

Coordinated adipose–liver metabolic remodelling reveals sex-specific peripheral adaptations during amyotrophic lateral sclerosis progression in TDP-43^{A315T} mice

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Abstract

Amyotrophic lateral sclerosis (ALS) is increasingly recognised as a systemic disorder associated with metabolic dysfunction, although the contribution of peripheral metabolic organs and sex-dependent responses remains unclear. We investigated metabolic remodelling across white adipose tissue (WAT), brown adipose tissue (BAT) and liver, during disease progression in the TDP-43^{A315T} mouse model and assessed its relevance in ALS patients. Lipidomic profiling was performed in WAT from male mice, while metabolic and endocrine pathways were evaluated by RT-qPCR in BAT and liver from male and female mice. Plasma cholesterol levels were determined in ALS patients at diagnosis. The results revealed that WAT exhibited marked alterations that were detectable at the asymptomatic stage and persisted throughout disease progression. Lipid remodelling was characterised by coordinated changes affecting lipid classes, with cardiolipins predominating at early stages and triacylglycerols becoming increasingly altered as disease progressed. BAT displayed predominantly late-stage transcriptional changes in male mice, characterised by increased expression of genes associated with brown adipocyte identity, metabolic regulation and leptin signalling. Female mice developed a distinct hepatic transcriptional signature characterised by increased expression of genes associated with fatty acid uptake and utilisation, together with reduced expression of genes involved in de novo lipogenesis. Furthermore, ALS patients revealed sex-associated differences in circulating cholesterol levels at diagnosis. Overall, ALS progression is associated with extensive metabolic remodelling in peripheral tissues, with marked tissue-specific and sex-dependent responses. These findings identify peripheral metabolism as a potential contributor to disease heterogeneity and highlight metabolic pathways as promising sources of translational biomarkers and therapeutic targets.

Key points

- Lipid alterations in WAT from male TDP-43 mice precede ALS symptom onset.
- BAT and liver show distinct metabolic adaptations in male and female ALS mice.
- Sex-related differences in cholesterol are present at ALS at diagnosis.
- Peripheral metabolism may contribute to ALS heterogeneity and future treatments.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disorder characterised by the selective degeneration of upper and lower motor neurons, ultimately leading to muscle weakness, paralysis and respiratory failure [22]. Accumulating evidence indicates that ALS extends beyond the nervous system and should be considered a multisystem disorder involving profound alterations in peripheral tissues and whole-body metabolism [39]. Metabolic disturbances frequently occur during disease progression and may precede the onset of overt neurological symptoms. Furthermore, metabolic status has emerged as an important determinant of disease progression and survival [39, 44].

Among the systemic abnormalities described in ALS, disturbances in lipid metabolism have attracted increasing attention. Clinical studies have reported alterations in circulating cholesterol, triglycerides and lipoproteins, although findings remain heterogeneous across cohorts [8, 11, 32]. Epidemiological and experimental evidence further suggests that body composition and adiposity influence disease progression [25, 29], supporting an active contribution of peripheral metabolic tissues to ALS pathogenesis.

Adipose tissue has emerged as a key regulator of systemic metabolism in ALS [12, 29, 34]. White adipose tissue (WAT) functions as both an energy reservoir and an endocrine organ through the secretion of adipokines such as leptin and adiponectin, whereas brown adipose tissue (BAT) primarily regulates energy expenditure through thermogenesis. Together with the liver, these organs form an integrated network that maintains systemic energy and lipid homeostasis [5, 38, 41]. Consistent with their metabolic relevance, ALS patients frequently exhibit reduced fat mass, altered body composition and hypermetabolism [25, 29], while experimental studies have identified substantial alterations in adipose tissue metabolism and adipokine signalling in ALS models [14, 18]. Furthermore, genetic manipulation of *TARDBP*, the gene encoding TDP-43, has been shown to alter body composition, adiposity and glucose homeostasis, indicating that TDP-43-dependent pathways can regulate peripheral metabolism independently of motor neuron degeneration [7]. However, the molecular mechanisms underlying these alterations and their contribution to disease pathogenesis remain poorly understood.

Mutations in *TARDBP* represent one of the major genetic causes of familial ALS [43], while pathological TDP-43 aggregation is observed in the vast majority of sporadic cases [2, 33]. Beyond its central role in RNA metabolism, accumulating evidence suggests that TDP-43 influences systemic metabolic processes, affecting adiposity, glucose regulation and lipid metabolism [4, 7]. Nevertheless, most investigations have focused on individual tissues or specific metabolic endpoints, leaving the integrated effects of disease progression on metabolically interconnected organs largely unexplored.

An additional aspect that remains insufficiently characterised is the influence of biological sex on peripheral metabolic responses during ALS progression. Indeed, biological sex has emerged as an important modifier of ALS susceptibility, clinical presentation and metabolic regulation [31]. However, whether males and females exhibit distinct peripheral metabolic adaptations during disease progression remains largely unknown.

Given the close physiological interactions between adipose tissue and liver in the regulation of systemic energy and lipid homeostasis [38, 41], we hypothesised that ALS progression is associated with coordinated metabolic remodelling across multiple peripheral organs. Therefore, the aim of the present study was to characterise metabolic alterations in WAT, BAT and liver during disease progression in the TDP-43^{A315T} mouse model, with particular emphasis on lipid metabolism and sex-dependent responses. To address this, we combined lipidomic and transcriptional approaches to identify tissue-specific metabolic adaptations and subsequently explored the translational relevance of these findings through the analysis of circulating cholesterol levels in a cohort of ALS patients at diagnosis. By integrating

experimental and clinical data, this study seeks to provide a comprehensive view of peripheral metabolic dysfunction in ALS and to identify pathways that may contribute to disease progression and metabolic heterogeneity.

MATERIALS AND METHODS

Experimental Animals

A total of 60 mice (30 males and 30 females) were included in this study, including transgenic (Tg) TDP-43^{A315T} mice [46] and wild-type (WT) non-transgenic littermates (C57Bl/6). WT and Tg animals were age-matched across all experimental groups. Lipidomic analyses were performed exclusively in male mice, whereas molecular biology studies included samples from both sexes. Tissue samples were collected at three distinct stages of ALS disease progression, according to the clinical progression observed previously in male TDP-43^{A315T} mice [17] to ensure consistency (n = 5/stage): 1) at 42 days, 2) at 65 days and 3) at 95 days. Independent cohorts of animals were euthanized at each time point. The presence of the human TDP-43 (hTDP-43) transgene was confirmed by PCR according to the distributor's protocol.

To monitor disease onset and progression, all mice were weighed and assessed three times per week until the disease onset-stage, after which they were checked daily in the morning until the disease end-stage. The maintenance and use of mice and all experimental procedures were approved by the Animal Ethics Committee of the Hospital Nacional de Paraplégicos, Toledo (Spain) (Approval No 36-OH/2019) in accordance with the Spanish Guidelines for the Care and Use of Animals for Scientific Purposes. All analyses were conducted by personnel blinded to the animal genotype.

Perfusion and tissue collection

Animals were deeply anesthetized with sodium pentobarbital (140 mg/kg, i.p.) and subsequently perfused transcardially with 0.01 M phosphate buffered saline (PBS; pH 7.4). Following perfusion, perigonadal WAT, interscapular BAT and liver samples were rapidly collected, immediately frozen on dry ice and stored at -80 °C until further analysis. WAT was subsequently used for lipidomic analysis, whereas BAT and liver samples were used for real-time qPCR analysis (RT-qPCR).

Lipidome analysis

WAT samples (~10 mg) were subjected to lipid extraction following a chloroform/methanol-based protocol. Briefly, 100 µL of ice-cold 0.88% NaCl solution, EquiSPLASH internal standard (10 µL) and 500 µL of chloroform/methanol (2:1, v/v), both pre-cooled, were added to the samples. Homogenization was performed for 1 min using a Potter homogenizer. The homogenized suspensions were incubated at -40°C for 15 min and subsequently sonicated at 80% amplitude for 30 s. Samples were then incubated

again at -40°C for 15 min. This cycle of homogenization, incubation, and sonication was repeated three times to ensure efficient lipid extraction.

Samples were centrifuged at 14,000 rpm for 15 min at 4 °C. The top phase (aqueous mixture and methanol) was transferred to a clean vial and maintained at -40 °C. The bottom organic phase was transferred to a clean vial and evaporated under a nitrogen gas flow. It was then preserved at -40 °C until injection. Internal standard contained a mixture of 13 deuterated lipidic standards at 100 µg mL⁻¹ each: 15:0–18:1(d7) PC, 18:1(d7) Lyso PC, 15:0–18:1(d7) PE, 18:1(d7) Lyso PE, 15:0–18:1(d7) PG (Na Salt), 15:0–18:1(d7) PI (NH₄ Salt), 15:0–18:1(d7) PS (Na Salt), 15:0–18:1(d7)-15:0 TAG, 15:0–18:1(d7) DAG, 18:1(d7) MAG, 18:1(d7) Chol Ester, d18:1–18:1(d9) SM, C15 Ceramide-d7. Lipid extracts were resuspended in isopropanol:acetonitrile:ammonium acetate (10 mM) (50:30:20 vol) and diluted 100x in this same solution. Diluted extracts (500 µL) were then analysed through direct infusion in an ESI qQTOF (TripleTOF 6600+, Sciex) mass spectrometer equipped with a DuoSprayTM source (Sciex). Samples were analysed in both positive and negative ions mode with the MSMSALL acquisition mode, consisting of a TOF Ms scanning mode and a series of MSMS scanning modes stepped in mass intervals of 200–1200 umas. The conditions for the ionization source were optimized for both acquisition modes (nebulization gases GS1 10 psi and GS2 15 psi, curtain gas 20 psi, ion spray voltage + 5000/-4500 V, temperature 125 °C and declustering potential of 80 V). MSMSALL acquisition mode was controlled by the Analyst software (Sciex). All precursors were selected in Q1, with an isolation window of 1Da in the MS interval of 200 S12 to 1200 umas, where the precursor ions were equally distributed in the isolation windows. Collision energy for lipids in positive mode was CE 25 ± 15 V and CE -40 ± 15 V for those in negative mode. Data processing was carried out by using the LipidView v1.3 software. Lipid classes and species were identified based on m/z exacta and fragmentation patterns.

Data were normalized using multiple linear regression and imported into MetaboAnalyst v6.0.

RNA Isolation and qPCR analysis

Total RNA was isolated from BAT and liver using the NZY Total RNA Isolation Kit (NZYtech), according to the manufacturer's protocol. For BAT, complementary DNA (cDNA) was synthesized from 1 µg of total RNA and the relative quantification of *Adipoq*, *Fabp4*, *Glut4*, *Leptin*, *ObRb*, *Ppargc1a*, *Pparg*, *Prdm16*, *Ucp1* and *Tardbp* were determined as previously described [16]. For liver, cDNA synthesis was performed using 0.5 µg of total RNA and relative gene expression of *Acox1*, *Cd36*, *Cpt1a*, *Fabp1*, *Fasn*, *Fgf21*, *Ppara*, *Scd1* and *Srebf1* were quantified. *Actin* and *36b4* were used as housekeeping genes for normalization of gene expression levels [15, 21]. Relative quantification was calculated using the $\Delta\Delta C_t$ method [30]. Reactions were run in duplicates on a CFX96 Real-Time System instrument and software (CFX Manager 3.0) (BioRad). Primers were designed using NCBI/Primer-BLAST software and sequences are presented in Table 1.

Human plasma samples

All procedures performed in studies involving human samples (plasma) were in accordance with the Ethics Committee (783/23/98) of the Universidad San Pablo-CEU, Madrid, Spain. Human plasma samples from ALS patients and controls were provided by the Biobank HUB-ICO-IDIBELL (B.0000609), a member of the Xarxa de Bancs de Tumors de Catalunya (XBTC), supported by the Pla Director d'Oncologia de Catalunya. They were processed following standard operating procedures with the appropriate approval of the Ethics and Scientific Committees.

Patients were eligible for inclusion if they had diagnosis of ALS based on Gold Coast criteria [42]. Samples were collected at the hospital using Lithium heparin collecting tubes at the time of diagnosis. Samples were centrifuged at 10,000 rpm for 10 min. The supernatant was collected and frozen at -80 °C.

ALS patients were classified by sex, body mass index (BMI) and survival. Patients were classified as non-obese (BMI < 25) and obese (BMI ≥ 25) as previously described [4]. BMI was calculated at the time of diagnosis. Patients were classified as slow progressors when survival was higher than 5 years, as normal progressors when survival was between 3 and 5 years, and as fast progressors when survival was less than 3 years as previously described [4]. All the human samples included were of patients who were already deceased at the time of the study.

Measurement of plasma total cholesterol by ELISA

Total cholesterol plasma levels were quantified using a TC (Total cholesterol) ELISA Kit (FineTest®). Each patient's samples were processed in duplicate.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software v11.0.2. Outliers identified by the ROUT method (Q = 1%) were removed prior to statistical analysis. Data normality was assessed using the Shapiro-Wilk test. Statistical analysis of molecular biology data was performed using three-way ANOVA followed by Tukey's multiple-comparison test to evaluate the effect of genotype, disease stage and sex. Male and female cohorts were then analysed independently using two-way ANOVA followed by Bonferroni's post hoc test. Statistical analysis of ELISA data was performed using the Mann-Whitney U test and two-way ANOVA followed by Bonferroni's multiple-comparison test. Data are presented as mean ± standard error of the mean (SEM) and statistical significance was set at $p < 0.05$.

Spearman correlation coefficients (r_s) were calculated using IBM SPSS Statistics version 29.0.2.0.

RESULTS

Disease progression is associated with marked lipid remodelling in WAT in TDP-43^{A315T} mice

Previous work from our group demonstrated marked histological and proteomic alterations in WAT during disease progression in the TDP-43^{A315T} mouse model [4]. Given these findings, we next investigated whether disease progression was also associated with changes in tissue lipid composition. As the most pronounced alterations were observed in male TDP-43^{A315T} mice in our previous analyses, subsequent lipidomic studies were performed exclusively in this sex.

Untargeted lipidomic profiling revealed distinct lipidomic signatures between WT and TDP-43^{A315T} mice throughout disease progression. Partial least squares discriminant analysis (PLS-DA) demonstrated a clear separation between the two genotypes at the asymptomatic stage (Fig. 1A), disease onset (Fig. 1B) and end stage (Fig. 1C). These findings were further supported by hierarchical clustering analysis of the 25 most differentially abundant lipid species, which consistently segregated WT and TDP-43^{A315T} mice across all disease stages (Fig. 1D-F). Notably, genotype-dependent differences were already detectable at the asymptomatic stage, indicating that lipid remodelling occurs before the appearance of overt disease manifestations.

To further characterize these alterations, volcano plot analysis was performed using a fold change (FC) threshold of > 2.0 or < 0.5 and a p -value < 0.05 . This analysis identified 63 differentially abundant lipid species at the asymptomatic stage (Fig. 1G), 181 at disease onset (Fig. 1H) and 63 at the end stage (Fig. 1I). Cardiolipins (CL) represented the predominant altered lipid class during the asymptomatic phase (Fig. 1J). As disease progressed to symptom onset, lipid remodelling expanded to include a substantial number of triacylglycerol (TAG) species in addition to CL (Fig. 1K). By the end stage, CL, TAG and phosphatidylinositols (PI) were the lipid classes containing the largest number of altered species (Fig. 1L). Together, these findings indicate a progressive and dynamic reorganization of the WAT lipidome throughout disease progression in TDP-43^{A315T} mice.

Because CL and TAG represented the lipid classes containing the highest number of differentially abundant species across disease progression, these classes were analysed separately using heatmap visualization (Fig. 2). This approach aimed to determine whether the observed differences between WT and TDP-43^{A315T} mice were driven by individual lipid species or reflected coordinated remodelling of entire lipid classes. At the asymptomatic stage, the top 25 altered CL species clearly separated WT and TDP-43^{A315T} mice (Fig. 2A), whereas TAG species showed limited discrimination between groups (Fig. 2D). At disease onset, both CL and TAG profiles segregated WT and TDP-43^{A315T} mice into distinct clusters (Fig. 2B, E). This stage was characterized by a marked reduction in CL abundance in TDP-43^{A315T} mice accompanied by a substantial increase in TAG levels. At the end stage, TAG profiles continued to effectively discriminate between genotypes (Fig. 2F), with most TAG species showing increased abundance in TDP-43^{A315T} mice. Similarly, CL species were predominantly elevated in TDP-43^{A315T} mice at this stage (Fig. 2C).

Interestingly, no individual lipid species remained consistently altered between WT and TDP-43^{A315T} mice throughout all stages of disease progression. Instead, disease progression was characterized by

coordinated remodelling of entire lipid classes, with distinct stage-dependent patterns affecting predominantly CL and TAG metabolism.

To gain insight into the metabolic pathways potentially associated with this lipid remodelling, BioPAN analysis was performed using the LIPID MAPS platform (Table 2). At the asymptomatic stage, the analysis predicted increased activity of the phosphatidylcholine (PC) biosynthesis pathway, with *PEMT* identified as a putative enzyme involved in the phosphatidylethanolamine (PE) to PC conversion, together with the suppression of fatty acid elongation reactions associated with *ELOVL2*, *ELOVL3* and *ELOVL5*. At disease onset, activation of both PE and PC biosynthetic pathways were predicted. This stage was also associated with *PISD* and *PEMT*, together with suppression of fatty acid elongation and desaturation reactions associated with *ELOVL2*, *ELOVL5*, *ELOVL6*, *FADS1*, *FADS2* and *SCD3*. At the end stage, PC biosynthesis remained the main pathway predicted to be activated, with *PEMT* identified as a putative associated enzyme, whereas fatty acid elongation reactions associated with *ELOVL1*, *ELOVL3* and *ELOVL7* were predicted to be suppressed.

Late-stage metabolic and endocrine remodelling of BAT in male TDP-43^{A315T} mice

Adipose tissue homeostasis depends on the coordinated regulation of energy storage and energy expenditure. Given the marked alterations previously identified in WAT in the TDP-43^{A315T} mouse model [4], we next investigated whether BAT was also affected during disease progression. To address this, we examined the expression of genes associated with thermogenic function, brown adipocyte identity, metabolic activity and endocrine signalling in both sexes.

No significant differences were detected in the expression of the canonical thermogenic markers *Ucp1* and *Ppargc1a* at any disease stage examined (Fig. 3A-B). In contrast, *Prdm16*, a key regulator of brown adipocyte identity, mRNA levels (Fig. 3C) were significantly higher in male TDP-43^{A315T} mice at 95 days than those observed in age-matched WT males ($p < 0.0001$), age-matched female TDP-43^{A315T} mice ($p < 0.0001$), and male TDP-43^{A315T} mice at both 60 ($p = 0.0001$) and 42 days of age ($p < 0.0001$).

Consistent with this finding, genes associated with adipocyte differentiation and lipid handling were also upregulated at 95 days of age. *Pparg* mRNA levels (Fig. 3D) were significantly higher in male TDP-43^{A315T} mice at 95 days compared with age-matched female TDP-43^{A315T} mice ($p = 0.047$) and Tg males analysed at 42 days of age ($p = 0.0063$). Similarly, *Fabp4* mRNA levels (Fig. 3E) were increased relative to age-matched WT males ($p = 0.007$), as well as to male TDP-43^{A315T} mice at 60 ($p = 0.0006$) and 42 days ($p < 0.0001$).

A comparable pattern was observed for genes involved in metabolic regulation. *Glut4* mRNA levels (Fig. 3F) were significantly elevated in male TDP-43^{A315T} mice at 95 days compared with Tg males at 60 ($p = 0.045$) and 42 days of age ($p = 0.003$). Likewise, *Adipoq* mRNA levels (Fig. 3G) were significantly

higher than those detected in age-matched WT males ($p = 0.035$), as well as in Tg males at 60 ($p < 0.001$) and 42 days ($p = 0.003$).

Given the emerging evidence linking adipose tissue dysfunction and endocrine signalling in ALS, we next examined genes associated with leptin signalling. Both *leptin* and *ObRb* were markedly upregulated in male TDP-43^{A315T} mice at 95 days of age. *Leptin* mRNA levels (Fig. 3H) were significantly higher than those observed in age-matched WT males ($p = 0.0013$) and Tg mice at 60 ($p = 0.0006$) and 42 days of age ($p < 0.0001$). In addition, *leptin* expression was substantially higher than that detected in age-matched female TDP-43^{A315T} mice ($p < 0.0001$). Similarly, *ObRb* mRNA levels (Fig. 3I) were significantly increased relative to Tg males at 60 ($p = 0.025$) and 42 days of age ($p < 0.001$).

Finally, endogenous *Tardbp* mRNA expression was evaluated in BAT given our previous observations of altered *Tardbp* levels in WAT [4]. As shown in Fig. 3J, *Tardbp* mRNA levels were significantly elevated in male TDP-43^{A315T} mice at 95 days compared with age-matched WT males ($p = 0.0046$) and Tg males at 42 days of age ($p = 0.0022$).

Collectively, these findings indicate that BAT undergoes marked transcriptional remodelling at the end stage of disease progression in male TDP-43^{A315T} mice. This response is characterised by increased expression of genes associated with brown adipocyte identity, adipocyte metabolism and leptin signalling, whereas canonical thermogenic markers remained unchanged.

Hepatic metabolism is altered in female TDP-43^{A315T} mice

Given the close metabolic interplay between BAT and liver, we next investigated whether disease progression was associated with transcriptional changes in hepatic metabolic pathways. Analysis of liver gene expression revealed a distinct profile in female TDP-43^{A315T} mice at 95 days of age (Fig. 4).

Regarding lipid uptake and transport, *Cd36* mRNA levels (Fig. 4A) were significantly increased in female TDP-43^{A315T} mice at 95 days compared with age-matched WT females ($p = 0.0008$), age-matched male TDP-43^{A315T} mice ($p < 0.0001$), and female TDP-43^{A315T} mice at 60 ($p = 0.0059$) and 42 days of age ($p < 0.0001$). Similarly, *Cpt1a* mRNA levels (Fig. 4B) were significantly elevated compared with age-matched male TDP-43^{A315T} mice ($p < 0.0001$) and Tg females at 60 ($p = 0.0063$) and 42 days ($p = 0.0173$). In contrast, no significant differences were observed in *Acox1* expression (Fig. 4C), a gene involved in peroxisomal fatty acid oxidation.

Markers associated with de novo lipogenesis exhibited an opposite pattern. *Srebf1* mRNA levels (Fig. 4D), encoding a major transcriptional regulator of lipogenic pathways, were significantly reduced in female TDP-43^{A315T} mice at 95 days compared with age-matched WT females ($p = 0.0287$) and Tg females at 60 ($p = 0.0107$) and 42 days ($p = 0.0254$). Consistent with this finding, expression of its downstream target *Fasn* was also decreased. *Fasn* mRNA levels (Fig. 4E) were significantly lower in female TDP-43^{A315T} mice at 95 days compared with Tg females at 60 days of age ($p = 0.0195$). In

addition, *Fasn* expression in male TDP-43^{A315T} mice at 60 days was significantly lower than that observed in age-matched WT females ($p = 0.0478$). Likewise, *Scd1* mRNA levels (Fig. 4F), another target gene of *Srebf1*, were significantly decreased in females TDP-43^{A315T} mice at 95 days compared with Tg females at 60 days ($p = 0.0390$), while expression at 60 days was significantly higher than that observed at 42 days ($p = 0.0475$).

Finally, genes involved in metabolic and endocrine regulation were examined. Consistent with the observed changes in lipid metabolic pathways, *Ppara* mRNA levels (Fig. 4G) were significantly increased in female TDP-43^{A315T} mice at 95 days compared with age-matched WT females ($p = 0.0051$), age-matched male TDP-43^{A315T} mice ($p < 0.0001$), and Tg females at both 60 ($p = 0.0014$) and 42 days of age ($p = 0.0056$). This increase was accompanied by elevated expression of two established *Ppara* target genes, *Fabp1* and *Fgf21*. *Fabp1* mRNA levels (Fig. 4H), a marker involved in hepatic fatty acid transport and intracellular trafficking, were significantly higher in female TDP-43^{A315T} mice at 95 days than in age-matched male TDP-43^{A315T} mice ($p = 0.0093$). Similarly, *Fgf21* mRNA levels (Fig. 4I), encoding a key hepatokine involved in systemic energy homeostasis, were markedly increased compared with age-matched male TDP-43^{A315T} mice ($p < 0.0001$) and Tg females at 60 ($p = 0.0003$) and 42 days of age ($p < 0.0001$).

Collectively, these findings indicate that female TDP-43^{A315T} mice develop a distinct hepatic metabolic signature at 95 days of age, characterised by increased expression of genes involved in fatty acid uptake and oxidation together with repression of de novo lipogenesis pathways. The concomitant induction of *Ppara*, *Fabp1* and *Fgf21* further suggests a shift towards enhanced lipid utilization and systemic metabolic adaptation.

Profile of total cholesterol in plasma of ALS patients at the time of diagnosis

Given the coordinated alterations in lipid metabolic pathways observed across adipose tissue and liver in the TDP-43^{A315T} mouse model, we next investigated whether evidence of systemic metabolic dysregulation was also detectable in ALS patients at diagnosis. To assess the translational relevance of our experimental findings, circulating total cholesterol levels were measured as a clinically accessible marker of lipid homeostasis in a previously characterised cohort in which altered leptin levels had been reported [4]. Since both leptin and cholesterol metabolism are closely linked to adipose tissue function and systemic energy balance [38, 41], we further explored whether cholesterol profiles varied according to sex and body mass index. Total cholesterol was determined in plasma samples from 57 ALS patients and 15 age-matched healthy controls.

Total plasma cholesterol levels at diagnosis showed a tendency towards lower values in ALS patients compared with healthy controls (Fig. 5A). When data were stratified by sex, this trend was observed in men patients but not in women (Fig. 5B). Further stratification according to disease progression rate

revealed a sex-dependent pattern in circulating cholesterol levels (Fig. 5C). In men patients, total cholesterol levels tended to increase with faster disease progression. In contrast, women in the normal-progression group exhibited significantly higher total cholesterol levels than men ($p = 0.018$).

To further investigate the influence of metabolic status, patients were stratified according to BMI. Interestingly, the increase in total cholesterol levels associated with faster disease progression in men patients was observed exclusively in the obese subgroup (Fig. 5D). No comparable trend was identified among non-obese patients (Fig. 5E).

Finally, we assessed the relationship between total cholesterol levels and clinical or metabolic variables using Spearman correlation analysis. This analysis included total cholesterol concentrations determined in the present study together with circulating leptin levels previously reported by our group in the same cohort [4]. The only statistically significant association identified was a positive correlation between total cholesterol levels and sex ($p = 0.007$, $r_s = 0.356$) (Table 3). Although no significant correlations were detected between total cholesterol levels and BMI or disease progression rate, a trend towards a positive association between total cholesterol and leptin levels was observed ($p = 0.094$, $r_s = 0.224$).

Collectively, these findings suggest the presence of sex-dependent alterations in circulating cholesterol levels in ALS patients at the time of diagnosis. Moreover, the association between increased cholesterol levels and faster disease progression observed specifically in obese men patients highlight the potential contribution of systemic lipid metabolism to disease heterogeneity.

DISCUSSION

ALS is increasingly recognised as a systemic disorder in which metabolic dysfunction accompanies and potentially contributes to neurodegeneration. Indeed, in addition to the progressive loss of upper and lower motor neurons, ALS patients frequently display hypermetabolism [13], weight loss [24], alterations in body composition [28] and dysregulated lipid homeostasis [8], often preceding the appearance of overt neurological symptoms. Recent evidence suggests that peripheral metabolic tissues actively participate in disease progression [39], highlighting the need to better understand the molecular mechanisms linking systemic metabolism and ALS pathophysiology.

In the present study, we demonstrate that disease progression in the TDP-43^{A315T} mouse model is associated with profound remodelling of peripheral metabolic tissues, affecting WAT, BAT and liver in a tissue-specific and sex-dependent manner (see Supplementary Tables S1 and S2). Furthermore, we provide evidence of altered circulating cholesterol profiles in ALS patients at the time of diagnosis. Collectively, these findings support the concept that metabolic dysregulation represents an integral component of ALS pathogenesis and identify peripheral lipid metabolism as a potential contributor to disease heterogeneity.

One of the most striking observations was the early and progressive lipid remodelling detected in WAT from male TDP-43^{A315T} mice. Lipidomic profiling revealed clear segregation between male WT and TDP-

43^{A315T} mice from the asymptomatic stage onwards, indicating that WAT lipids alterations are already detectable before the onset of clinically overt disease in males. Interestingly, the observed lipid remodelling was characterised by coordinated changes affecting entire lipid classes rather than persistent dysregulation of individual lipid species. CL emerged as the most prominently altered lipid class during the asymptomatic stage in TDP-43^{A315T} mice. Given their essential role in mitochondrial membrane architecture, respiratory chain assembly and oxidative phosphorylation, early CL alterations may indicate disturbances in mitochondrial homeostasis within WAT [10, 35]. Importantly, these lipidomic findings are supported by our previous proteomic analysis of WAT performed in the same cohort of male TDP-43^{A315T} mice, which identified mitochondrial dysfunction as a prominent early molecular alteration [4]. The convergence of protein and lipid signatures associated with mitochondrial biology strongly suggests that mitochondrial impairment is already present in WAT before the onset of overt clinical symptoms in TDP-43^{A315T} mice. In this context, the CL remodelling observed here may represent a lipidomic manifestation of impaired mitochondrial integrity and bioenergetic adaptation. This interpretation is consistent with extensive evidence implicating mitochondrial dysfunction as a central pathogenic mechanism in ALS and TDP-43 proteinopathy, where altered mitochondrial dynamics, impaired oxidative phosphorylation and defective energy metabolism contribute to disease progression [6, 27]. Furthermore, the detection of these alterations during the asymptomatic stage is consistent with growing evidence indicating that metabolic disturbances can precede motor symptom onset by several years in ALS, supporting the notion that peripheral metabolic dysfunction represents an early component of disease pathophysiology rather than merely a consequence of advanced neurodegeneration [9, 45]. Together, these findings suggest that mitochondrial dysfunction within WAT may constitute an early peripheral manifestation of the broader metabolic remodelling associated with ALS progression.

As disease advanced, TAG became increasingly dysregulated, suggesting progressive alterations in lipid storage and utilisation pathways. Together with our previous histological and proteomic observations [4], these data indicate that WAT undergoes extensive metabolic remodelling throughout disease progression. Importantly, because these alterations occur before symptom onset, they raise the possibility that WAT dysfunction contributes actively to the development of the systemic metabolic phenotype characteristic of ALS.

In contrast to WAT, BAT exhibited a predominantly late-stage transcriptional response. Although the canonical thermogenic markers *Ucp1* and *Ppargc1a* remained unchanged, end-stage male TDP-43^{A315T} mice displayed increased expression of genes associated with brown adipocyte identity (*Prdm16*), adipocyte differentiation (*Pparg*), lipid handling (*Fabp4*), glucose uptake (*Glut4*) and endocrine activity (*Adipoq*). These findings do not support a classical activation of thermogenesis but instead suggest a transcriptional programme compatible with metabolic adaptation. Recent work has demonstrated pathological alterations of BAT in ALS mouse models [40], including mitochondrial dysfunction and impaired differentiation capacity, supporting the notion that BAT participates in systemic metabolic responses during disease progression.

Particularly noteworthy was the coordinated upregulation of *leptin* and *ObRb* in BAT from end-stage male TDP-43^{A315T} mice. Beyond its established role in appetite regulation and energy expenditure, leptin is increasingly recognised as a modulator in adipose tissue plasticity and BAT activity, and as a mediator in inter-organ metabolic communication. Previous studies have demonstrated extensive interactions between leptin signalling, adipose tissue remodelling and the pathways governing systemic energy homeostasis and thermogenic regulation [38, 41]. These observations suggest that altered leptin responsiveness may represent an adaptive mechanism aimed at preserving metabolic homeostasis under conditions of energetic stress. Although the functional consequences of increased BAT leptin signalling remain to be determined, our findings support the existence of altered endocrine communication within adipose tissue during ALS progression. This interpretation is further supported by previous reports of altered circulating leptin levels in ALS patients [1, 4, 36] and by the growing recognition that adipose tissue-derived signalling pathways contribute to the systemic metabolic abnormalities associated with the disease [12, 34].

Another major finding of this study is the pronounced sexual dimorphism observed across peripheral tissues (see Supplementary Tables S1 and S2). Whereas male TDP-43^{A315T} mice exhibited prominent WAT lipidomic alterations and a marked late-stage transcriptional response in BAT, females developed a distinctive hepatic metabolic signature. Specifically, female TDP-43^{A315T} mice displayed increased expression of genes involved in fatty acid uptake and utilisation (*Cd36*, *Cpt1a* and *Ppara*) together with suppression of de novo lipogenesis pathways (*Srebf1*, *Fasn* and *Scd1*). Collectively, these changes suggest a metabolic shift favouring lipid utilisation over lipid synthesis, potentially reflecting a compensatory adaptation aimed at maintaining systemic energy homeostasis. The marked induction of *Fgf21* further supports this interpretation. FGF21 is a liver-derived endocrine factor that plays a central role in the regulation of lipid metabolism, adipose tissue function and whole-body energy homeostasis [19, 20, 26]. Hepatic FGF21 expression is robustly induced under conditions of fasting, nutrient deprivation and metabolic stress through activation of PPAR α -dependent pathways, where it coordinates adaptive responses that promote fatty acid oxidation, ketogenesis and energy conservation [3, 23, 37]. In addition, FGF21 functions as a key mediator of liver-adipose tissue communication, facilitating metabolic adaptation across peripheral tissues and contributing to the maintenance of systemic energy balance during periods of increased energetic demand [19, 20]. Accordingly, the concomitant upregulation of *Ppara*, *Fabp1* and *Fgf21* identified in female TDP-43^{A315T} mice is consistent with activation of a coordinated hepatic adaptive programme aimed at enhancing lipid utilisation, maintaining metabolic flexibility and preserving systemic energy homeostasis. The absence of a comparable response in males further suggests the existence of sex-specific mechanisms regulating hepatic metabolic resilience during ALS progression. The tissue distribution of these alterations suggests that males and females TDP-43^{A315T} mice may engage distinct peripheral adaptive mechanisms during disease progression. Although the molecular basis of this sexual dimorphism remains unclear, increasing evidence indicates that sex influences both disease presentation and metabolic regulation in ALS. Our findings therefore highlight the importance of considering sex as a biological variable when investigating metabolic pathways and developing therapeutic strategies.

To explore the translational relevance of these findings, we analysed circulating total cholesterol levels in ALS patients at diagnosis. Although the overall reduction in cholesterol levels was modest, sex-stratified analyses revealed distinct patterns between men and women, while additional stratification according to BMI uncovered associations that were primarily observed in obese male patients. Previous studies investigating cholesterol and other lipid biomarkers in ALS have produced inconsistent results, with some reports identifying altered cholesterol levels and others finding limited prognostic value [8, 11, 32]. Nevertheless, recent large-scale analyses support the notion that circulating lipid profiles reflect disease-associated metabolic alterations and may provide insights into disease heterogeneity [32, 45].

Importantly, cholesterol did not emerge as a robust standalone prognostic biomarker in our cohort. However, the observed sex- and BMI-dependent patterns reinforce the concept that metabolic heterogeneity is a key feature of ALS. Moreover, the tendency towards a positive association between cholesterol and leptin levels is consistent with the broader metabolic alterations identified across adipose tissues and liver, further supporting the existence of systemic metabolic remodelling in ALS.

In conclusion, our study demonstrates that disease progression in the TDP-43^{A315T} model is associated with extensive metabolic remodelling across multiple peripheral organs. WAT exhibits early lipidomic alterations before symptom onset, BAT develops a late-stage adaptive transcriptional response predominantly in males, and female TDP-43^{A315T} mice engage a distinct hepatic programme characterised by activation of lipid utilisation pathways and induction of *Fgf21*. Together with the sex-dependent cholesterol profiles observed in ALS patients, these findings provide further evidence that peripheral metabolic dysfunction is a fundamental component of ALS biology and highlight metabolic pathways as promising sources of translational biomarkers and therapeutic targets. Although the TDP-43^{A315T} model is extensively characterised and recapitulates key clinical, pathological and metabolic features of ALS progression [4, 17, 46] the present study primarily provides a molecular characterisation of peripheral metabolic remodelling. Consequently, the transcriptional and lipidomic alterations identified across adipose tissues and liver cannot, by themselves, distinguish between pathogenic, compensatory or adaptive responses.

Declarations

Ethics approval and consent to participate

All animal procedures were performed in accordance with the Animal Ethics Committee of the Hospital Nacional de Paraplégicos (Approval No 36-OH/2019) (Spain) in accordance with the European Communities Council Directive (86/609/EEC) for the Care and Use of Animals for Scientific Purposes.

Human samples described in this manuscript were collected under protocols that were reviewed and approved by the Functional Unit of Amyotrophic Lateral Sclerosis (UrFELA), Service of Neurology, Bellvitge University Hospital, Hospitalet de Llobregat, (Spain). Subjects underwent informed consent prior to participating in research studies. This study was performed in line with the principles of the

Declaration of Helsinki. Approval was granted by the Ethics Committee of the Universidad San Pablo-CEU (783/23/98) (Spain).

Consent for publication

All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization: C-FM; Methodology: C-BC, A-FD, G-DV; Formal analysis and investigation: C-BC, A-FD, G-DV; Writing - original draft preparation: C-BC, C-FM; Writing - review and editing: C-BC, G-DV, R-DM, MP, C-FM; Funding acquisition: C-FM; Resources: R-DM, MP, C-FM; Supervision: C-FM.

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Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

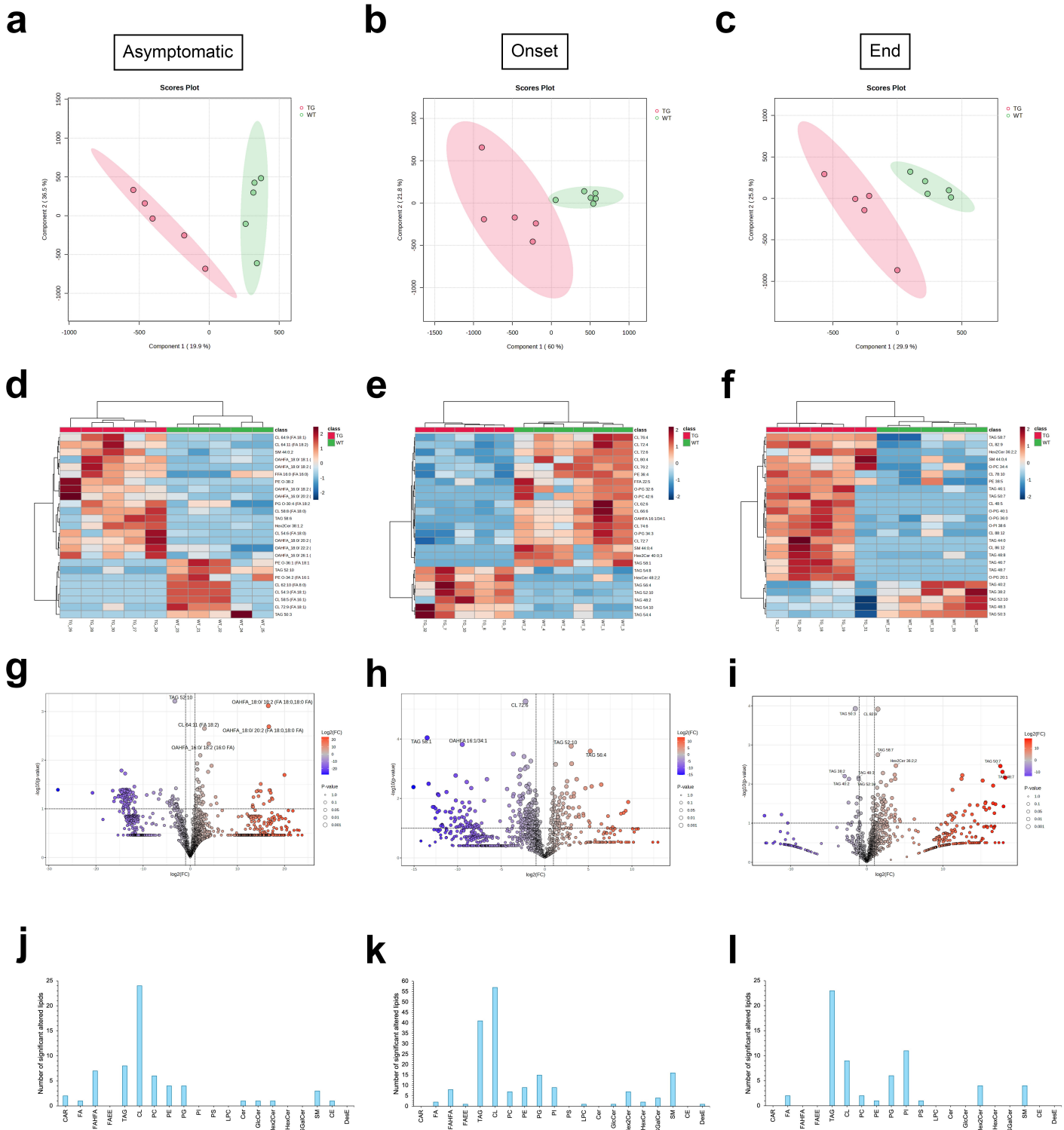


Figure 1

Lipidomic analysis of WAT from male TDP-43^{A315T} mice. (a, b, c) Partial least square discriminant analysis (PLS-DA) showing the separation of lipid profiles between WT and Tg mice at asymptomatic (a), onset (b) and end stage (c). (d, e, f) Heatmap of the top 25 differentially expressed lipids at asymptomatic (d), onset (e) and end stage (f). Colour intensities are based on Z-scores, where red indicates upregulation and blue downregulation. (g, h, i) Volcano plot showing significantly altered lipids

at asymptomatic (g), onset (h) and end stage (i). Red dots indicate lipids upregulated in the Tg mice, whereas blue dots indicate downregulated lipids. (j, k, l) Number of significantly altered lipid species by lipid class identified in the volcano plot analysis at asymptomatic (j), onset (k) and end stage (l). The lipid classes are abbreviated as: acylcarnitines (CAR), free fatty acids (FA), fatty acid esters of hydroxy fatty acids (FAHFA), fatty acid ethyl esters (FAEE), triacylglycerols (TAG), cardiolipins (CL), phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG), phosphatidylinositols (PI), phosphatidylserines (PS), lysophosphatidylcholines (LPC), ceramides (Cer), glucosylceramides (GlcCer), hexosylceramides (HexCer), dihexosylceramides (Hex2Cer), sulfogalactosylceramides (SGalCer), sphingomyelins (SM), cholesteryl esters (CE) and desmosteryl esters (DesE)

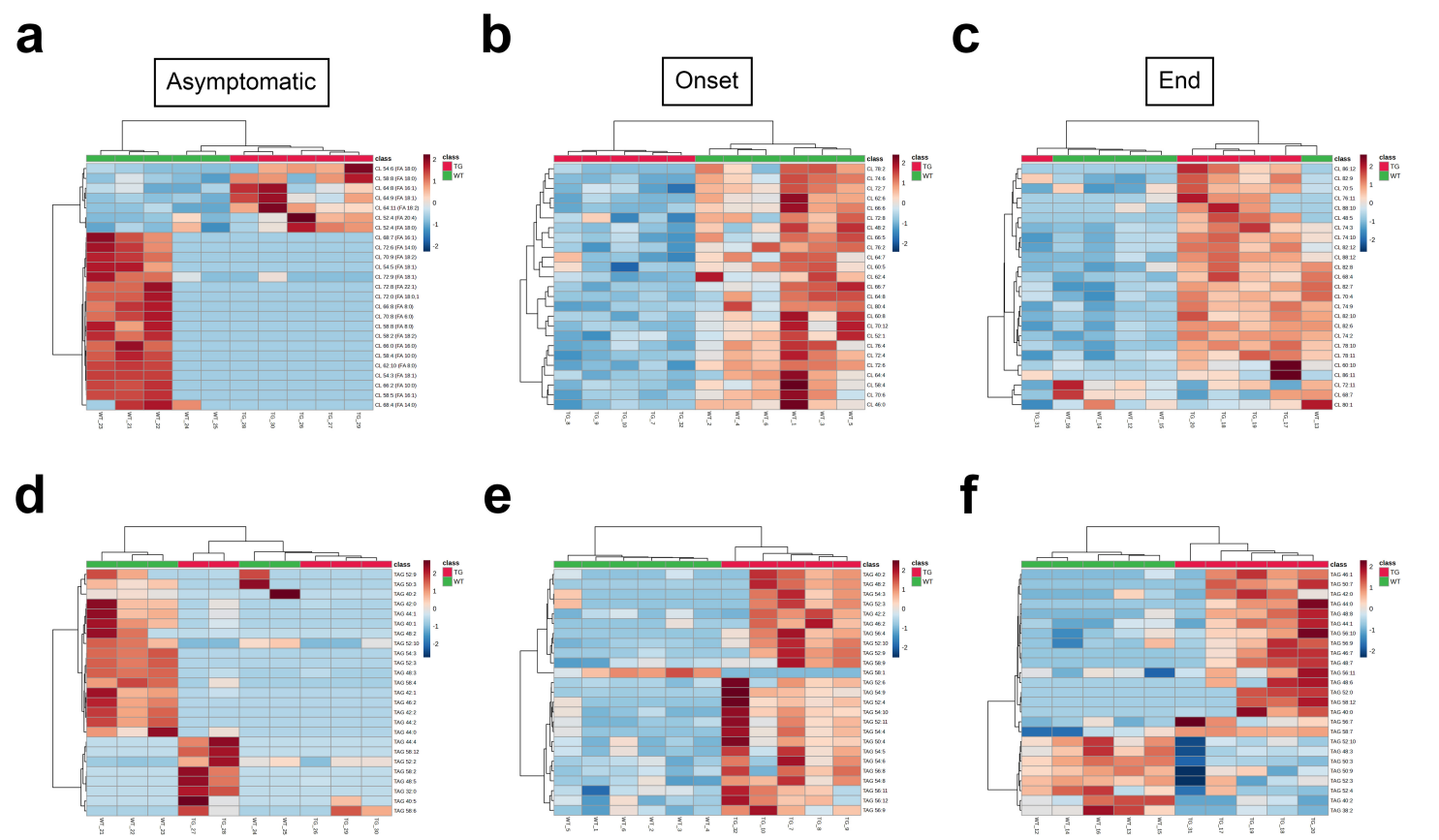


Figure 2

Heatmap of CL and TAG from lipidomic analysis of WAT from male TDP-43^{A315T} mice. (a, b, c) Heatmap of the 25 top differentially expressed CL at asymptomatic (a), onset (b) and end stage (c). (d, e, f) Heatmap of the 25 top differentially expressed TAG at asymptomatic (d), onset (e) and end stage (f). Colour intensities are based on Z-scores, where red indicates upregulation and blue downregulation

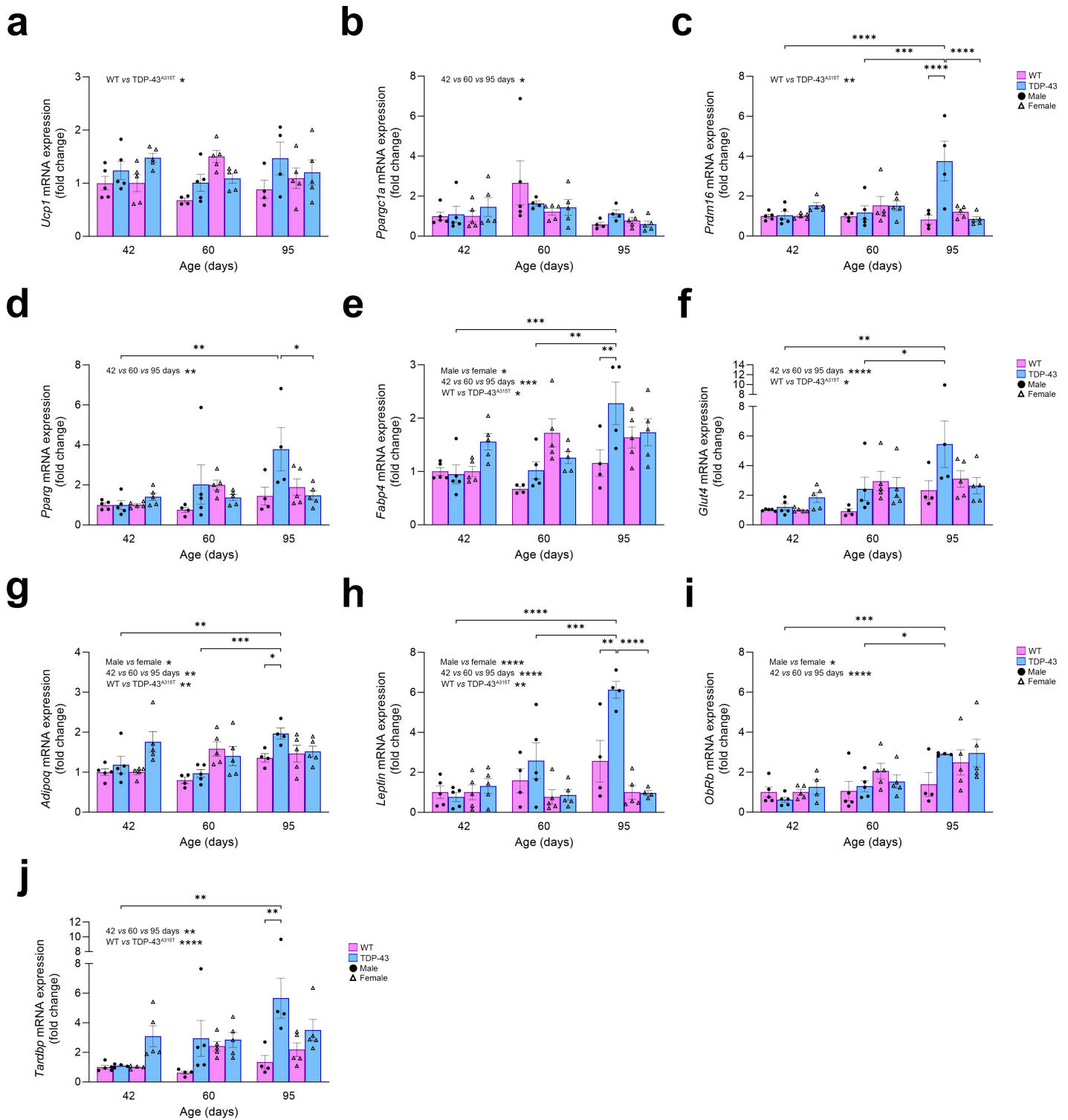


Figure 3

Differential expression at mRNA level in BAT of male and female TDP-43^{A315T} mice. (a) *Ucp1*, (b) *Ppargc1a*, (c) *Prdm16*, (d) *Pparg*, (e) *Fabp4*, (f) *Glut4*, (g) *Adipoq*, (h) *Leptin*, (i) *ObRb* and (j) *Tardbp*. Transcripts were assessed by RT-qPCR at 42, 60 and 95 days. Values are expressed as mean $\pm$ SEM for each group. The significant effects derived from the three-way ANOVA are directly annotated within each plot and detailed in Supplementary Table S1. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

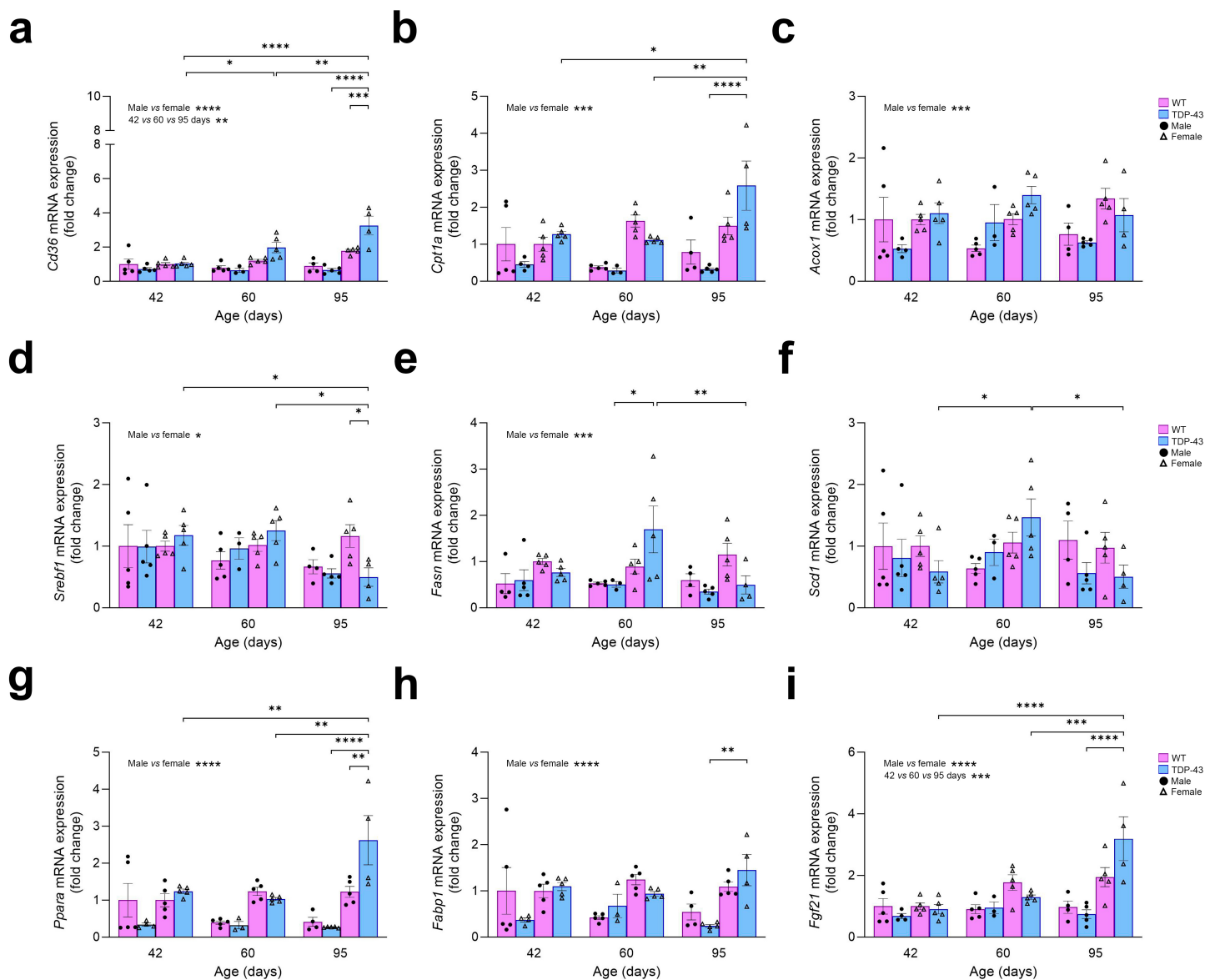


Figure 4

Differential expression at mRNA level in liver of male and female TDP-43^{A315T} mice. (a) *Cd36*, (b) *Cpt1a*, (c) *Acox1*, (d) *Srebf1*, (e) *Fasn*, (f) *Scd1*, (g) *Ppara*, (h) *Fabp1* and (i) *Fgf21*. Transcripts were assessed by RT-qPCR at 42, 60 and 95 days. Values are expressed as mean $\pm$ SEM for each group. The significant effects derived from the three-way ANOVA are directly annotated within each plot and detailed in Supplementary Table S2. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

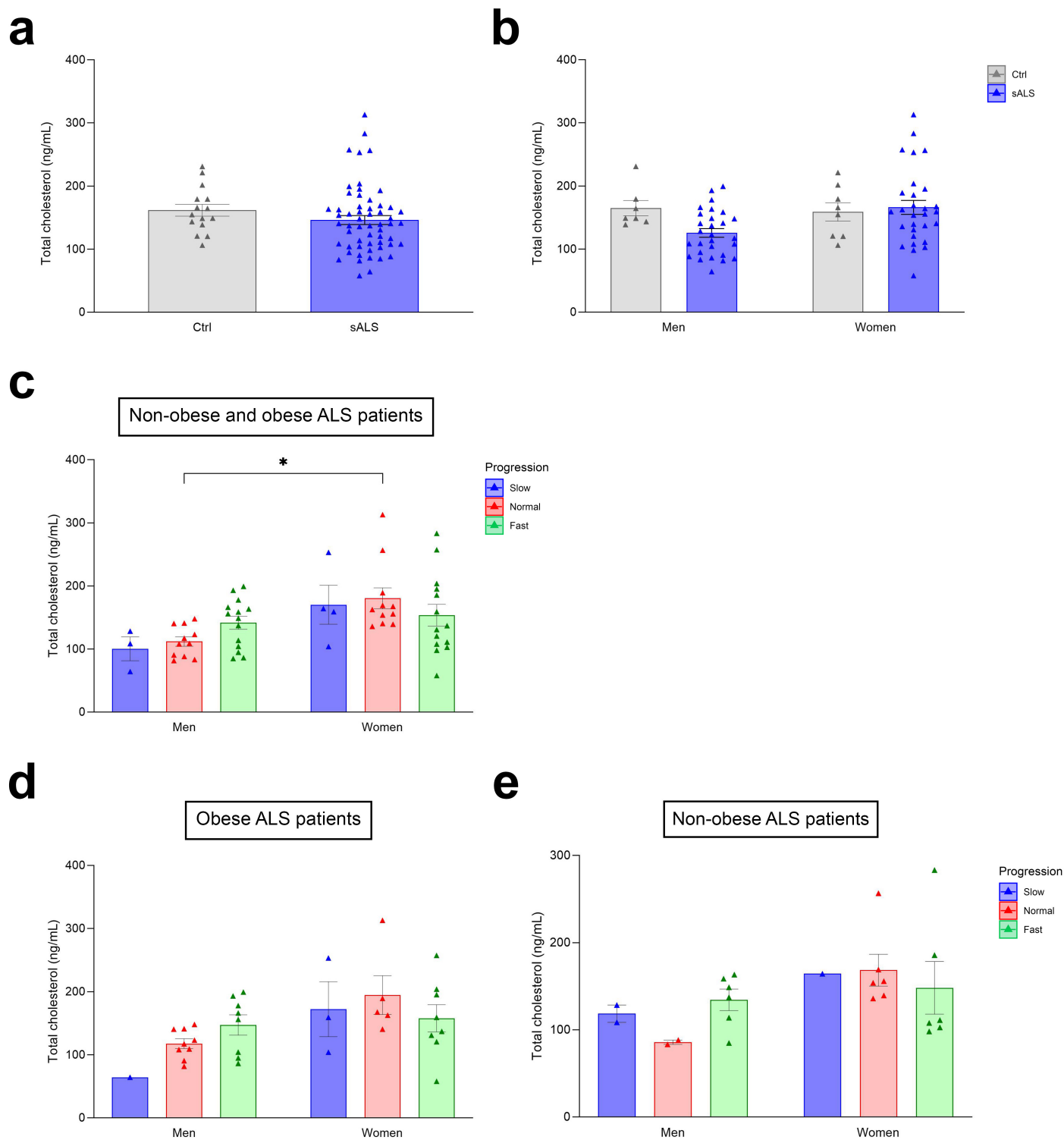


Figure 5

Analysis of total cholesterol in plasma of ALS patients at the time of diagnosis. (a) Concentration of total cholesterol levels at diagnosis measured by ELISA in healthy controls and ALS subgrouped. (b) Concentration of total cholesterol levels at diagnosis in ALS and controls subgrouped by sex. (c) Concentration of total cholesterol at diagnosis in ALS subgrouped by sex and rate of progression. (d) Concentration of total cholesterol at diagnosis in ALS subgrouped by sex and rate of progression in

obese patients. (e) Concentration of total cholesterol at diagnosis in ALS subgrouped by sex and rate of progression in non-obese patients. * $p < 0.05$

Supplementary Files

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