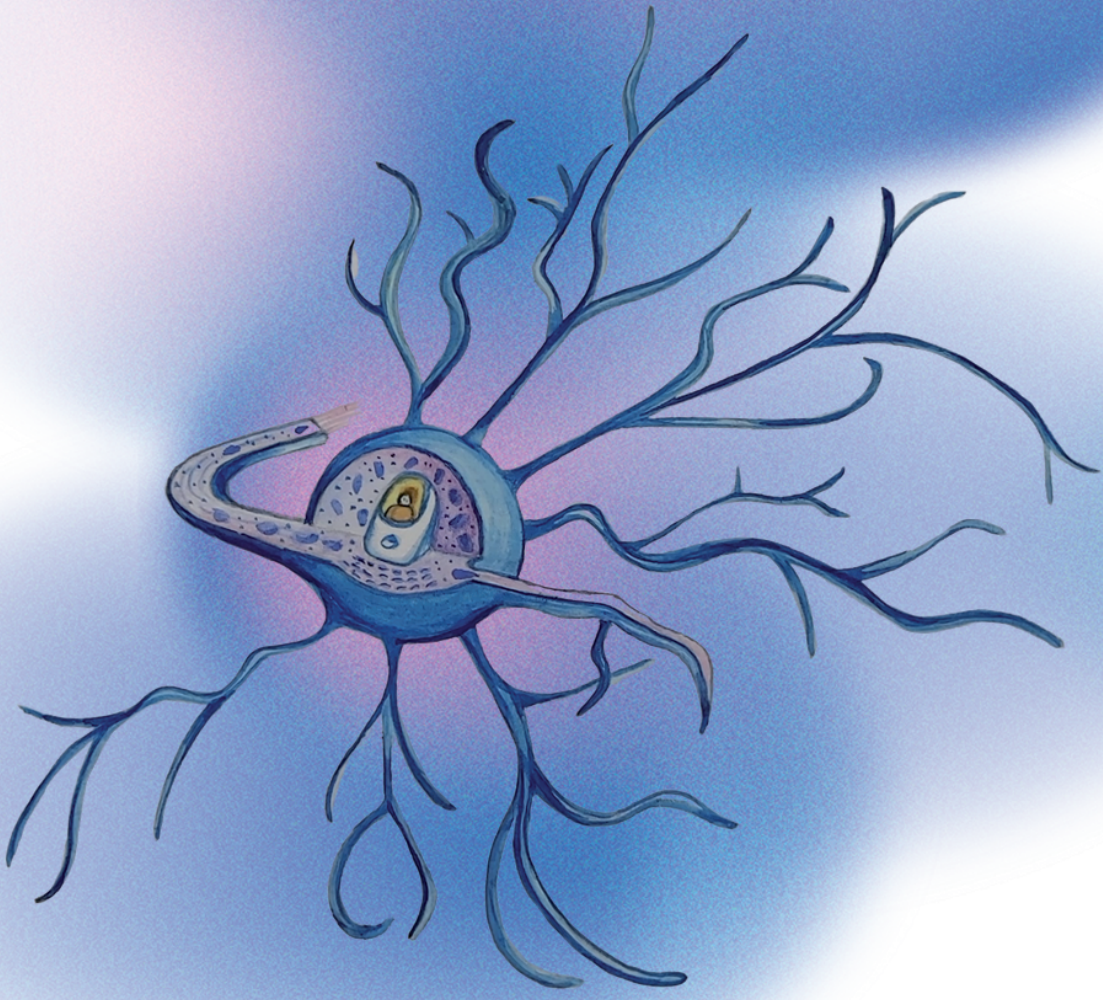
General conclusion

This thesis provides an insight into metabolic disturbances in patients with ALS (most of our cases), but also in patients with PMA and PLS. We show that body weight – most importantly fat-free mass – and serum creatinine might be useful clinical biomarkers for assessing disease progression, and, moreover, might predict prognosis more accurately than contemporary methods. A patient's body composition should, therefore, be assessed in order to determine the proportion of FFM and FM, and assessments should continue throughout the disease course. In addition, we have shown that serum cholesterol values seem to reflect changes in body composition. It would seem, therefore, not worthwhile to use this as a biomarker to predict survival. Bio-electrical impedance analysis would be an easy and low-cost method for assessing a patient's body composition in future studies. However, it is recommended that future studies use either BIA or ADP to assess the effect of nutritional management on disease course in patients with ALS, BIA being easier to apply. Researchers should be aware of the overestimation of FFM by BIA. The metabolic index can be used to determine a patient's metabolic state (i.e. hypo-, normo-, hypermetabolic). This might provide valuable information, which might even be helpful in distinguishing between MND-subtypes (i.e. ALS, PMA, or PLS). As we have shown, researchers should be aware of the limitations in determining the metabolic index and should be consistent in the operational procedures applied to determine FFM and assess the pREE. Adequate nutritional management might, therefore, prove to be a valuable therapeutic strategy in patients with ALS.

REFERENCES

1. Ludolph A, Dupuis L, Kasarskis E, Steyn F, Ngo S, McDermott C. Nutritional and metabolic factors in amyotrophic lateral sclerosis. Nat Rev Neurol 2023;(Online ahead of print)
2. Peter RS, Rosenbohm A, Dupuis L, et al. Life course body mass index and risk and prognosis of amyotrophic lateral sclerosis: results from the ALS registry Swabia. Eur J Epidemiol 2017;32(10):901–908.
3. Bouteloup C, Desport JC, Clavelou P, et al. Hypermetabolism in ALS patients : an early and persistent phenomenon. J Neurol 2009;256:1236–1242.
4. Westeneng HJ, Debray TPA, Visser AE, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. Lancet Neurol 2018;17(5):423–433.
5. De Aguilar JLG, Dupuis L, Oudart H, Loeffler JP. The metabolic hypothesis in amyotrophic lateral sclerosis: Insights from mutant Cu/Zn-superoxide dismutase mice. Biomedicine and Pharmacotherapy 2005;59(4):190–196.
6. Dupuis L, Pradat PF, Ludolph AC, Loeffler JP. Energy metabolism in amyotrophic lateral sclerosis. Lancet Neurol 2011;10(1):75–82.
7. Barone C, Qi X. Altered Metabolism in Motor Neuron Diseases: Mechanism and Potential Therapeutic Target. Cells 2023;12(11):1536.
8. Westeneng HJ, van Veenhuijzen K, van der Spek RA, et al. Associations between lifestyle and amyotrophic lateral sclerosis stratified by C9orf72 genotype: a longitudinal, population-based, case-control study. Lancet Neurol 2021;20(5):373–384.
9. Ludolph AC, Dorst J, Dreyhaupt J, et al. Effect of High-Caloric Nutrition on Survival in Amyotrophic Lateral Sclerosis. Ann Neurol 2020;87(2):206–216.
10. Dorst J, Cypionka J, Ludolph AC. High-caloric food supplements in the treatment of amyotrophic lateral sclerosis: A prospective interventional study. Amyotroph Lateral Scler Frontotemporal Degener 2013;14(7–8):533–536.
11. Altinkaynak M, Gok GK, Ozmen B, et al. Prognostic Value of Regular Nutritional Treatment in Patients With Amyotrophic Lateral Sclerosis. Neurologist 2023;28(3):166–172.
12. Castanheira A, Swash M, De Carvalho M. Percutaneous gastrostomy in amyotrophic lateral sclerosis: a review. Amyotroph Lateral Scler Frontotemporal Degener 2022;23(3–4):176–189.
13. Shamai L, Lurix E, Shen M, et al. Association of body mass index and lipid profiles: Evaluation of a broad spectrum of body mass index patients including the morbidly obese. Obes Surg 2011;21(1):42–47.
14. Abdel-Khalik J, Yutuc E, Crick PJ, et al. Defective cholesterol metabolism in amyotrophic lateral sclerosis. J Lipid Res 2017;58(1):267–278.
15. Cutler RG, Pedersen WA, Camandola S, Rothstein JD. Evidence that accumulation of ceramides and cholesterol esters mediates oxidative stress-induced death of motor neurons in amyotrophic lateral sclerosis. Ann Neurol 2002;52(4):448–457.
16. Henriques A, Croixmarie V, Bouscary A, et al. Sphingolipid metabolism is dysregulated at transcriptomic and metabolic levels in the spinal cord of an animal model of amyotrophic lateral sclerosis. Front Mol Neurosci 2018;10:433.
17. Blasco H, Veyrat-Durebex C, Bocca C, et al. Lipidomics Reveals Cerebrospinal-Fluid Signatures of ALS. Sci Rep 2017;7(1):17652.

18. Van Eijk RPA, Eijkemans MJC, Ferguson TA, Nikolakopoulos S, Veldink JH, Van Den Berg LH. Monitoring disease progression with plasma creatinine in amyotrophic lateral sclerosis clinical trials. J Neurol Neurosurg Psychiatry 2018;89(2):156–161.
19. Steyn FJ, Ioannides ZA, Van Eijk RPA, et al. Hypermetabolism in ALS is associated with greater functional decline and shorter survival. J Neurol Neurosurg Psychiatry 2018;89(10):1016–1023.
20. Desport J, Lacoste M, Preux P, Couratier P. Hypermetabolism in ALS : Correlations with Clinical and Paraclinical Parameters. Neurodegener Dis 2005;2(3–4):202–207.
21. Desport JC, Preux PM, Magy L, et al. Factors correlated with hypermetabolism in patients with amyotrophic lateral sclerosis. American Journal of Clinical Nutrition 2001;74(3):328–334.
22. He J, Fu J, Zhao W, et al. Hypermetabolism associated with worse prognosis of amyotrophic lateral sclerosis. J Neurol 2022;269(3):1447–1455.
23. Jésus P, Fayemendy P, Nicol M, et al. Hypermetabolism is a deleterious prognostic factor in patients with amyotrophic lateral sclerosis. Eur J Neurol 2018;25(1):97–104.
24. Weijs PJM. Hypermetabolism, is it real? The example of amyotrophic lateral sclerosis. J Am Diet Assoc 2011;111(11):1670–1673.
25. Fayemendy P, Marin B, Labrunie A, et al. Hypermetabolism is a reality in amyotrophic lateral sclerosis compared to healthy subjects. J Neurol Sci 2021;420:1–5.
26. Shefner JM, Musaro A, Ngo ST, et al. Skeletal muscle in amyotrophic lateral sclerosis. Brain 2023;(Online ahead of print)
27. Flack KD, Siders WA, Johnson LA, Roemmich JN. Cross-Validation of Resting Metabolic Rate Prediction Equations. J Acad Nutr Diet 2016;116(9):1413–1422.
28. Saberi S, Stauffer JE, Schulte DJ, Ravits J. Neuropathology of Amyotrophic Lateral Sclerosis and Its Variants. Neurol Clin 2015;33(4):855–876.
29. De Vries BS, Rustemeijer LMM, Bakker LA, et al. Cognitive and behavioural changes in PLS and PMA: challenging the concept of restricted phenotypes. J Neurol Neurosurg Psychiatry 2019;90(2):141–147.
30. McCluskey G, Morrison KE, Donaghy C, et al. Serum Neurofilaments in Motor Neuron Disease and Their Utility in Differentiating ALS, PMA and PLS. Life 2023;13(6):1301.



Appendices

Summary
Summary in Dutch (Nederlandse samenvatting)
Acknowledgements (Dankwoord)
List of publications
Curriculum Vitae of the author

ENGLISH SUMMARY

AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS) is a neurological condition which affects motor neurons are. These are present in the central and the peripheral nervous systems. In ALS, both systems are affected. When only the peripheral nervous system is affected, we refer to the condition, progressive spinal muscular atrophy (PMA). When only the central nervous system is affected, we refer to the disease, primary lateral sclerosis (PLS). The three conditions together are called the motor neuron diseases (MND), ALS being the most common. As motor neurons control the muscles, their degeneration results in weakness in bulbar and/or spinal muscles. Eventually, the respiratory muscles become affected, causing patients to develop progressive respiratory symptoms which, in turn, result in death. The initial symptoms vary; some patients develop swallowing and/or speech difficulties (bulbar symptoms), while others develop weaknesses in arms and/or legs or respiratory symptoms (spinal symptoms). The diversity of symptoms can be measured using the ALS Functional Rating Scale-Revised (ALSFRS-R) questionnaire: a lower score reflecting a more progressive disease. The disease can occur at any age, but most patients develop the first symptoms between the ages of 50 and 65 years old. Until now, no cure has been found. Survival time varies, but is, on average, approximately three years after symptom onset. Average survival in PMA is similar to that in ALS, whereas patients with PLS have a survival time of up to more than 15 years, albeit with significant functional limitations. The variation in symptoms and differences in disease progression between these three conditions, but mainly within ALS itself, have led to an increased number of studies investigating the potential causes of these variations. In the patients with ALS, this has resulted in a number of specific subgroups. Every subgroup has specific characteristics, which may prove to be a biomarker for prognosis and/or disease progression. Identifying these subgroups with (a) similar biomarker(s) is important in predicting the disease course of a patient. In addition, this may help elucidate the etiology of the disease and may serve as a target for future therapeutic interventions.

METABOLISM AS A BIOMARKER

ALS would appear to be caused by a variety of pathophysiological mechanisms. There is evidence of a disturbed metabolic state in patients with ALS, giving rise to the metabolic hypothesis. Previous researches have shown that metabolic changes may result in different disease courses. The metabolism may be disrupted due to 1) an altered and insufficient intake of a patient, for example due to swallowing difficulties, and/or 2) changes in the metabolic state. Changes in the metabolic state have been found on a cellular level – including motor neurons – in some patients with ALS; in turn, these may result in a general disruption of metabolism. Whether this is a cause or consequence of the disease is not known. However, in either situation, the metabolic state might function as a biomarker for disease progression. An altered metabolism can result in various disturbed processes, for which there are a variety

of biomarkers, including cholesterol, body weight and the individual components of body weight: fat-free mass and fat mass. In addition, the measurement of the metabolic state itself might serve as a potential biomarker. Although determining the metabolic state may be challenging, previous studies have demonstrated its importance; A subgroup of patients with ALS do have an increased metabolic state – also known as hypermetabolism – which has proved to result in a shorter survival. Therefore, it is important to apply a uniform method when determining the metabolic state and the metabolic biomarkers in the attempts to predict the disease course and prognosis. This could inform better counselling of patients and their relatives regarding disease course and survival.

FINDINGS

This thesis focusses on various factors of metabolism with a view to increasing the amount of evidence for an altered metabolic state in patients with ALS, and to determining their prognostic value. Body weight is an easily measurable factor; changes in body weight can reflect changes in a body's metabolism. In **chapter 2** we determined the effect of weight loss on survival. The amount of weight loss was measured between symptom onset and date of diagnosis of ALS. We included 2,420 patients of whom 67% reported weight loss, averaging 5.1 kg in 9.4 months. Remarkably, we did not find weight loss only in those patients who had symptoms of swallowing difficulties, but also in those without, suggesting other factors might cause weight loss. Furthermore, we found that with each additional kilo of weight loss, the risk of dying during follow-up increased by 3%. This effect persisted when our model was corrected for important prognostic factors in ALS, such as: decreased respiratory function, bulbar onset, and lower score on the ALSFRS-R. This suggests weight loss is an independent variable of survival prediction. It is important, therefore, to discover the underlying cause in order, eventually, to prevent weight loss by a specific (dietary) intervention.

Cholesterol is a type of lipid. Lipids act as structural components of neuronal membranes, signaling molecules and energy substrates required for normal functioning of neurons. As serum cholesterol levels might function as metabolic biomarkers, in **chapter 3,** we investigated the prognostic value of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides on survival in 1,324 patients with ALS. We firstly looked into the results of previous studies by performing a systematic review, which revealed heterogeneity between studies. This was mainly due to differences in study design, sample size of the study population, and the correction of specific known prognostic factors in ALS. Secondly, our data showed that serum cholesterol levels change as the disease progresses. Additionally, we found that higher serum HDL-cholesterol levels were associated with a worse prognosis. This might, however, be due to the association between BMI and HDL-cholesterol: higher HDL-cholesterol levels are associated with a lower number in BMI. Therefore, HDL-cholesterol may be related to the weight loss, as found in **chapter 2**. Albeit speculative, one could hypothesize that the prognostic association might partially reflect a pre-manifest or prodromal sign of ALS. These

findings suggest longitudinal studies are necessary to show the relationship between cholesterol, disease progression and weight loss.

Given the fact that patients with ALS develop atrophy and muscle weakness, it seems worthwhile to distinguish total body weight into fat-free mass (i.e., muscle mass) and fat mass. These variables are both used in formulae for calculating resting energy expenditure. In **Chapter 4** we performed a longitudinal study including 107 Dutch and Australian patients. We showed that the decrease in fat-free mass correlates with serum creatinine concentration. This is important for severely affected patients who are, for example, not able to be weighed on a scale. This study presents an easy and inexpensive biomarker to evaluate whether fat-free mass remains stable during the disease course. Whether creatinine is an adequate biomarker for monitoring fat-free mass in, for example, a dietary therapeutic intervention should be investigated further.

Fat-free mass and fat mass are variables used in various formulae to determine a person's resting energy expenditure, i.e. the energy expenditure when awake and not performing any activity. Several formulae have been validated in specific populations worldwide. These can be used to calculate predicted resting energy expenditure which can subsequently be compared to the measured resting energy expenditure, resulting in the metabolic index. This index indicates whether someone has a decreased, normal, or increased resting metabolism. In **chapter 5**, we studied the geographical differences in resting energy expenditure and compared a variety of formulae in geographically different study populations, including 286 patients with ALS from three countries: Australia, China and the Netherlands. The metabolic state of these patients was compared to 320 controls. The study showed that the identification of hypermetabolism in ALS is influenced by the formulae and demographic-specific prediction thresholds used to define alterations in metabolic rate. There does not seem to be an 'ideal' formula for determining predicted resting energy expenditure. However, if required, the Sabounchi Structure 4 formula was found to be the most applicable. This was applied in **chapters 6** and **7**. In **chapter 6**, we show that changes in fat-free mass in patients with ALS seem to determine whether someone has decreased, normal or increased resting metabolism. In addition, we show that this depends on the method used: bioelectrical impedance analysis (BIA) or air displacement plethysmography (ADP). We demonstrate that BIA can be used effectively in daily practice to measure fat-free mass. However, researchers should be aware that BIA overestimates fat-free mass by an average of 2.5 kg. Therefore, determining the metabolic index based on the values provided by BIA can affect a patient's metabolic state (i.e. hypo-, normo- or hypermetabolic). In **Chapter 7**, therefore, we determined the metabolic index in 79 ALS patients, 33 patients with PMA and 30 patients with PLS using the values provided by ADP. These values were applied in the Sabounchi Structure 4 formula. Until now, the metabolic index has only been examined in patients with ALS, while PMA and PLS seem to have a shared pathophysiological background. In the present study,

hypermetabolism was observed in all three disorders: 55.7% of the patients with ALS, 27.3% of the patients with PMA and 26.7% of the patients with PLS. Hence, the altered metabolic rate was most commonly observed in patients with ALS, but was also present in patients with PMA and PLS. We found no relationship with specific known disease characteristics. This implies that an altered metabolic state might be due to a specific pathophysiology, which may be a shared pathophysiology in ALS, PMA and PLS.

In conclusion, this dissertation focusses on metabolic changes in ALS, the prognostic value of various metabolic biomarkers and, moreover, on fat-free mass. We 1) compare different techniques for measuring fat-free mass and 2) assess the effect of several formulae on the determining resting energy expenditure. These results were used to investigate the metabolic state of the patients by determining the metabolic index. We found that hypermetabolism is not a specific phenomenon in ALS. This dissertation supports the need for further (longitudinal) studies to gain clarity on changes in fat-free mass and metabolic state during the disease course. Moreover, the effect of specific therapies on maintaining fat-free mass and/or altering the metabolic index and, subsequently, determine their prognostic value is worthwhile, as shown in this dissertation.

SUMMARY IN DUTCH (Nederlandse samenvatting)

AMYOTROFISCHE LATERALE SCLEROSE

Amyotrofische laterale sclerose (ALS) is een neurologisch ziektebeeld, waarbij de motorische zenuwcellen – of motorische neuronen – zijn aangedaan. De motorische neuronen zijn gelegen in zowel het centrale als perifere zenuwstelsel; bij ALS is er een probleem in beide zenuwstelsels. Wanneer alleen het perifere zenuwstelsel is aangedaan, spreken we van de ziekte progressieve spinale musculaire atrofie (PMA). Wanneer alleen het centrale zenuwstel aangedaan is, spreken we van primaire laterale sclerose (PLS). Deze ziektebeelden bij elkaar noemen we de motorneuronziekten (MND), waarbij ALS de meest voorkomende variant is. De motorische neuronen zorgen voor het aansturen van de bewegingsspieren. Aantasting van deze motorische neuronen leidt tot spierzwakte, wat over het gehele lichaam voor kan komen. Uiteindelijk raken de ademhalingsspieren aangedaan, waardoor patiënten progressieve benauwdheidsklachten krijgen en komen te overlijden. De eerste symptomen variëren; bij de één zijn de eerste symptomen slik- en/of spraakproblemen (bulbaire symptomen), terwijl de ander eerst krachtsverlies ervaart in armen en/of benen of juist benauwdheidsklachten (spinale symptomen). De verscheidenheid aan klachten wordt gemeten middels de ALSFRS-R vragenlijst: hoe lager de score, hoe erger de symptomen zijn. De ziekte kan ontstaan op iedere leeftijd, maar de meeste mensen krijgen de eerste symptomen tussen de 50 en 65 jaar oud. ALS is een ziektebeeld waar patiënten nog niet van kunnen genezen. De overleving varieert, maar uiteindelijk overlijden alle patiënten aan deze ziekte, waarbij de gemiddelde overleving na het ontstaan van de eerste klachten ongeveer drie jaar is. Bij PMA is de gemiddelde overleving ongeveer gelijk aan die van ALS, maar patiënten met PLS hebben gemiddeld een langere overleving, tot meer dan 15 jaar. Hetzij wel met, soms forse, functionele beperkingen. De variatie in symptomen en beloop tussen deze ziektebeelden en tevens binnen de ziekte ALS, heeft gezorgd voor meer onderzoek naar de oorzaken van deze variatie. Dit heeft onder andere binnen de groep van patiënten met ALS laten zien, dat er groepen zijn met dezelfde kenmerken, die zich weer onderscheiden van een andere subgroep. Zo'n specifiek kenmerk noemen we een biomarker. Het identificeren van deze subgroepen met dezelfde biomarker(s) is belangrijk om te kunnen inschatten wat het te verwachte ziektebeloop is bij verschillende patiënten. Daarnaast kan het iets zeggen over de etiologie van de ziekte en is het van belang om te onderzoeken of een specifieke therapie op zo'n biomarker effectief kan zijn voor een subgroep.

HET METABOLISME ALS BIOMARKER

ALS lijkt veroorzaakt te worden door een verscheidenheid aan pathofysiologische mechanismen. Bij een deel van de patiënten met ALS zijn er aanwijzingen dat de stofwisseling – of het metabolisme – verstoord is. Deze 'metabole hypothese' wordt al langere tijd onderzocht. Hierbij heeft eerder onderzoek onder andere aangetoond dat metabole veranderingen mogelijk zorgen voor een ander beloop van de ziekte.

Dit aangetoonde verstoorde metabolisme kan – simpelweg – op twee manieren veroorzaakt worden: 1) doordat de patiënt te weinig energie tot zich nemen, door bijvoorbeeld slikstoornissen, en 2) doordat het metabolisme in het lichaam verandert, waardoor er dus meer verbranding is van (voedings)stoffen. Er zijn reeds duidelijke aanwijzingen gevonden dat er veranderingen zijn in het metabolisme van de lichaamscellen – waaronder de zenuwcellen – van een deel van de patiënten met ALS, wat mogelijk leidt tot een algeheel verstoord metabolisme. Het is onbekend of dit de oorzaak of het gevolg is van de ziekte, maar in beiden gevallen kan het metabolisme een biomarker zijn voor het verloop van de ziekte. Om dit te kunnen meten, kent het metabolisme verschillende biomarkers, zoals: de cholesterol, het lichaamsgewicht, en de afzonderlijke componenten van het lichaamsgewicht, namelijk: de spiermassa en vetmassa. Daarnaast is het mogelijk om het metabolisme van het lichaam te meten; hierbij wordt op basis van de zuurstofopname en koolstofdioxide uitscheiding het metabolisme bepaald. Deze meting kan uitdagend zijn, maar uit voorgaand onderzoek is gebleken dat deze meting wel belangrijk is. Voorgaand onderzoek heeft aangetoond dat patiënten met ALS die een verhoogd metabolisme hebben – ook wel hypermetabolisme – een sneller ziektebeloop kennen en, daardoor, een kortere overleving. Het toepassen van een uniforme methode in het meten van dit metabolisme en de metabole biomarkers is daardoor van belang om het beloop en de ernst van de symptomen/ziekte te kunnen voorspellen. Op basis hiervan kunnen de patiënten en hun familieleden zo volledig mogelijk geïnformeerd worden en kunnen, derhalve, betere conclusie(s) getrokken worden.

BEVINDINGEN

Om verdere en betere uitspraken te kunnen doen over het metabolisme in patiënten met ALS, heeft dit proefschrift zich gericht op verschillende factoren die bij het metabolisme betrokken zijn. Het lichaamsgewicht is een makkelijk meetbare factor bij mensen en veranderingen in het lichaamsgewicht kunnen een weerspiegeling zijn van veranderingen in het metabolisme. In **hoofdstuk 2** hebben we gekeken naar het effect van gewichtsverlies op de overleving, waarbij het gewichtsverlies gemeten is tussen het moment van ontstaan van eerste symptomen tot aan het moment dat de diagnose ALS werd gesteld. In dit onderzoek hebben we 2,420 patiënten geïncludeerd, waarbij ruim 67% van de patiënten meldden dat ze gewichtsverlies hadden met een gemiddelde van 5,1 kg over 9,4 maanden. Opmerkelijk was dat we dit niet alleen vonden bij de patiënten met slik- of kauwproblemen, maar ook bij de patiënten zonder deze problemen. Dit suggereert dat er (ook) een ander proces gaande moet zijn waardoor het gewichtsverlies is ontstaan. Daarnaast bleek de hoeveelheid gewichtsverlies een negatief effect te hebben op de overleving; iedere kilo extra aan gewichtsverlies gaf een toename van het risico op overlijden van 3%. Dit effect hield stand wanneer we ons model corrigeerde voor belangrijke factoren waarvan al bekend is dat ze bepalend zijn voor de overleving, zoals: slechtere longfunctie, bulbaire start van de ziekte en een lagere score op de ALSFRS-R. Dit suggereert dat gewichtsverlies een onafhankelijke variabele is om de overleving te kunnen voorspellen. Daaropvolgend is het dus belangrijk

om te achterhalen waar dit gewichtsverlies vandaan komt en aanvullend wat eraan gedaan zou kunnen worden.

Om te bepalen waar de oorzaak zou kunnen liggen, hebben we in **hoofdstuk 3** cross-sectioneel gekeken naar de cholesterolgehalten in het bloed als prognostische factor op de overleving. Cholesterol is een lipide en wordt voor verschillende processen in het lichaam gebruikt. Het is onder andere onderdeel van de celmembraan van neuronen, maar wordt door deze neuronen ook gebruikt als energie substraat. In dit hoofdstuk hebben we gekeken naar het effect van totaal cholesterol, LDL-cholesterol, HDL-cholesterol en triglyceriden op de overleving in 1,324 patiënten met ALS. Hierbij hebben we de resultaten vergeleken van voorgaand internationaal onderzoek. Hierin vonden we een variatie van resultaten. Dit leek vooral het gevolg te zijn van verschillen in de onderzoeksopzet, de grootte van de studie-populatie en het betrekken van andere, reeds bekende, prognostische factoren. Uit onze data bleek dat de cholesterol in het serum verandert wanneer de symptomen van de ziekte meer en heviger aanwezig zijn. Daarnaast vonden we dat hogere serum HDL-cholesterol waarden geassocieerd zijn met een slechtere prognose. Gezien HDL-cholesterol het 'goede' cholesterol wordt genoemd, was dit een opvallende en ook nieuwe bevinding. Dit leek echter ook het gevolg te zijn van de associatie die BMI kent met het HDL-cholesterol: hoe hoger het HDL-cholesterol, hoe lager het BMI. Hierdoor kan het HDL-cholesterol gerelateerd zijn aan het in **hoofdstuk 2** gevonden gewichtsverlies. Hoewel speculerend, een hoger HDL-cholesterol zou het gevolg kunnen zijn van neuronale schade door veranderingen in het lipiden metabolisme ten gevolge van de ziekte, waardoor het een biomarker is voor een slechtere prognose. Dit dient wel nog verder onderzocht te worden in longitudinale onderzoeken, zodat de relatie tussen cholesterol en ziekteprogressie beter gelegd kan worden.

Gezien ALS gepaard gaat met atrofie en spierzwakte lijkt het van toegevoegde waarde om het lichaamsgewicht te splitsen, te analyseren en te vervolgen in de vetvrije massa (i.e. spiermassa) en de vetmassa. Dit is mede omdat deze variabelen bepalend zijn voor o.a. het berekenen van het metabolisme. In **hoofdstuk 4** hebben we aangetoond dat de vetvrije massa een correlatie heeft met de creatinine concentratie in het bloed. Dit is van belang voor de patiënten die bijvoorbeeld niet meer op een weegschaal kunnen staan. In dit longitudinale onderzoek vonden we in 107 Nederlandse en Australische patiënten dat het creatinine lager werd naarmate de vetvrije massa ook steeds lager werd. Hierdoor is er in het bloed een makkelijke en goedkope marker meetbaar om te evalueren of de vetvrije massa stabiel blijft. Of het creatinine vervolgens ook een goede marker is voor het vervolgen van de vetvrije massa bij bijvoorbeeld een therapeutische interventie, moet in verder onderzoek uitgezocht worden.

De vetvrije massa en vetmassa zijn variabelen die gebruikt worden in verschillende formules om het rustmetabolisme van een mens te kunnen bepalen. Het rustmetabolisme is het

metabolisme dat een persoon in een wakkere toestand heeft wanneer hij/zij geen activiteit verricht. Er zijn meerdere formules over de tijd gevalideerd in specifieke populaties wereldwijd. Middels deze formules kan het verwachte rustmetabolisme berekend worden. Deze kan vervolgens afgezet worden tegen het gemeten rustmetabolisme. Het meten van het metabolisme gebeurt in liggende positie in een gesloten ruimte met een stabiele temperatuur, waarbij middels de zuurstofinname en koolstofdioxide afgifte het rustmetabolisme bepaald wordt. Het berekende *vs.* het gemeten rustmetabolisme geeft de 'metabolic index'. Deze index geeft aan of iemand een verlaagd, normaal, of verhoogd rustmetabolisme heeft. In **hoofdstuk 5** hebben we gekeken naar de geografische verschillen van dit rustmetabolisme en de toepasbaarheid van de verschillende formules op verschillende populaties. In dit onderzoek hebben we 286 patiënten met ALS uit drie landen geïncludeerd, namelijk: Australië, China, en Nederland. Het metabolisme van deze patiënten werd vergeleken met 320 controles uit deze landen. In dit onderzoek kwam naar voren dat het berekenen van het rustmetabolisme en de afkapwaarden die je gebruikt in een bepaalde populatie steeds opnieuw bepaald moet worden en waarschijnlijk geografisch bepaald zijn. Daarnaast lijkt er niet een 'ideale' formule te zijn die wereldwijd gebruikt kan worden, maar dient voor iedere populatie bepaald te worden wat de best-fitting formule is aan de hand van een controle populatie. De Sabounchi Structure 4 formule bleek uit dit onderzoek het best toepasbaar te zijn. Deze formule hebben we toegepast in **hoofdstuk 6** en **hoofdstuk 7**. In **hoofdstuk 6** laten we zien dat de verandering in vetvrije massa bij de patiënten met ALS van invloed is op het bepalen of iemand een verlaagd, normaal, of verhoogd rustmetabolisme heeft. Tevens laten we zien dat dit afhankelijk is van de methode die je gebruikt. Onderzoekers dienen zich hier bewust van te zijn, omdat het categoriseren van patiënten in een verlaagd, normaal, of verhoogd metabolisme consequenties kan hebben bij toekomstige (medicamenteuze) interventies. We stellen dat het belangrijker is om de vetvrije massa bij de patiënten te vervolgen in plaats van het totale lichaamsgewicht. Hierbij is een nauwkeurige meting van de vetvrije massa van belang. In ditzelfde onderzoek hebben we twee methoden vergeleken: de bio-elektrische impedantie analyse (BIA) en de air displacement plethysmography (ADP). De ADP brengt grotere kosten met zich mee en het gebruiksgemak is lager dan bij de BIA. In dit onderzoek laten we zien dat de BIA goed gebruikt kan worden in de dagelijkse praktijk om vetvrije massa te meten, maar stellen wel dat deze methode de vetvrije massa overschat met gemiddeld 2,5 kg. Het bepalen van de metabolic index met de verkregen waarden van de BIA kan daardoor riskant zijn indien je patiënt categoriseert op basis van de metabolic index. Vanwege de bevindingen in **hoofdstuk 5** en **hoofdstuk 6** bepalen we de metabolic index in **hoofdstuk 7** bij 79 patiënten met ALS, 33 patiënten met PMA en 30 patiënten met PLS. In dit onderzoek bepaalden we de metabolic index door de waarden uit de ADP te gebruiken in de Sabounchi Structure 4 formule. Tot nog toe is er wereldwijd enkel gekeken naar de metabolic index bij patiënten met ALS, terwijl de ziektebeelden PMA en PLS een gedeelde pathofysiologie hebben met ALS. We laten in dit onderzoek zien dat hypermetabolisme bij alle drie de ziektebeelden gezien wordt: in 55.7% van de patiënten

met ALS, in 27.3% van de patiënten met PMA, en in 26.7% van de patiënten met PLS. De hoogste gemiddelde metabolic index wordt tevens gevonden bij de patiënten met ALS. We vonden geen relatie met specifieke bekende ziekte karakteristieken. Dit impliceert dat een veranderd metabolisme door een specifieke pathofysiologie komt, wat tevens een gedeelde pathofysiologie kan zijn in ALS, PMA, en PLS.

Concluderend, dit proefschrift richt zich op de metabole veranderingen bij ALS, waarbij we gekeken hebben naar de prognostische waarden van bepaalde metabole biomarkers en ingezoomd hebben op de vetvrije massa, waarbij we 1) verschillende technieken vergelijken om de vetvrije massa te meten en 2) het effect hiervan bepalen op verschillende formules voor het berekenen van het rustmetabolisme. Vervolgens hebben we de resultaten van deze onderzoeken gebruikt om de metabole status van de patiënten te onderzoeken middels het bepalen van de metabolic index. Hieruit bleek dat het hypermetabolisme niet een specifiek verschijnsel is voor ALS. Dit proefschrift geeft aanleiding om verdere (longitudinale) onderzoeken te verrichten om meer duidelijkheid te krijgen over de veranderingen van de vetvrije massa en de metabole status gedurende het ziektebeloop. Simultaan kan onderzocht worden wat het effect is van een specifieke therapie op het behouden van vetvrije massa en/of een (medicamenteuze) therapie op het verlagen van de metabolic index en het effect hiervan op de overleving.

ACKNOWLEDGEMENTS (Dankwoord)

Dit proefschrift was niet tot stand gekomen zonder de hulp van en fijne samenwerking met vele mensen. Iedereen die een bijdrage heeft geleverd wil ik bedanken, waarbij ik een aantal personen wil uitlichten.

De samenstelling van dit proefschrift zou niet gelukt zijn zonder de hulp van de patiënten. De welwillendheid om deel te nemen aan mijn onderzoek naar de rol van het metabolisme in de ziekte ALS was groot, hoe progressief de ziekte soms ook was. Ik ben enorm dankbaar dat de patiënten keer op keer weer terugkwamen om deel te nemen aan het onderzoek. Ook wil ik hun familieleden bedanken voor hun toewijding. Jullie geleverde inspanningen waardeer ik enorm.

Beste prof. dr. L.H. van den Berg, beste Leonard, ik wil je bedanken voor alle vrijheid, mogelijkheden en begeleiding die je hebt gegeven om dit proefschrift tot een succes te brengen. De gedrevenheid waarmee jij je inzet om op alle vlakken onderzoek op te zetten en uit te voeren is groot. Dat waardeer ik enorm in je. Zowel in het werk als in de persoonlijke contacten heb je mij geholpen en heb ik veel van je geleerd. Bedankt!

Beste dr. R.P.A. van Eijk, beste Ruben, wat hebben wij veel meetings met elkaar gehad en wat heb jij veel bijgedragen aan mijn groei binnen het onderzoek. In mijn eerste maanden duizelde de statistiek, maar dankzij jou kreeg ik, naarmate de tijd vorderde, 'R' eindelijk onder de knie. Jouw inzichten en ideeën waren soms buitengewoon en vol bewondering heb ik dat mogen aanschouwen. Bedankt voor al je hulp die je hebt gegeven om dit proefschrift, de MEASURE en MetFlex studie tot een succes te brengen.

Dear dr. Ngo, dear Shyuan, it is such an honor to have you as a member of my PhD supervisory team. I enjoyed our regularly online meetings. Thank you for all your input and our interesting discussions. It was very inspiring to hear your ideas about metabolism in MND and your further thoughts on setting up new researches. Additionally, I would like to thank Frederik Steyn, as he was part of our interesting discussions. Dear Shu and Derik, thank you for all your efforts.

De leden van de beoordelingscommissie, te weten prof. dr. R.A.H. Adan, prof. dr. B. van Engelen, prof. dr. L.J. Kappelle, prof. dr. N.C. Notermans en prof. dr. F.L.J. Visseren, bedank ik voor de tijd en interesse die jullie hebben gestoken in de beoordeling van dit proefschrift.

Lieve Myrte, een hele grote dank gaat uit naar jou. De MEASURE en de MetFlex studie waren zonder jou geen succes geweest. Je toewijding, betrokkenheid en inzet bij de studie waren groot. We hadden dezelfde instelling en dat zorgde ervoor dat we op één lijn zaten. Gedu-

rende de COVID pandemie moesten we op een andere manier vormgeven aan de studies. Mede dankzij jouw flexibiliteit hebben we de metingen volgens de COVID voorschriften kunnen uitvoeren. Een uitzonderlijke situatie waar we ons goed doorheen hebben geslagen. Onze samenwerking was uniek en daar wil ik je enorm voor bedanken. Je regelde alles tot in de laatste details, wat de studiepatiënten ten goede kwam. Zonder jou waren verscheidene data voor dit proefschrift niet beschikbaar geweest. Naast de goede samenwerking hebben we enorm kunnen lachen, samen met de patiënten. Je hebt een hele mok met allerlei uitspraken weten samen te stellen, omdat de BodPod toch wel wat teweegbracht bij de studiepatiënten, zoals: "Waar ga ik heen met deze ruimtecapsule?" en "Tijdens de meting wil ik u vragen om niet naar uw familielid te zwaaien."

Beste collega's van de diëtetiek, te weten Anne, Annemieke, Elles, Jade, Francis en Sytske, bedankt voor jullie tijd, hulp en expertise bij het uitvoeren van de metingen voor de MEASURE en MetFlex studie. Keer op keer stonden jullie weer klaar, waarbij COVID de gehele planning in de war gooide. Door jullie flexibiliteit konden de metingen desondanks doorgaan, waardoor veel data verkregen is voor de onderzoeken. Daarnaast hebben we als 'MEASURE-team' hardgelopen voor de ALS Lenteloop, wat een groot succes was. Bedankt!

Lieve collega's van het ALS/NMZ lab, te weten Adriaan, Annebelle, Bálint, Boudewijn, Bram, Camiel, Diederik, Eva, Eveline, Ewout, Féline, Hannelore, Harold, Henk-Jan, Ingrid, Iris, Janna, Jeroen, Kevin, Leandra, Lisa, Loes, Koen, Louise, Madde, Maria, Marieke, Sean, Stefan en alle anderen, bedankt voor de leerzame en gezellige tijd op het lab. De dagelijkse meermaals voorbijtrekkende geur van vers gezette koffie heeft er uiteindelijk toe geleid dat ik koffie ben gaan drinken. Ik denk dat mede door deze 'huiselijke sfeer' dit proefschrift is afgekomen. Ik wil jullie bedanken voor de tijd die jullie namen voor de R support, waarbij de verkregen informatie vervolgens verwerkt kon worden in onze lunch van 11:30 uur, om daarna de overgebleven frustratie met wat tafelvoetbal er nog uit te rammen. Deze traditie werd verstoord door de COVID pandemie, maar het contact als collega's hebben we online goed onderhouden. Bedankt voor al jullie steun, lachmomenten, hulp en het aanhoren van mijn frustratiemomenten.

Beste Annemarie, wat had ik zonder jou gemoeten? Ik hoefde je maar te mailen of even langs te lopen als ik iets nodig had. Op korte termijn had je dan een afspraak met Leonard gepland, of je had het al geregeld. Bedankt daarvoor! Het zal je misschien verbazen, maar dat houten krukje wat achter je staat op jouw kamer, was mijn favoriete plek om even te gaan zitten in afwachting van mijn afspraak met Leonard. Bedankt voor de gesprekken die we toen steeds met elkaar hadden, die waren vertrouwd en nuttig.

Beste prof. dr. Veldink, beste Jan, beste dr. Van Es, beste Michael en beste dr. Van Rheenen, beste Wouter, bedankt voor jullie aanvullingen in de manuscripten en onderzoeken. Het was

een eer om onderdeel te zijn van een team waar zoveel kennis zit, waardoor ik veel van jullie heb geleerd.

Lieve collega's van het medicijnonderzoek, het huidige D3, en in het bijzonder Arianne, Bianca en Tommy, bedankt voor de leerzame en leuke tijd met elkaar. Wat hebben wij veel meegemaakt. Als beginnend onderzoeker ging een hele andere wereld voor mij open: de wereld van medicijnstudies. Hierbij kwamen vele facetten kijken: de wet- en regelgeving, de ethische aspecten, de controle op de studies van audits en monitoring en ga zo maar door. Jullie hebben mij daar snel in doen groeien, waardoor de samenwerking soepel en vlot verliep. Het was prettig om te zien hoe jullie positieve instelling keer op keer de overhand nam om gemotiveerd toe te werken naar een oplossing voor de ziekte ALS. Als we er even doorheen zaten, soms mede veroorzaakt door het progressieve beloop van de ziekte, konden we elkaar weer uit deze negatieve spiraal halen. Bianca, ik zal het huisbezoek naar één van onze studiepatiënten nooit vergeten. Bedankt voor alles.

Beste collega's van H2, te weten Hermieneke, Lisanne, Malu, Melissa, Nesrin, Nienke, Simone, Ummihan, verschillende studenten en alle anderen, bedankt voor jullie ondersteuning, begeleiding en het meedenken bij de verschillende onderzoeken. Femke wil ik ook graag bedanken voor de hulp bij de datamanagement.

Beste verpleegkundig specialisten van de MND-poli, in het bijzonder Kim, bedankt voor jullie ondersteuning en hulp bij het goed laten verlopen van het spreekuur, maar ook bedankt voor het creëren van ruimte voor de uitvoering van het wetenschappelijk onderzoek.

Lieve Inez, samen zijn wij gestart aan de opleiding tot neuroloog. Bij de start bleek dat we ongeveer op hetzelfde punt stonden in ons promotietraject. Dit heeft ervoor gezorgd dat we over alles konden spuien (ook wel: klagen) en elkaar konden helpen als we ergens tegen aan liepen. Mattie, bedankt en goed gedaan. We zijn er bijna!

Dear Brenda, without your help my manuscripts would not be grammatically perfect! Many thanks.

Dear other co-authors of the articles presented in this thesis, thanks for all your efforts.

Lieve zus, lieve paranimf, wat ben ik blij dat jij aan mijn zijde staat op de dag van mijn verdediging. Je kent mijn gedachtegangen als geen ander en de controle overgeven aan jou is dan ook gemakkelijk. Het is fijn om een zus te hebben met een positieve instelling, waarbij de eventuele beren op de weg makkelijk te trotseren zijn (of je moet je er gewoon niet druk om maken). Jouw nuchterheid is fijn en verhelderend. Ik ben als broertje trots op je en ben

blij met mijn grote zus. Je steunt mij in alles, in goede en in slechte tijden. Daar ben ik je enorm dankbaar voor.

Beste Simba, beste paranimf, vijftien jaar na de start van onze vriendschap sta ook jij naast mij op de dag van mijn verdediging. Jouw nuchterheid, positieve instelling en kritische blik hebben ertoe geleid dat ik dit promotietraject tot een goed einde kon brengen. Je bent altijd geïnteresseerd geweest in mijn onderzoek en manuscripten en je volgde deze dan ook op de voet. Juist omdat onze promotietrajecten volledig anders verliepen – jij binnen de chirurgie en aan een andere universiteit – konden we onze ervaringen goed aan elkaar kwijt. Bedankt voor je luisterend oor en je adviezen en opmerkingen; deze waren verhelderend, zowel werk- als niet-werk gerelateerd. Nu we allebei dit jaar onze verdediging hebben, kunnen onze weekenden voorlopig weer échte vrije weekenden zijn (tenzij we dienst hebben!).

Lieve Rieke, onze telefoongesprekken duren tegenwoordig anderhalf uur, omdat we veel bij te praten hebben. Ondanks jouw verhuizing naar Wenen, praten we altijd meteen verder waar we gebleven waren. Het relativeerde en motiveerde altijd goed, omdat – gezien er meestal enkele maanden tussen onze telefoongesprekken zaten – vaak toch wel bleek dat mijn onderzoek of manuscript best opgeschoten was. Bedankt voor deze mooie en al hele lange vriendschap!

Lieve vrienden, bedankt dat jullie altijd voor mij klaarstaan. In het bijzonder wil ik benoemen Bob, Max, Thomas en Wouter. Onze vriendschap heeft al voor vele mooie momenten gezorgd. Vele weekendjes weg om de wereld te verkennen, zowel binnen als buiten Nederland. Daarnaast genieten we van goede etentjes, een bezoekje aan het casino en kunnen we een 'klein' pilsje op zijn tijd goed waarderen. Daarnaast, lieve Eline, wil ik jou bedanken voor de gesprekken tijdens onze wandelingen met koffie. Dit was altijd een goede start van de dag.

Lieve familie, dank voor al jullie steun, betrokkenheid en interesse in mijn werk. Gedurende de COVID pandemie was de tuin in Zevenhoven een aantal keer mijn werkplek. Lieve Dineke en Arnold, bedankt voor deze gastvrijheid.

Lieve oma Janse van Mantgem†, u was een oma om nooit te vergeten. De start van mijn promotietraject heeft u meegemaakt, waarbij u altijd vol interesse en verwondering luisterde naar mijn verhalen. Het leven werd u te zwaar, waardoor u het einde van het promotietraject helaas niet meemaakt. Ik mis nog altijd mijn bezoekjes aan uw warme huis en de etentjes die wij samen hadden. Ik weet dat u enorm trots geweest zou zijn; dat gevoel van trots voelde ik.

Lieve oma Verdonk†, lieve opa Verdonk, een voorbeeld van een koppel wat elkaar op alle vlakken aanvulde. Vol interesse luisterden jullie naar mijn verhalen, waarbij het niet gedetailleerd genoeg kon. Té medisch of té ingewikkeld, dat bestaat niet. Dan moest ik het maar op

een andere manier uitleggen, totdat duidelijk was, wat ik nou eigenlijk precies gedaan had. Dat was voor mijzelf enorm verhelderend, omdat ik op sommige momenten ook even de draad kwijt kon zijn. Helaas werd, lieve opa, oma in de zomer van 2023 van de ene op de andere dag, zonder enige aankondiging, uit het leven gegrepen. Uw aanwezigheid op de dag van de verdediging, zal ik, namens jullie, als enorme steun ervaren.

Lieve Moes, mijn tijdelijke stille aanbidder, jouw bemoedigende woorden hebben dit promotietraject tot een goed einde gebracht. Uit twee compleet andere werelden trokken wij naar elkaar toe. De stress en drukte die ik gedurende mijn promotietraject heb gekend, waren niet altijd makkelijk voor jou. Je bent mij blijven steunen, waar ik je dankbaar voor ben. Nu wordt het tijd dat we jouw studie tot een goed einde gaan brengen. Met elkaar is het leven leuker en enerverender. Met jou sta ik sterker en ik kijk uit naar onze toekomst samen!

Lieve papa en mama, wie had gedacht dat ik zou promoveren. Om eerlijk te zijn: ik niet! Maar jullie stimulatie, motivatie, steun, vertrouwen en liefde, zorgden ervoor dat dit mogelijk werd. Jullie waren en zijn mijn voorbeeld: hard werken wordt beloond. Jullie hebben altijd achter mijn keuzes gestaan en hebben deze mogelijk gemaakt. Het is niet te beschrijven hoe dankbaar ik jullie daarvoor ben. We hebben de afgelopen jaren veel meegemaakt, waarbij ik de onvoorwaardelijke steun gevoeld heb en nog steeds voel. Ik hou van jullie!

LIST OF PUBLICATIONS

M.R. Janse van Mantgem, R.P.A. van Eijk, H.K. van der Burgh, H.H.G. Tan, H.J. Westeneng, M.A. van Es, J.H. Veldink, L.H. van den Berg, Prognostic value of weight loss in patients with amyotrophic lateral sclerosis: a population-based study, Journal of Neurology, Neurosurgery and Psychiatry (JNNP), 2020;91(8):867-875

M.O. Mol[1], S.W.R Nijmeijer[1], J.G.J. van Rooij, R. van Spaendonk, Y.A.L. Pijnenburg, S.J. van der Lee, R. van Minckelen, L. Donker Kaat, A.J.M. Rozemuller, **M.R. Janse van Mantgem**, W. van Rheenen, M.A. van Es, J.H. Veldink, F.A.M. Hennekam, M. Vernooij, J.C. van Swieten, P.E. Cohn-Hokke, H. Seelaar, E.G.P. Dopper, Distinctive pattern of temporal atrophy in patients with frontotemporal dementia and the I383V variant in TARDBP, Journal of Neurology, Neurosurgery and Psychiatry (JNNP), 2021;92(7):787-789

C.J. Holdom, **M.R. Janse van Mantgem**, R.P.A. van Eijk, S.L. Howe, L.H. van den Berg, P.A. McCombe, R.D. Henderson, S.T. Ngo, F.J. Steyn, Venous creatinine as a biomarker for loss of fat-free mass and disease progression in patients with amyotrophic lateral sclerosis, European Journal of Neurology, 2021;28(11):3615-3625

M.R. Janse van Mantgem, R.P.A. van Eijk, A. Hackeng, W. van Rheenen, M.A. van Es, J.H. Veldink, L.H. van den Berg, The Association between Serum Lipids and Survival in Patients with Amyotrophic Lateral Sclerosis: a Meta-Analysis and Population-Based Study, Neurology, 2023;100(10):e1062-e1071

Cory J. Holdom, **Mark R. Janse van Mantgem**, Ji He, Stephanie L. Howe, Pamela A. McCombe, Dongsheng Fan, Robert D. Henderson, Leonard H. van den Berg, Ruben P.A. van Eijk, Frederik J. Steyn, Shyuan T. Ngo, Variation in Resting Metabolic Rate Impacts Identification of Metabolic Change in Geographically Distinct Cohorts of Amyotrophic Lateral Sclerosis Patients, Neurology, 2024;102(5):e208117

Mark R. Janse van Mantgem*, Maaike L. Soors d'Ancona*, Myrte Meyjes, Leonard H. van den Berg, Elles Steenhagen, Annemieke Kok, Ruben P.A. van Eijk, Bioelectrical Impedance Analysis is a valid Alternative to Air-Displacement Plethysmography for assessment of Fat-free Mass in Patients with Motor Neuron Diseases, Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2024;1-10, online ahead of print

Mark R. Janse van Mantgem, Ruben P.A. van Eijk, Cory J. Holdom, Myrte Meyjes, Shyuan T. Ngo, Frederik J. Steyn, Leonard H. van den Berg, Assessing the Metabolic Rate in Patients with Amyotrophic Lateral Sclerosis, Progressive Muscular Atrophy, and Primary Lateral Sclerosis, Submitted at Journal of Neurology

Jordi W.J. van Unnik, Myrte Meyjes, **Mark R. Janse van Mantgem**, Leonard H. van den Berg, Ruben P.A. van Eijk, Remote monitoring of amyotrophic lateral sclerosis using wearable sensors detects differences in disease progression and survival: a prospective cohort study, eBioMedicine, 2024;103:1-13

Annebelle Michielsen*, Kevin van Veenhuijzen*, **Mark R. Janse van Mantgem***, Michael A. van Es, Jan H. Veldink, Ruben P.A. van Eijk, Leonard H. van den Berg, Henk-Jan Westeneng, Hypothalamic correlates of metabolism and cognition in amyotrophic lateral sclerosis**,** in preparation

CURRICULUM VITAE

PERSONAL DETAILS	
Name:	Mark Robert Janse van Mantgem
Date of birth:	4th of February, 1991
Place of birth:	Amstelveen
Nationality:	Dutch
BIG number:	1 9922 8769 01

EDUCATION	
Oct 2018	**University Medical Center Utrecht, Utrecht, The Netherlands** BROK (Basiscursus Regelgeving en Organisatie Klinisch Onderzoekers – ICH GCP guidelines)
June 2014 – July 2017	**Amsterdam University Medical Center – University of Amsterdam (AMC-UvA), Amsterdam, The Netherlands** Master Medicine
Sep 2010 – Aug 2013	**Amsterdam University Medical Center – University of Amsterdam (AMC-UvA), Amsterdam, The Netherlands** Bachelor Medicine
Sep 2009 – Aug 2010	**Vrije University Amsterdam, Amsterdam, The Netherlands** Propaedeutic year in 'Medische Natuurwetenschappen'
Sep 2003 – Aug 2009	**Hermann Wesselink college Amstelveen, Amstelveen, The Netherlands** Gymnasium

EMPLOYEMENT	
April 2022 - Present	**University Medical Center Utrecht, Utrecht, The Netherlands** Neurology resident – Arts in opleiding tot specialist (AIOS) – Department of Neurology
May 2018 - Present	**University Medical Center Utrecht, Utrecht, The Netherlands** PhD-student – Clinical and Experimental Neuroscience – Department of Neurology
May 2018 - Present	**University Medical Center Utrecht, Utrecht, The Netherlands** Research physician – Department of Neurology/Neuromuscular disorders
Aug 2017 – May 2018	**Medical Center Slotervaart, Amsterdam, The Netherlands** Arts niet in opleiding tot specialist (ANIOS) – Department of Neurology

July 2013 – June 2016	**Onze Lieve Vrouwe Hospital (OLVG), location Oost, Amsterdam, The Netherlands** Front office employee and triagist on the emergency room

RESEARCH SKILLS	
Oct 2020 - Present	**Metabolic flexibility in ALS (MetFlex)** Sub-investigator of this investigator-initiated phase II trial
Mei 2018 - Present	**Metabolism and Survival in ALS (MEASURE)** Sub-investigator of this multicenter study
Mei 2018 - Present	**Research physician of several medicine trials in patients with amyotrophic lateral sclerosis** Genentech (phase 1), ORARIALS (phase 3), RefALS (phase 3), Biogen, 245AS101 – ALS study (phase 1 and 2), Biogen, 275AS101 – ALS study (phase 1 and 2)
Jan 2017 – Juni 2017	**University of Oxford, John Radcliffe Hospital, Oxford Parkinson's Disease Centre (OPDC), United Kingdom** Scientific research project: 'Neuropsychological predictors and longitudinal changes of cognition in Parkinson's disease patients.' Supervision by dr. M.T.H. Hu, Associate Professor and Consultant Neurologist

SIDE ACTIVITIES	
May 2018 - Present	**Supervision of medical students** - Janneke Drost – *Prevalence of autoimmune disease in patient with ALS: A case-control study*
Oct 2017 - Present Nov 2013 – Aug 2015	- Anemone Hackeng – *The prognostic value of serum cholesterol in patients with ALS: a systematic review and population-based study*
	Chair of Vereniging van Eigenaren, Olympia – Amsterdam **Nationaal Coassistenten Congres (NCC)** Board member

OTHER SKILLS	
Languages:	Dutch (Native), English (Advanced)
Statistical packages:	The R Project for Statistical Computing (Intermediate)

UMC Utrecht

Universiteit Utrecht

ISBN 978-90-393-7663-8