REVIEW

Dysregulation of energy homeostasis in amyotrophic lateral sclerosis

Simon J. Guillot[a], *Matei Bolborea*[a,b,*], *and Luc Dupuis*[a,*]

Purpose of review
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease targeting upper and lower motor neurons, inexorably leading to an early death. Defects in energy metabolism have been associated with ALS, including weight loss, increased energy expenditure, decreased body fat mass and increased use of lipid nutrients at the expense of carbohydrates. We review here recent findings on impaired energy metabolism in ALS, and its clinical importance.

Recent findings
Hypothalamic atrophy, as well as alterations in hypothalamic peptides controlling energy metabolism, have been associated with metabolic derangements. Recent studies showed that mutations causing familial ALS impact various metabolic pathways, in particular mitochondrial function, and lipid and carbohydrate metabolism, which could underlie these metabolic defects in patients. Importantly, slowing weight loss, through high caloric diets, is a promising therapeutic strategy, and early clinical trials indicated that it might improve survival in at least a subset of patients. More research is needed to improve these therapeutic strategies, define pharmacological options, and refine the population of ALS patients that would benefit from these approaches.

Summary
Dysfunctional energy homeostasis is a major feature of ALS clinical picture and emerges as a potential therapeutic target.

Keywords
amyotrophic lateral sclerosis, diet, energy metabolism, hypothalamus, weight loss

INTRODUCTION

Until recently, amyotrophic lateral sclerosis (ALS) was considered a disease restricted to the motor system. The simultaneous degeneration of upper motor neurons, in the motor cortex, and of lower motor neurons, in the brainstem and spinal cord, appeared sufficient to explain the clinical phenotype of patients. Recent years have however demonstrated that ALS signs and symptoms are not restricted to the motor system, but also involve cognitive and metabolic alterations. This idea is consistent with the notion that ALS is part of a continuum with frontotemporal dementia (FTD) [1,2].

More than two decades ago, Couratier *et al.* observed that malnutrition correlated with worsened survival of ALS patients [3]. These observations, underpinned by studies in animal models [4], highlighted the alteration of systemic energy homeostasis in ALS patients. In recent years, an important body of evidence documented the clinical importance of the dysfunctional energy homeostasis observed in ALS. Anatomical and cellular substrates for these mechanisms are still poorly understood. Here, we review details of these emerging concepts in ALS.

EVIDENCE OF SYSTEMIC ENERGY HOMEOSTASIS ABNORMALITIES IN AMYOTROPHIC LATERAL SCLEROSIS

The occurrence of premorbid weight loss in ALS is the most evident symptom of defective energy

[a]University of Strasbourg, INSERM, UMR-S1118, Central and Peripheral Mechanisms of Neurodegeneration, Strasbourg, France and [b]School of Life Sciences, University of Warwick, Coventry, UK

Correspondence to Luc Dupuis, University of Strasbourg, INSERM, UMR-S1118, Central and Peripheral Mechanisms of Neurodegeneration, Biomedical Research Centre in Strasbourg, CRBS, 1 rue Eugène Boeckel, Strasbourg, France. Tel: +33 3 68 85 34 57;
e-mail: ldupuis@unistra.fr

*Senior authors.

Curr Opin Neurol 2021, 33:000–000
DOI:10.1097/WCO.0000000000000982

KEY POINTS

- Weight loss is a major symptom of ALS, occurring early, independently of dysphagia and not fully explained by denervation-induced muscle atrophy.
- Causes of weight loss are incompletely characterized and include alterations in metabolic fluxes in various tissues including skeletal muscle.
- Hypothalamic atrophy, as well as alterations in hypothalamic peptides controlling energy metabolism, have been associated with metabolic derangements in ALS.
- High caloric diet is a promising therapeutic strategy, with preliminary evidence of efficacy in fast progressing ALS patients.

metabolism. ALS patients usually display normal to low body mass index at onset [5], and typically lose weight and body fat with the progression of the disease [3]. However, weight loss is not observed in all ALS patients, affecting between one and two-thirds of individuals [6▪,7,8]. Consistent with the importance of weight loss in the disease process, the risk of developing ALS has been repeatedly shown to increase with lower premorbid body fat [9–11] and is also correlated with lower levels of leptin, an adipocyte-derived hormone reflecting adipose energy stores [12]. The process of weight loss in ALS appears to precede the onset of motor symptoms as presymptomatic ALS patients begin losing weight several years before the disease onset and diagnosis [13]. The systemic metabolic impairment in ALS remains incompletely characterized, and several studies have documented paradoxical glucose intolerance [14], insulin resistance [15] and, at least in some studies, increased circulating lipids [16,17] as well as redistribution of adipose tissue towards more visceral fat [18].

Weight loss appears to affect patients with spinal and bulbar onset of symptoms [6▪], and, importantly (see later) occurs also in a significant proportion of patients who do not have the problem of dysphagia [6▪,19]. Weight loss is also not fully explained by denervation-induced muscle atrophy. This reinforces weight loss is not a mere consequence of disease progression but rather an initial symptom. Furthermore, the weight alteration (as low as 5% of initial body weight) is adversely associated with the survival of ALS patients either in the total ALS population [6▪,20–22] or after invasive ventilation [23]. This deleterious effect of weight change is observed in various genetic backgrounds, either European or Asian populations [19,24], which makes it an important prognostic factor [6▪].

MECHANISMS OF SYSTEMIC ENERGY METABOLISM IMPAIRMENT

What causes weight loss in ALS patients? Weight loss could be caused either by increased energy expenditure or decreased energy intake [4] (Fig. 1). Interestingly, premorbid physical activity, that increases energy expenditure, has been repeatedly associated with ALS risk [25,26].

Besides, a large proportion of ALS patients display higher than predicted resting energy expenditure before any intervention with ventilatory support [27,28]. In 2021, it is accepted that hypermetabolism is observed in ALS patients [29] and mouse models [30,31], and is correlated with greater functional decline [32,33]. On a theoretical note, however, it is important to realize that the concept of hypermetabolism has been discussed for many years and has to be considered with caution, particularly in animal models. The relationship between energy expenditure and body weight has to be used with particular caution [34]. The traditional normalisation by body weight in small mammals is questionable, whereas the Harris and Benedict equation, used in humans, considers several physiological parameters and describes more accurately energy expenditure [35,36]. A re-evaluation of hypermetabolism in ALS is therefore necessary.

Decreased energy intake is a mechanism observed during the progression of ALS symptoms, which ultimately causes difficulties in eating and changes in dietary habits, resulting from dysphagia (Fig. 1). However, more recent studies converge to show that weight alteration occurs in patients even in the absence of early dysphagia [6▪,19]. Studies quantitating the appetite of ALS patients using appetite questionnaires demonstrated that ALS patients display loss of appetite [37], which is unrelated to bulbar symptoms and dysphagia [38]. Most importantly, this loss of appetite worsens with disease progression and is correlated with loss of weight and fat mass [38,39]. These changes in eating behaviour are related to cognitive defects and distinguish between patients with pure ALS and those with cognitive impairment [40]. It cannot be excluded that neuromuscular respiratory failure could also in itself, possibly through CO_2 retention or hypoxia, cause loss of appetite [41].

Weight and appetite are controlled centrally and these pathways are affected in ALS [42,43▪]. The key brain region in the process is the hypothalamus which finely controls the energy homeostasis [44] (Fig. 1). Arcuate nucleus neurons such as pro-

CE: D.C.; WCO/340511; Total nos of Pages: 8;
WCO 340511

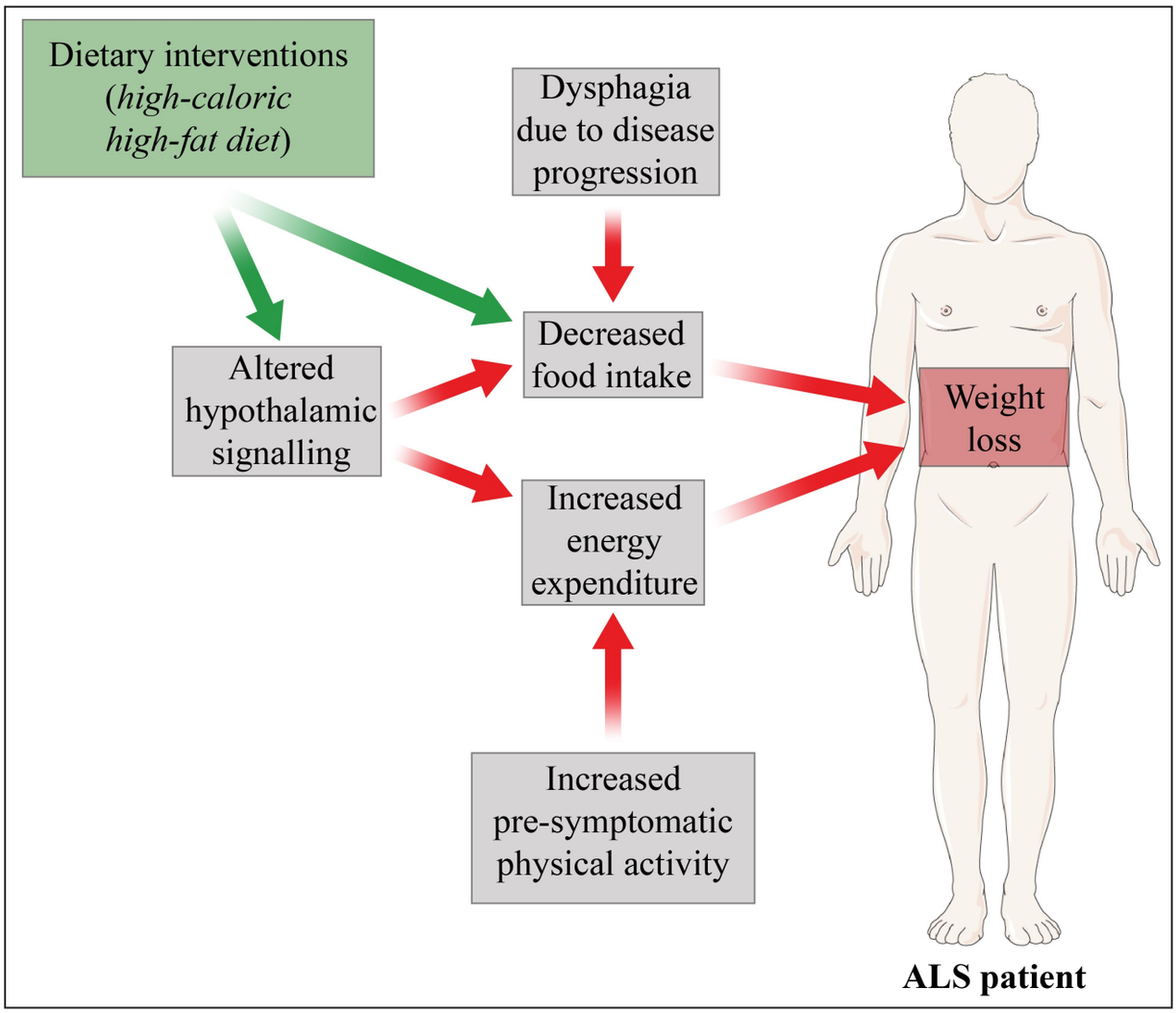

FIGURE 1. Mechanisms of weight loss in ALS patients. ALS, amyotrophic lateral sclerosis.

opiomelanocortin (POMC)- and agouti-related peptide (AgRP)-expressing cells are the first-order neurons responsive to energy status, and respectively decrease or increase food intake. Interestingly several studies showed that the hypothalamus accumulates TDP-43 lesions [45,46▪▪], especially the lateral hypo-thalamic area [46▪▪] (Fig. 2). Indeed, the hypothala-mus appears atrophied, as measured using Magnetic resonance imaging [47], and hypothalamic connec-tivity is altered in both patients and ALS mice [48▪▪]. Functional evidence linking hypothalamic neuro-modulators to weight loss remains scarce. Indirect evidence pointed out that the hypothalamus of ALS patients does not adequately respond to food intake inducing cues. ALS patients exposed to the antidiabetic drug, pioglitazone, did not gain weight, whereas this drug acts selectively on hypothalamic POMC neurons to increase body weight. Consistently, pioglitazone did not increase food intake in ALS mice [49]. Such blunted pioglitazone response is likely due to a defect in melanocortinergic neurons, as POMC expression is decreased and AgRP expression is increased in ALS mice [49]. Independent studies showed increased levels of neuropeptide Y, a neuropeptide co-expressed with AgRP, in ALS patients [15].

The hypothalamic involvement in ALS remains, however, to be disentangled, and a recent study showed a decreased orexin expression in lateral hypothalamus neurons, that could contribute to weight loss [46▪▪] (Fig. 2). Further functional investigation of these hypothalamic neuronal subtypes in ALS weight loss is warranted [50]. However, ablation of leptin, an adipocyte-derived hormone acting on the hypothalamus to induce satiety, in mutant mice expressing the *SOD1* ALS-related mutation, increased lifespan and improved muscle function

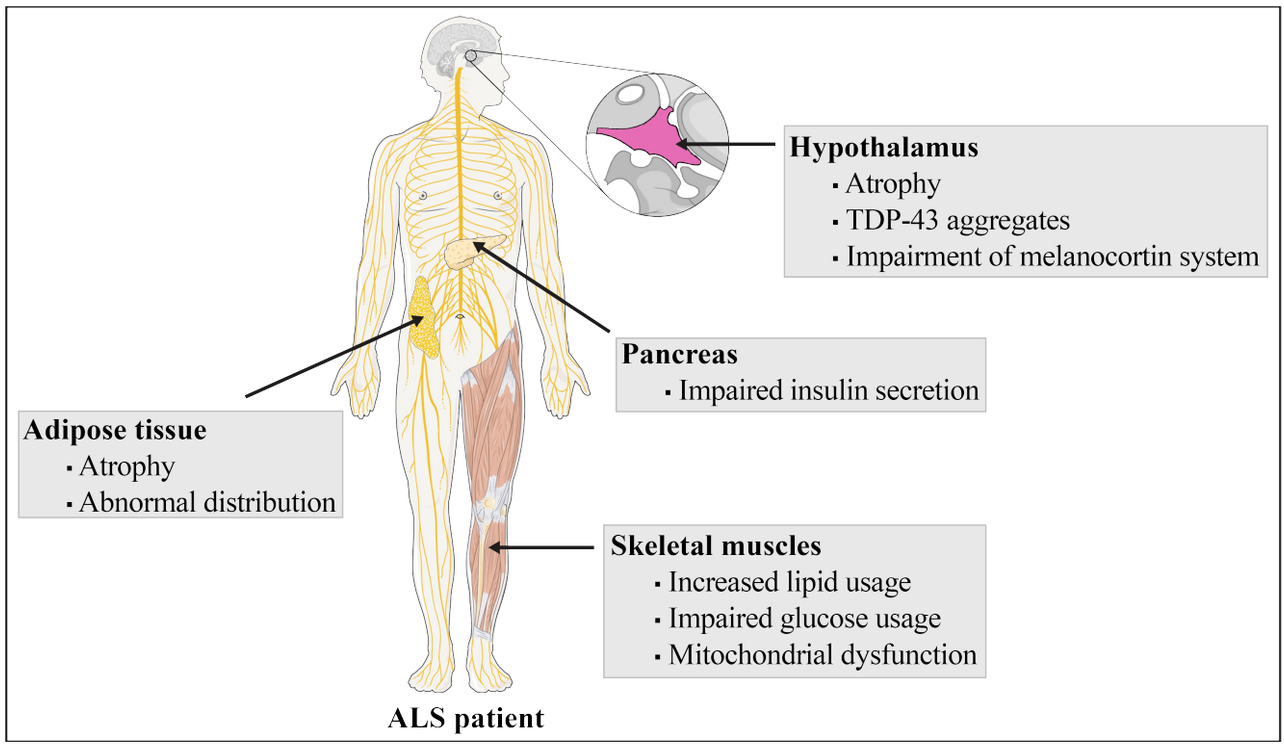

FIGURE 2. Systemic metabolic alterations in ALS patients and models. ALS, amyotrophic lateral sclerosis.

[31], indirectly suggesting the relevance of the hypothalamus to ALS progression.

ABNORMAL METABOLIC FLUXES IN AMYOTROPHIC LATERAL SCLEROSIS

Abnormal usage of nutrients might underlie weight loss. In the skeletal muscle of a mouse model expressing mutant SOD1, Palamiuc *et al.* showed that lipid metabolism was favoured over glucose at an early stage of the disease onset (Fig. 2). Consistently, mutant SOD1 mice showed an increased mitochondrial β-oxidation, indicative of increased lipid catabolism [51▪]. Early in the disease progression a decreased respiratory exchange ratio [30,51▪], altered gene expression [30,51▪,52] and an increased oxidative capacity [51▪] were described. The increased lipid catabolism is conversely associated with decreased glucose oxidation. Thus, mutant SOD1 mice develop progressive glucose intolerance as a consequence of reduced usage of muscle glucose [51▪,52,53]. Interestingly, *SOD1* is not the only ALS related gene whose manipulation in transgenic animals modifies the usage of metabolic fuels. The overexpression of mutant A315T TDP-43 leads to increased adipose tissue and abnormal muscle response to insulin [54], whereas conditional knock-out of TDP-43 dramatically increased the usage of lipids. The latter results in loss of adipose tissue and death within days [55]. Such alterations in fuel usage may be relevant to ALS patients since a similar increase in β-oxidation was observed in patients-derived primary myotubes [56▪▪] and in patient-derived fibroblasts [57]. Further-more, these altered metabolic fluxes would be con-sistent with the trend of ALS patients to develop paradoxical insulin resistance as well as with the protective effects of increased blood lipids in ALS [17,58]. Moreover, cells carrying the *C9ORF72* muta-tion also exhibited substrate-specific alterations of bioenergetic substrates, in particular for pyruvate and glycogen [59]. These altered metabolic fluxes could constitute a compensatory response to abnormal mitochondrial function [30,51▪]. Multiple pieces of evidence point to mitochondrial damage in response to mutant proteins associated with ALS, such as SOD1, TDP-43 and Fused in sarcoma (FUS) [60–62], particularly in muscles and axons. These impaired mitochondria in muscles are located at the neuromuscular junctions [63–65] which suggest that they participate in neuromuscular junction demise [61,66,67].

HOW COULD ALTERED METABOLIC FLUXES PARTICIPATE IN AMYOTROPHIC LATERAL SCLEROSIS?

Pharmacological evidence suggests that the metabolic shift towards lipid use participates in disease

progression. Dichloroacetate, an inhibitor of pyruvate dehydrogenase kinase, improves mitochondrial functions in mutant *SOD1* mice resulting in a slower weight loss [52]. Furthermore, ranolazine, a compound known to decrease β-oxidation was able to temporarily restore metabolic homeostasis, although it did not improve survival [51▪]. Strikingly, downregulating the key enzyme responsible for fatty acid import into mitochondria, carnithine palmitoyltransferase 1, was able to slow down disease progression [68▪▪].

Genetic interventions aiming at genetically boosting mitochondrial biogenesis, through overexpression of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α), was able to prevent muscle atrophy and improve mitochondrial function by restoration of muscle signalling and ATP consumption [69]. Conversely, knocking out a major muscle isoform of PGC-1α was detrimental to male mutant SOD1 mice [70]. The effects of PGC-1α overexpression appear however discordant across studies, and, more recent evidence suggests that overexpressing PGC-1α would not prevent or delay the onset of the disease [30,71].

An alternative strategy to manipulate metabolic fluxes could be to favour glucose usage. A recent study demonstrated that increased expression of GLUT-3 (glucose transporter 3) and phosphofructokinase in TDP-43 fly models was neuroprotective in the nervous system, but not in muscles [72]. Similar results were obtained in fly and cell models of *C9ORF72* ALS through the improvement of insulin signalling [73]. This indirectly suggests that improvement of glucose metabolism could modify disease progression. Such therapeutic strategies aiming at reorientating energy metabolism to glucose usage have however not yet been translated through the clinic.

DIETARY MODULATION OF METABOLISM IS EFFICIENT IN MODELS AND PATIENTS

Dietary improvement of patients could also potently modify energy metabolism (Fig. 1). In 2004, we reported that a high-fat diet could improve metabolic status, prevent the loss of motor neurons, and extend survival in mutant SOD1 mice [30]. More recently, similar results were established in TDP-43 mutant mice using a high-fat diet to show reduced oxidative stress and improved presymptomatic state [74]. It was shown recently however that very high-fat content was associated with poorer outcomes in mutant *SOD1* mice [68▪▪], suggesting a bell-shaped curve in the protection mediated by high caloric intake in mouse models.

The first clinical trial was performed by Wills and collaborators, which observed improved survival in gastrostomised patients fed with high caloric diets as compared with an isocaloric diet [75,76]. Lately, the lipid supplementation in LIPCAL-ALS trial observed a protective effect of dietary lipid supplementation on weight loss and survival of fast progressing ALS patients [77▪▪], although the trial did not meet its primary endpoint on the total ALS population. This dietary intervention decreased circulating neurofilament levels, consistent with a neuroprotective action [78]. Further studies, in particular with prespecification of the analysis of fast-progressing patients, are warranted to confirm these initial studies. Other dietary interventions could prove useful in ALS but have not reached the stage of clinical studies. Ketone bodies or ketogenic diets have shown potential in mutant SOD1 mice [79] and fly models of TDP-43 ALS [80], and remain to be further tested.

Several studies have suggested that intake of polyunsaturated fatty acids could protect against ALS [81,82]. However, no clinical trial has yet tested whether there could be differences in efficacy depending on the degree of unsaturation.

HOW ARE AMYOTROPHIC LATERAL SCLEROSIS-RELATED MUTATIONS INTERFERING WITH ENERGY METABOLISM?

ALS is a disease with a prominent genetic contribution, with 4 major genes (*SOD1, C9ORF72, TARDBP* encoding TDP-43 and *FUS*). How these genetic mutations cause or contribute to metabolic defects remain incompletely characterized. Mutations in *SOD1* were the first genetic causes of ALS identified, and most experimental studies still use transgenic mice expressing mutant forms of *SOD1*. How mutant SOD1s might precipitate weight loss and shift energy metabolism remains unclear. Several lines of evidence suggest that *SOD1* mutant proteins might affect mitochondrial function, through interaction with major mitochondrial proteins such as VDAC1 [83] or Bcl-2 [84], leading to oxidative stress, damage to mitochondrial RNA [85] and mitochondrial damage [86–90]. Further work should dissect the relative contributions of these different mechanisms, and their respective contributions in specific cell types.

As for *SOD1*, mutations in *TARDBP*, encoding TDP-43 appear to have important effects on mitochondrial physiology, that could possibly underlie dysfunction in energy homeostasis. Indeed, aggregation of TDP-43 has been reported to sequester several key mRNAs which encode nuclear-encoded mitochondrial proteins [91]. A fraction of TDP-43 enters the mitochondria and affects mitochondrial function through different mechanisms including

alterations in mitochondrial transcript metabolism, activation of the mitochondrial transition pore or the mitochondrial unfolded protein response [92–94]. TDP-43 might also have effects on energy metabolism beyond mitochondria and has been shown to directly regulate insulin secretion (Fig. 2) through regulation of a circular RNA encoded by the insulin gene [95▪] and through regulation of the expression of the calcium channel CaV1.2 in pancreatic β-cells [96▪].

Like TDP-43, FUS is an RNA binding protein involved in multiple aspects of RNA metabolism in both ALS and ALS-FTD. FUS has been reported to regulate the stress response and mitochondrial functions, such as transport and autophagy processes [97]. FUS modulates mitochondrial oxidative stress in muscles by inhibiting the action of the heat shock protein 60 [97,98]. The *FUS* gene appears to encode for a second polypeptide, called altFUS, that is mitochondrially targeted and required for the toxic effects in *FUS* mutants [99].

Expansion of a hexanucleotide repeat in the *C9ORF72* gene is associated with both ALS and ALS-FTD and leads to motor neuron toxicity through multiple mechanisms, including haploinsufficiency of the *C9ORF72* gene product and toxicity of RAN-translated di-peptide protein repeats. Although defective mitochondria have been found in both mutant models and ALS and ALS-FTD patients carrying the *C9ORF72* mutation [100,101], knowledge about those mitochondrial alterations are sparse [102]. It has been shown that the *C9ORF72* protein might be involved in stabilizing mitochondrial complex I [103]. It remains however unclear how the hexanucleotide repeat expansion leads to mitochondrial defects.

Beyond familial ALS, a large proportion of ALS cases do not have familial history. *ACSL5* was recently highlighted in a genome-wide association study as a genetic factor increasing ALS risk. *ACLS5* is known to convert free long-chain fatty acids into fatty-acid-coenzyme A, thereby, playing a key role in lipid biosynthesis and fatty acids degradation [61]. Overexpression of *ACSL5* was associated with rapid weight loss, as has *GPX3*, another ALS-related gene [104,105,106▪▪]. Additional loci, including *B4GALNT1*, *G2E3-SCFD1* and *TRIP11-ATXN3*, have been identified with a gene-based analysis as disease-associated genes [106▪▪]. This study suggests that there are genetic contributions to the observed alterations in energy metabolism in ALS patients, beyond familial ALS. Further investigations are required to identify additional mechanisms and decipher their role and contributions to the observed metabolic defects.

CONCLUSION

Weight loss has emerged in the last ten years as a strong prognostic marker in ALS. Clinical, epidemiological, and experimental evidence all converge to highlight the importance of this process in disease progression. The recent clinical studies are very encouraging in pursuing nutritional therapeutic trials and identifying anatomical and biological elements involved in weight loss, as well as subpopulations of patients that might benefit from dietary adaptations. Dietary correction of the dysregulated energy metabolism in ALS is likely to emerge as a future disease-modifying approach in ALS.

Acknowledgements

Substantial contribution beyond the authors: none.

Financial support and sponsorship

Work related to the topic of this review was supported by Fondation pour la recherche médicale (FRM, DEQ20180339179), Axa Research Funds (rare diseases award 2019, to L.D.), Fondation Thierry Latran (HypmotALS, to L.D.). L.D. laboratory is also supported by MNDA (Dupuis/Apr16/852-791 to L.D.), Agence Nationale de la Recherche (ANR-16-CE92-0031, ANR-16-CE16-0015, ANR-19-CE17-0016, ANR-20-CE17-0008). L.D. is USIAS fellow 2019. S.J.G. is funded by a PhD fellowship from Inserm and the Région Grand Est (France).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

1. Brown RH, Al-Chalabi A. Amyotrophic lateral sclerosis. N Engl J Med 2017; 377:162–172.
2. van Es MA, Hardiman O, Chio A, *et al.* Amyotrophic lateral sclerosis. Lancet 2017; 390:2084–2098.
3. Desport JC, Preux PM, Truong TC, *et al.* Nutritional status is a prognostic factor for survival in ALS patients. Neurology 1999; 53:1059–1063.
4. Dupuis L, Pradat PF, Ludolph AC, Loeffler JP. Energy metabolism in amyotrophic lateral sclerosis. Lancet Neurol 2011; 10:75–82.
5. Desport JC, Preux PM, Truong CT, *et al.* Nutritional assessment and survival in ALS patients. Amyotroph Lateral Scler Other Motor Neuron Disord 2000; 1:91–96.
6. ▪ 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; 91:867–875.

This study provides a very comprehensive characterization of weight loss in an extended population of patients.

7. Wei Q, Ou R, Chen Y, *et al.* RNM-01 Weight stability is associated with longer survival in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2019; 20(sup1):309–326.
8. Wei QQ, Ou R, Cao B, *et al.* Early weight instability is associated with cognitive decline and poor survival in amyotrophic lateral sclerosis. Brain Res Bull 2021; 171:10–15.

9. Gallo V, Wark PA, Jenab M, *et al.* Prediagnostic body fat and risk of death from amyotrophic lateral sclerosis: the EPIC cohort. Neurology 2013; 80:829–838.

10. O'Reilly EJ, Wang H, Weisskopf MG, *et al.* Premorbid body mass index and risk of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2013; 14:205–211.

11. O'Reilly EJ, Wang M, Adami HO, *et al.* Prediagnostic body size and risk of amyotrophic lateral sclerosis death in 10 studies. Amyotroph Lateral Scler Frontotemporal Degener 2018; 19:396–406.

12. 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; 7:4374.

13. 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; 32:901–908.

14. Pradat PF, Bruneteau G, Gordon PH, *et al.* Impaired glucose tolerance in patients with amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2010; 11:166–171.

15. Ahmed RM, Phan K, Highton-Williamson E, *et al.* Eating peptides: biomarkers of neurodegeneration in amyotrophic lateral sclerosis and frontotemporal dementia. Ann Clin Transl Neurol 2019; 6:486–495.

16. Dorst J, Kuhnlein P, Hendrich C, *et al.* Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. J Neurol 2011; 258:613–617.

17. Dupuis L, Corcia P, Fergani A, *et al.* Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. Neurology 2008; 70:1004–1009.

18. Lindauer E, Dupuis L, Muller HP, *et al.* Adipose tissue distribution predicts survival in amyotrophic lateral sclerosis. PLoS One 2013; 8:e67783.

19. 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; 90:666–673.

20. Marin B, Arcuti S, Jesus P, *et al.* Population-based evidence that survival in amyotrophic lateral sclerosis is related to weight loss at diagnosis. Neurodegener Dis 2016; 16:225–234.

21. Mariosa D, Beard JD, Umbach DM, *et al.* Body mass index and amyotrophic lateral sclerosis: a study of US Military Veterans. Am J Epidemiol 2017; 185:362–371.

22. Shimizu T, Nakayama Y, Matsuda C, *et al.* Prognostic significance of body weight variation after diagnosis in ALS: a single-centre prospective cohort study. J Neurol 2019; 266:1412–1420.

23. Nakayama Y, Shimizu T, Matsuda C, *et al.* Body weight variation predicts disease progression after invasive ventilation in amyotrophic lateral sclerosis. Sci Rep 2019; 9:12262.

24. Dorst J, Chen L, Rosenbohm A, *et al.* Prognostic factors in ALS: a comparison between Germany and China. J Neurol 2019; 266:1516–1525.

25. Harwood CA, Westgate K, Gunstone S, *et al.* Long-term physical activity: an exogenous risk factor for sporadic amyotrophic lateral sclerosis? Amyotroph Lateral Scler Frontotemporal Degener 2016; 17:377–384.

26. Lacorte E, Ferrigno L, Leoncini E, *et al.* Physical activity, and physical activity related to sports, leisure and occupational activity as risk factors for ALS: a systematic review. Neurosci Biobehav Rev 2016; 66:61–79.

27. 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.

28. 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.

29. Bouteloup C, Desport JC, Clavelou P, *et al.* Hypermetabolism in ALS patients: an early and persistent phenomenon. J Neurol 2009; 256: 1236–1242.

30. Dupuis L, Oudart H, Rene F, *et al.* Evidence for defective energy homeostasis in amyotrophic lateral sclerosis: benefit of a high-energy diet in a transgenic mouse model. Proc Natl Acad Sci USA 2004; 101:11159–11164.

31. Lim MA, Bence KK, Sandesara I, *et al.* Genetically altering organismal metabolism by leptin-deficiency benefits a mouse model of amyotrophic lateral sclerosis. Hum Mol Genet 2014; 23:4995–5008.

32. Ferri A, Coccurello R. What is 'Hyper' in the ALS hypermetabolism? Mediators Inflamm 2017; 2017:7821672.

33. 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.

34. Tschop MH, Speakman JR, Arch JR, *et al.* A guide to analysis of mouse energy metabolism. Nat Methods 2011; 9:57–63.

35. Weijs PJ. Hypermetabolism, is it real? The example of amyotrophic lateral sclerosis. J Am Diet Assoc 2011; 111:1670–1673.

36. Ellis AC, Rosenfeld J. Which equation best predicts energy expenditure in amyotrophic lateral sclerosis? J Am Diet Assoc 2011; 111:1680–1687.

37. Holm T, Maier A, Wicks P, *et al.* Severe loss of appetite in amyotrophic lateral sclerosis patients: online self-assessment study. Interact J Med Res 2013; 2:e8.

38. Mezoian T, Belt E, Garry J, *et al.* Loss of appetite in amyotrophic lateral sclerosis is associated with weight loss and decreased calorie consumption independent of dysphagia. Muscle Nerve 2020; 61:230–234.

39. Steyn FJ, Ngo ST. Prognostic value of weight loss in patients with amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2020; 91:813.

40. Ahmed RM, Caga J, Devenney E, *et al.* Cognition and eating behavior in amyotrophic lateral sclerosis: effect on survival. J Neurol 2016; 263: 1593–1603.

41. Matu J, Gonzalez JT, Ispoglou T, *et al.* The effects of hypoxia on hunger perceptions, appetite-related hormone concentrations and energy intake: a systematic review and meta-analysis. Appetite 2018; 125:98–108.

42. Abdalla MM. Central and peripheral control of food intake. Endocr Regul 2017; 51:52–70.

43. ▪ 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; 20:497–505.

This study relates loss of appetite to weight loss and fat mass in patients.

44. Zeltser LM, Seeley RJ, Tschop MH. Synaptic plasticity in neuronal circuits regulating energy balance. Nat Neurosci 2012; 15:1336–1342.

45. Cykowski MD, Takei H, Schulz PE, *et al.* TDP-43 pathology in the basal forebrain and hypothalamus of patients with amyotrophic lateral sclerosis. Acta Neuropathol Commun 2014; 2:171.

46. ▪▪ Gabery S, Ahmed RM, Caga J, *et al.* Loss of the metabolism and sleep regulating neuronal populations expressing orexin and oxytocin in the hypothalamus in amyotrophic lateral sclerosis. Neuropathol Appl Neurobiol 2021. doi: 10.1111/nan.12709.

A first study documenting pathological changes in specific neuronal populations in ALS hypothalamus.

47. Gorges M, Roselli F, Muller HP, *et al.* Functional connectivity mapping in the animal model: principles and applications of resting-state fMRI. Front Neurol 2017; 8:200.

48. ▪▪ Bayer D, Antonucci S, Muller HP, *et al.* Disruption of orbitofrontal-hypothalamic projections in a murine ALS model and in human patients. Transl Neurodegener 2021; 10:17.

The first evidence in mouse models and patients of altered hypothalamic connectivity in ALS.

49. Vercruysse P, Sinniger J, El Oussini H, *et al.* Alterations in the hypothalamic melanocortin pathway in amyotrophic lateral sclerosis. Brain 2016; 139(Pt 4):1106–1122.

50. Ahmed RM, Steyn F, Dupuis L. Hypothalamus and weight loss in amyotrophic lateral sclerosis. Handb Clin Neurol 2021; 180:327–338.

51. ▪ Scaricamazza S, Salvatori I, Giacovazzo G, *et al.* Skeletal-muscle metabolic reprogramming in ALS-SOD1(G93A) mice predates disease onset and is a promising therapeutic target. iScience 2020; 23:101087.

A thorough study documenting alterations in muscle metabolic pathways in mouse models and providing potential pharmacological strategies.

52. Palamiuc L, Schlagowski A, Ngo ST, *et al.* A metabolic switch toward lipid use in glycolytic muscle is an early pathologic event in a mouse model of amyotrophic lateral sclerosis. EMBO Mol Med 2015; 7:526–546.

53. McDonald TS, Kumar V, Fung JN, *et al.* Glucose clearance and uptake is increased in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis through an insulin-independent mechanism. FASEB J 2021; 35:e21707.

54. Stallings NR, Puttaparthi K, Dowling KJ, *et al.* TDP-43, an ALS linked protein, regulates fat deposition and glucose homeostasis. PLoS One 2013; 8:e71793.

55. Shan X, Chiang PM, Price DL, Wong PC. Altered distributions of Gemini of coiled bodies and mitochondria in motor neurons of TDP-43 transgenic mice. Proc Natl Acad Sci USA 2010; 107:16325–16330.

56. ▪▪ Steyn FJ, Li R, Kirk SE, *et al.* Altered skeletal muscle glucose-fatty acid flux in amyotrophic lateral sclerosis. Brain Commun 2020; 2:fcaa154.

The first evidence in patients of altered metabolic fluxes in skeletal muscle.

57. Gerou M, Hall B, Woof R, *et al.* Amyotrophic lateral sclerosis alters the metabolic aging profile in patient derived fibroblasts. Neurobiol Aging 2021; 105:64–77.

58. Delaye JB, Patin F, Piver E, *et al.* Low IDL-B and high LDL-1 subfraction levels in serum of ALS patients. J Neurol Sci 2017; 380:124–127.

59. Allen SP, Hall B, Woof R, *et al.* C9orf72 expansion within astrocytes reduces metabolic flexibility in amyotrophic lateral sclerosis. Brain 2019; 142: 3771–3790.

60. Ravanidis S, Doxakis E. RNA-binding proteins implicated in mitochondrial damage and mitophagy. Front Cell Dev Biol 2020; 8:372.

61. Ruffoli R, Bartalucci A, Frati A, Fornai F. Ultrastructural studies of ALS mitochondria connect altered function and permeability with defects of mitophagy and mitochondriogenesis. Front Cell Neurosci 2015; 9:341.

62. Wang P, Deng J, Dong J, *et al.* TDP-43 induces mitochondrial damage and activates the mitochondrial unfolded protein response. PLoS Genet 2019; 15:e1007947.

63. Dupuis L, Loeffler JP. Neuromuscular junction destruction during amyotrophic lateral sclerosis: insights from transgenic models. Curr Opin Pharmacol 2009; 9:341–346.

64. Khalil B, Cabirol-Pol MJ, Miguel L, *et al.* Enhancing Mitofusin/Marf ameliorates neuromuscular dysfunction in Drosophila models of TDP-43 proteinopathies. Neurobiol Aging 2017; 54:71–83.

65. Smith EF, Shaw PJ, De Vos KJ. The role of mitochondria in amyotrophic lateral sclerosis. Neurosci Lett 2019; 710:132933.

66. Briese M, Saal-Bauernschubert L, Luningschror P, *et al.* Loss of Tdp-43 disrupts the axonal transcriptome of motoneurons accompanied by impaired axonal translation and mitochondria function. Acta Neuropathol Commun 2020; 8:116.

67. Oakes JA, Davies MC, Collins MO. TBK1: a new player in ALS linking autophagy and neuroinflammation. Mol Brain 2017; 10:5.

68. ■■ Trabjerg MS, Andersen DC, Huntjens P, *et al.* Downregulating carnitine palmitoyl transferase 1 affects disease progression in the SOD1 G93A mouse model of ALS. Commun Biol 2021; 4:509.

A comprehensive study showing that genetic interventions aiming at rewiring metabolic fluxes can be beneficial in a mouse model of ALS. This study also brings a note of caution on extreme high fat diets, that appear detrimental.

69. Da Cruz S, Parone PA, Lopes VS, *et al.* Elevated PGC-1alpha activity sustains mitochondrial biogenesis and muscle function without extending survival in a mouse model of inherited ALS. Cell Metab 2012; 15:778–786.

70. Eschbach J, Schwalenstocker B, Soyal SM, *et al.* PGC-1alpha is a male-specific disease modifier of human and experimental amyotrophic lateral sclerosis. Hum Mol Genet 2013; 22:3477–3484.

71. Sanchis-Gomar F, Garcia-Gimenez JL, Gomez-Cabrera MC, Pallardo FV. Mitochondrial biogenesis in health and disease. Molecular and therapeutic approaches. Curr Pharm Des 2014; 20:5619–5633.

72. Manzo E, Lorenzini I, Barrameda D, *et al.* Glycolysis upregulation is neuroprotective as a compensatory mechanism in ALS. Elife 2019; 8:e58565. doi: 10.7554/eLife.45114.

73. Atilano ML, Gronke S, Niccoli T, *et al.* Enhanced insulin signalling ameliorates C9orf72 hexanucleotide repeat expansion toxicity in Drosophila. Elife 2021; 10:e58565.

74. Coughlan KS, Halang L, Woods I, Prehn JH. A high-fat jelly diet restores bioenergetic balance and extends lifespan in the presence of motor dysfunction and lumbar spinal cord motor neuron loss in TDP-43A315T mutant C57BL6/J mice. Dis Model Mech 2016; 9:1029–1037.

75. Al-Chalabi A. High-calorie diets in amyotrophic lateral sclerosis. Lancet 2014; 383:2028–2030.

76. Wills AM, 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; 383:2065–2072.

77. ■■ Ludolph AC, Dorst J, Dreyhaupt J, *et al.* Effect of high-caloric nutrition on survival in amyotrophic lateral sclerosis. Ann Neurol 2020; 87:206–216.

Although this clinical trial did not meet its primary endpoint, posthoc analysis suggest efficacy of high caloric high lipid nutrition in a large population of fast progressing patients.

78. Dorst J, Schuster J, Dreyhaupt J, *et al.* Effect of high-caloric nutrition on serum neurofilament light chain levels in amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2020; 91:1007–1009.

79. Zhao Z, Lange DJ, Voustianiouk A, *et al.* A ketogenic diet as a potential novel therapeutic intervention in amyotrophic lateral sclerosis. BMC Neurosci 2006; 7:29.

80. Manzo E, O'Conner AG, Barrows JM, *et al.* Medium-chain fatty acids, beta-hydroxybutyric acid and genetic modulation of the carnitine shuttle are protective in a drosophila model of ALS based on TDP-43. Front Mol Neurosci 2018; 11:182.

81. Fitzgerald KC, O'Reilly EJ, Falcone GJ, *et al.* Dietary omega-3 polyunsaturated fatty acid intake and risk for amyotrophic lateral sclerosis. JAMA Neurol 2014; 71:1102–1110.

82. Veldink JH, Kalmijn S, Groeneveld GJ, *et al.* Intake of polyunsaturated fatty acids and vitamin E reduces the risk of developing amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2007; 78:367–371.

83. Israelson A, Arbel N, Da Cruz S, *et al.* Misfolded mutant SOD1 directly inhibits VDAC1 conductance in a mouse model of inherited ALS. Neuron 2010; 67:575–587.

84. Pedrini S, Sau D, Guareschi S, *et al.* ALS-linked mutant SOD1 damages mitochondria by promoting conformational changes in Bcl-2. Hum Mol Genet 2010; 19:2974–2986.

85. Chang Y, Kong Q, Shan X, *et al.* Messenger RNA oxidation occurs early in disease pathogenesis and promotes motor neuron degeneration in ALS. PLoS One 2008; 3:e2849.

86. Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? Nat Med 2004; 10(Suppl 7):S18–25.

87. Cui H, Kong Y, Zhang H. Oxidative stress, mitochondrial dysfunction, and aging. J Signal Transduct 2012; 2012:646354.

88. Ferraiuolo L, Higginbottom A, Heath PR, *et al.* Dysregulation of astrocyte-motoneuron cross-talk in mutant superoxide dismutase 1-related amyotrophic lateral sclerosis. Brain 2011; 134(Pt 9):2627–2641.

89. Kirkinezos IG, Bacman SR, Hernandez D, *et al.* Cytochrome c association with the inner mitochondrial membrane is impaired in the CNS of G93A-SOD1 mice. J Neurosci 2005; 25:164–172.

90. Mattiazzi M, D'Aurelio M, Gajewski CD, *et al.* Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. J Biol Chem 2002; 277:29626–29633.

91. Zuo X, Zhou J, Li Y, *et al.* TDP-43 aggregation induced by oxidative stress causes global mitochondrial imbalance in ALS. Nat Struct Mol Biol 2021; 28:132–142.

92. Izumikawa K, Nobe Y, Yoshikawa H, *et al.* TDP-43 stabilises the processing intermediates of mitochondrial transcripts. Sci Rep 2017; 7:7709.

93. Wang W, Wang L, Lu J, *et al.* The inhibition of TDP-43 mitochondrial localization blocks its neuronal toxicity. Nat Med 2016; 22:869–878.

94. Yu CH, Davidson S, Harapas CR, *et al.* TDP-43 Triggers Mitochondrial DNA Release via mPTP to Activate cGAS/STING in ALS. Cell 2020; 183:636–649. e18.

95. ■ Stoll L, Rodriguez-Trejo A, Guay C, *et al.* A circular RNA generated from an intron of the insulin gene controls insulin secretion. Nat Commun 2020; 11:5611.

Demonstrate an unexpected role for TDP-43 in the direct regulation of insulin secretion.

96. ■ Araki K, Araki A, Honda D, *et al.* TDP-43 regulates early-phase insulin secretion via CaV1.2-mediated exocytosis in islets. J Clin Investig 2019; 129:3578–3593.

Demonstrate an unexpected role for TDP-43 in the direct regulation of insulin secretion.

97. Birsa N, Bentham MP, Fratta P. Cytoplasmic functions of TDP-43 and FUS and their role in ALS. Semin Cell Dev Biol 2020; 99:193–201.

98. Deng J, Yang M, Chen Y, *et al.* FUS interacts with HSP60 to promote mitochondrial damage. PLoS Genet 2015; 11:e1005357.

99. Brunet MA, Jacques JF, Nassari S, *et al.* The FUS gene is dual-coding with both proteins contributing to FUS-mediated toxicity. EMBO Rep 2021; 22:e50640.

100. Balendra R, Isaacs AM. C9orf72-mediated ALS and FTD: multiple pathways to disease. Nat Rev Neurol 2018; 14:544–558.

101. Odnokoz O, Nakatsuka K, Wright C, *et al.* Mitochondrial redox signaling is critical to the normal functioning of the neuronal system. Front Cell Dev Biol 2021; 9:613036.

102. Onesto E, Colombrita C, Gumina V, *et al.* Gene-specific mitochondria dysfunctions in human TARDBP and C9ORF72 fibroblasts. Acta Neuropathol Commun 2016; 4:47.

103. Wang T, Liu H, Itoh K, *et al.* C9orf72 regulates energy homeostasis by stabilizing mitochondrial complex I assembly. Cell Metab 2021; 33: 531–546. e9.

104. Langhardt J, Flehmig G, Kloting N, *et al.* Effects of weight loss on glutathione peroxidase 3 serum concentrations and adipose tissue expression in human obesity. Obes Facts 2018; 11:475–490.

105. Teng AC, Adamo K, Tesson F, Stewart AF. Functional characterization of a promoter polymorphism that drives ACSL5 gene expression in skeletal muscle and associates with diet-induced weight loss. FASEB J 2009; 23:1705–1709.

106. ■■ 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; 33:108323.

A very first study seeking to identify the genetic basis of weight loss in ALS, and linking weight loss to previously identified ALS risk factors.