

Metabolic profile and pathological alterations in the muscle of patients with early-stage amyotrophic lateral sclerosis.

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Supplementary File S1.

1. Metabolomics Analysis.

An UPLC Ultimate WPS-3000 system (Dionex, Germany) coupled to a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Germany) and operated in positive (HESI+) and negative (HESI-) heated electrospray ionization modes (analysis for each ionization mode) was used. Liquid chromatography was performed using two different columns to increase the metabolic coverage. First, samples were injected on a Phenomenex Kinetex 1.7 μ m XB – C18 column (150 mm x 2.10 mm) maintained at 55°C. The mobile phase gradient (preceded by an equilibrium time of 3 min) was maintained at a flow rate of 0.4 mL/min. The multistep gradient was programmed as follows: 0–2 min, 0.1% B; 2–6 min, 0.1–25% B; 6–10 min, 25–80% B; 10–12 min, 80–90% B; 12–16.5 min, 90–99.9% B; 16.5–20 min, 99.9–0.1% B. The second analyze was realized on a hydrophilic interaction liquid chromatography (HILIC) column (150 mm x 2.10 mm, 100 Å). The gradient was maintained at a flow rate of 0.3 mL/min. The multistep gradient was programmed as follows: 0–1.5 min, 95% B; 1.5–8 min, 95–82% B; 8–15 min, 82–75% B; 15–15.5 min, 75–25% B; 15.5–16 min, 25–3% B; 16–22 min, 3–95% B. The volume of injection was 5 μ L. During the full-scan acquisition, which ranged from 58 to 870 m/z, the instrument operated at 70,000 resolution (m/z = 200). Source parameters were maintained as described previously¹.

Pre-analytical and analytical steps of the experiment were validated by Quality Control (QC) samples (mix of 20 samples). Coefficients of variation (CV% = (the standard deviation/ mean) x 100), were calculated from all metabolites data. Metabolites with a CV in QCs >30% were excluded from the final dataset. QCs were analyzed at the beginning of the run, every 10 samples and at the end of the run.

A targeted analysis was applied on the samples, based on a library of standard compounds (Mass Spectroscopy Metabolite Library (MSML®) of standards, IROA Technologies™). The following criteria were used to identify the metabolites: (1) retention time of the detected metabolite within ± 20 s of the standard reference, (2) exact measured molecular mass of the metabolite within a range of 10 ppm around the known molecular mass of the reference compound, and (3) correspondence between isotopic ratios of the metabolite and the standard reference. The signal value was calculated using Xcalibur® software (Thermo Fisher Scientific, San Jose, CA) by integrating the chromatographic peak area corresponding to the selected metabolite.

2. Statistical analysis.

2.1 Univariate analysis

Comparison of continuous parameters defining population characteristics and mitochondrial enzymes data was done by Student or Wilcoxon test, depending on the distribution's normality (according to Shapiro test) and qualitative data were compared by the Chi² test. All data were checked for outliers by the Grubb's test. Correlation between metabolome data, mitochondrial enzymes and antioxidant genes were performed by the Spearman test, while the relation between FVC and lauroylcarnitine levels was evaluated by Pearson correlation (GraphPad Software version 7; GraphPad Software Inc.)

Metabolomics data was non-normalized, log-transformed and auto-scaled. The univariate analysis of metabolites levels between groups (ALS/controls and groups related to disease evolution) was based on fold-change (FC) values and the threshold of significance after non-parametric Wilcoxon test. This analysis was represented on a volcano plot using Metaboanalyst, version 5.0 (www.metaboanalyst.ca/). The x-axis represents the fold change between the subject groups (log scale). The y-axis represents the p-value for t-tests of differences between samples (negative log scale). False Discovery Rate (FDR) was used to account for multiple tests, thus highlighting only the most discriminant parameters. We highlighted only metabolites with $p < 0.1$ and $FC(ALS/Controls) > 1.2$ or < 0.8 . In case of comparison between more than two groups, Kruskal Wallis test was performed.

2.2 Metabolomics multivariate analysis

Multivariate analysis was performed to evaluate the relation between metabolome (in muscle and serum) and diagnosis as well as clinical evolution within the ALS cohort (based on the percentage of variation of BMI, ALSRFS-r and disease evolution). Data were Unit variance (UV)-scaled and log-transformed and were analyzed using SIMCA (v15).

Classification was performed by unsupervised Principal Component Analysis (PCA) to evaluate distribution of samples and identify outsiders. Supervised analysis based on Orthogonal Partial Least-Squares Discriminant Analysis (OPLS-DA) or PLS-DA (Partial Least-Squares Discriminant Analysis) enabled the evaluation of discrimination (determined by groups or values of quantitative parameters). OPLS-DA was cross-validated by withholding one-seventh of the samples in seven successive simulations such that each sample was omitted once in order to guard against overfitting,

leading to OPLS-DA built from one so called “predictive” component and two or more orthogonal components. The quality of the models was described by the cumulative modeled variation in the X matrix R²X(cum), the cumulative modeled variation in the Y matrix R²Y(cum), and the cross validated predictive ability Q²(cum) values. OPLS-DA was represented as a scatter plot of the scores t₁ vs t_{1o}. This plot represents the separation of the two classes of observations that occurs in the horizontal (t₁) direction and the vertical (t_{1o}) direction expresses within class variability. The default OPLS-DA loadings scatter plot displays the relation between the X-variables and the Y-variables for the first predictive component and the first Y-orthogonal component. Values of Variable Influence on Projection (VIP) represent the importance of the metabolites for the models. We performed a model optimization by variable exclusion to retain the 15 most discriminant metabolites. The most relevant variables were determined from the VIP and loading values scaled as correlation coefficients (pcorr).

The significance of the OPLS-DA model was evaluated by: 1) the Q²Ycum (goodness of prediction, >0.4 being a good predictive model), and the R²Ycum (goodness of fit) values. Q² is the fraction of the total variation of X or Y that can be predicted by a component, as estimated by cross-validation and R²Y and R²X display the fraction of the sum of squares for the selected component, 2) CV-ANOVA (ANalysis Of VAriance testing of Cross-Validated predictive residuals) test that is a diagnostic tool for assessing the reliability of the models, 3) permutation test, that is based on the comparison of the goodness of fit (R² and Q²) of the original model with the goodness of fit of several models based on data where the order of the Y-observations has been randomly permuted, while the X-matrix has been kept intact. The permutation plot shows, for a selected Y-variable, on the vertical axis the values of R² and Q² for the original model (far to the right) and of the Y-permuted models further to the left. The horizontal axis shows the

correlation between the permuted Y-vectors and the original Y-vector for the selected Y. Considering the size of the cohort we did not validate metabolites/metabolome profile in an independent cohort to fulfill compulsory criteria of the use of biomarkers in routine practice, but we checked the reliability of the models to ensure robustness to pathway analysis.

2.3 Pathway Analysis

We constructed Venn diagrams to reveal the metabolites 1) in muscle and serum most significantly associated with the diagnosis or 2) associated with the four parameters chosen to evaluate disease evolution (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). Enrichment and pathway analysis were systematically performed from all discriminant metabolites highlighted in the OPLS or PLS-DA models. Metabolic pathway enrichment analysis and pathway topology analysis were conducted using MetaboAnalyst platform ², which computes a single *p* value for each metabolic pathway. Pathway topology analysis applies graph theory to measure a given experimentally identified metabolite's importance in a pre-defined metabolic pathway.

Pathway analysis calculated pathway impact that represents a combination of the centrality and pathway enrichment results; higher impact values represent the relative importance of the pathway, relative to all pathways included in the analysis. The pathway impact value was calculated as the sum of importance measures of the metabolites, normalized by the sum of importance measures of all metabolites in each pathway ³. Major criteria are used to perform an informative analysis regarding the quality of pathway data. The KEGG pathway database (<http://www.genome.jp/kegg/pathway.html>) was used to explore the highlighted metabolic pathways. We highlighted pathways that present *p*<0.05 and 2 or more hits between discriminant metabolites.

2.4 Survival

We performed a survival analysis to evaluate the influence of biochemical and clinical parameters on disease duration using the Cox proportional hazard model. After a collinearity check, multivariate Cox proportional hazard models explored factors significantly associated with survival. P values <0.05 were considered significant. Analyses were performed using the JMP statistical software version. 7.0.2 (SAS Institute, Cary, NC, USA).

References

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Supplementary file S2. Characteristics of ALS patients and controls included in this study.

| Identity | Group | Sex | Associated pathologies | treatments | Anesthesia | Anesthetics |
|----------|---------|-----|--|---|-------------------------|--|
| C01 | ALS | M | Arterial hypertension, dyslipidemia | Rilutek/Teglutik, Hexaqueine, Forlax, Topalgie, Atropine, Hydrochlorothiazide, Amlodipine, Kardegeic | Local | Lidocaïne |
| C02 | ALS | W | Dysthyroidism | unknown | Local | Lidocaïne |
| C03 | ALS | M | Dyslipidemia | unknown | Local | Lidocaïne |
| C04 | ALS | W | Arterial hypertension, Thromboembolic pathology | Kardégic, Liorésal + HCT, Telmisartan, Ixprim | Local | Lidocaïne |
| C05 | ALS | M | Depression | Rilutek, Tocopherol | Local | Lidocaïne |
| C06 | ALS | W | Arterial hypertension, Asthma | Temerit, Tahor, Coaprovel, Seretide, Alprazolam, Rilutek, Tocopherol, Adrénaline, Laroxyl | Local | Lidocaïne |
| C07 | ALS | W | unknown | Fluoxetine, Seroplex, Atropine collyre | Local | Lidocaïne |
| C08 | ALS | M | None | Paracetamol, Tocopherol, Rilutek, Debridat | Local | Lidocaïne |
| C09 | ALS | W | None | Rilutek, Keppra, Laroxyl, Atropine collyre, Inexium, Tocopherol, Triflucan | Local | Lidocaïne |
| C10 | ALS | W | Epilepsy | Rilutek, Topalgie, Transipeg, Atropine collyre | Local | Lidocaïne |
| C11 | ALS | M | Dyslipidemia | Aprazolam, Météospasmyl, Duphalac, Lercan, Kardégic, Inegy, Uvédose | Local | Lidocaïne |
| C12 | ALS | W | Arterial hypertension, Dyslipidemia | Topalgie | Local | Lidocaïne |
| C13 | ALS | W | Tumor pathology, Dyslipidemia | Tocopherol, Rilutek, Levothyrox, Lexomil, Okimus, Effexor, Tercian, Imovane | Local | Lidocaïne |
| C14 | ALS | M | None | | Local | Lidocaïne |
| C15 | ALS | W | Coronary artery disease | | Local | Lidocaïne |
| C16 | ALS | M | None | | Local | Lidocaïne |
| C17 | ALS | M | Arterial hypertension | | Local | Lidocaïne |
| T01 | Control | W | Hypothyroidism, arterial hypertension, Hypercholesterolemia | Amlodipine, Fenofibrate, Dosulepine, Pravastatine, Levothyrox, Esomeprazole, Zolpidem, Bromazepam, Hydroxyzine, Prégabaline, Ketoprofène, Paracétamol, Enoxaparine, Cafzoline, Morphine, Ondansetron, Polyéthylène glycol, Tramadol, Vitamine C | Loco-regional + general | Sufentanil, propofol, ketamine, ephedrine, phenylephrine, nefopam, ropivacaïne |
| T02 | Control | W | None | Prégabaline, Alprazolam, PEG, Ondansetron, Ketoprofene, Esomeprazole, Enoxaparine, Morphine, Paracetamol, Tramadol, Vitamine C, Cefazoline | Loco-regional + general | Midazolam, Sufentanil, Propofol, Ketamine, Atracurium, Nefopam, Droperidol, Dexamethasone, Ropivacaïne |
| T03 | Control | M | Arterial hypertension, atrial fibrillation, ischemic cardiopathy, aortic bioprosthetic, hypercholesterolemia | Trinitrine, Acetylsalicylate, rosuvastatine, Bisoprolol Ramipril, Pregabaline, Morphine, Ondansetron, Esomeprazole, Acupan, Paracetamol, Tramadol, Vit C | Loco-regional + general | Sufentanil, Ketamine, Propofol, Atracurium, Dexamethasone, Ephedrine, Droperidol, Ropivacaïne |

| | | | | | | |
|-----|---------|---|--|--|-------------------------|--|
| T04 | Control | M | Arterial hypertension, Diabetes (type 1) | Hydrochlorothiazide, Metformine, Ramipril, Cefazoline, Lyrica, Lantus, Zopiclone, Venofen, Macrogol, Ondansetron, Ketoprofene, Esomeprazole Morphine, Paracetamol, Tramadol Cefazoline, Tramadol, Lexomil, Loprazolam Etodolac, Fentanyl, Levothyroxine, Pregabalin Morphine, Macrogol, Ondansetron, Ketoprofene Esomeprazole, Paracetamol, Lamaline, VitC | Loco-regional + general | Sufentanil, Propofol, Atracurium, Dexamethasone, Morphine, droperidol, ketamine, Ropivacaine |
| T05 | Control | W | Ankylosing spondylitis, Hypothyroidism | Cefazoline, Lyrica, Ondansetron, Paracetamol Morphine, Imovane, Cymbalta, Lamaline, Dafalgan Tramadol, Diclofenac, Trandolapril, Bisoprolol, Lercanidipine, Temesta, Zolpidem, Omeprazole, Bromazepam, Enoxaparine, Vitamine C, Ketoprofene Bromazepam, Seropam, Morphine, Hydroxyzine Paracetamol, Ketoprofene, Pregabalin, Esomeprazole Ondansetron, Lamaline, Vitamine C, Amoxicilline (25/07/16) Pregabalin, Ketoprofene, Paracetamol, Tramadol Omeprazole, Vitamine C | Loco-regional + general | Midazolam, Sufentanil, Propofol, Atracurium, Dexamethasone, Ketamine, Neosynephrine, Ephedrine, Ropivacaine |
| T06 | Control | W | Arterial hypertension, Crohn's disease, bronchopneumopathie chronique obstructive, Breast cancer, hip sepsis | Cefazoline, Lyrica, Ondansetron, Paracetamol Morphine, Imovane, Cymbalta, Lamaline, Dafalgan Tramadol, Diclofenac, Trandolapril, Bisoprolol, Lercanidipine, Temesta, Zolpidem, Omeprazole, Bromazepam, Enoxaparine, Vitamine C, Ketoprofene Bromazepam, Seropam, Morphine, Hydroxyzine Paracetamol, Ketoprofene, Pregabalin, Esomeprazole Ondansetron, Lamaline, Vitamine C, Amoxicilline (25/07/16) Pregabalin, Ketoprofene, Paracetamol, Tramadol Omeprazole, Vitamine C | Loco-regional + general | Sufentanyl, ketamine, propofol, Atracurium, Lidocaine, Dexamethasone, Droperidol, Morphine |
| T07 | Control | W | Cholesterolemia, depression | Paracetamol, Ketoprofene, Pregabalin, Esomeprazole Ondansetron, Lamaline, Vitamine C, Amoxicilline (25/07/16) Pregabalin, Ketoprofene, Paracetamol, Tramadol Omeprazole, Vitamine C | Loco-regional + general | Droperidol, Dexamethasone, Sufentanil, Ketamine, Propofol, Atracurium, Ephedrine, Dalacine, Ropivacaine Sufentanyl, Propofol, Atracurium, Aphedrine, Atropine, Ropivacaine, Ketamine |
| T08 | Control | M | None | Pregabalin, Ketoprofene, Paracetamol, Lamaline, Vitamine C, Cefazoline | Loco-regional + general | Sufentanyl, Hypnovel, Propofol, Atracurium, Dexamethasone, Ketamine, Morphine, Droperidol |
| T09 | Control | M | None | Pregabalin, Ketoprofene, Paracetamol, Lamaline, Vitamine C, Cefazoline | Loco-regional + general | Hypnovel, Sufentanyl, Ketamine, Propofol, Atracurium, Ropivacaine, Cefazoline, Morphine, Tramadol |
| T10 | Control | M | None | Pregabalin, Ketoprofene, Ondansetron, Paracetamol Tramadol, Omeprazole, Vitamine C, Vancomycine Tazocilline, Cefazoline, Amoxicilline, Sufentanyl Popofol, Ephedrine, Ondansetron, Esomeprazole, Tramadol | Loco-regional + general | Sufentanly, Midazolam, Ketamine, Propofol, Atracurium, Dexamethasone, Ephedrine, Droperidol |
| T11 | Control | W | Hepatitis C (cured) | Exacyl, Morphine, Ketoprofene, Paracetamol Ondansetron, Pregabalin, Esomeprazole, Vitamine C Kardegec, Pravastatine, Diastabol, Bipreterax, Metformine , Lansoprazole, Glibenclamide, Allpurinol, Ranidipine Nebivolol, Levothyrox, Lyrica, Morphine, Enoxaparine, Insuline Lantus, Augmentin, Ipratrpium, Terbutaline Paracetamol, ketoprofene, Zophren, Tramadol, Vit C | Loco-regional + general | Hypnovel, Sufentanyl, Ketamine, Propofol, Exacyl, Salbutamol, Ropivacaine, Ephedrine, Atrovent, Bricanyl, Droperidol |
| T12 | Control | M | Arterial hypertension, Pulmonary embolism, Cholesterolemia, Diabetes (type 1) | Pregabalin, Paracetamol, Ketoprofene, Morphine Droperidol, Ondansetron, Esomeprazole, Tramadol, Vit C | Loco-regional + general | Hypnovel, Sufentanyl, Ketamine, Propofol, Atracurium, Dexamethasone, Ephedrine |
| T13 | Control | M | None | Pregabalin, Paracetamol, Ketoprofene, Morphine Droperidol, Ondansetron, Esomeprazole, Tramadol, Vit C | Loco-regional + general | Hypnovel, Sufentanyl, Ketamine, Propofol, Atracurium, Dexamethasone, Ephedrine |

| | | | | | | |
|-----|---------|---|--|---|-------------------------|--|
| T14 | Control | W | Arterial hypertension, breast cancer | Amlodipine, Valsartan, Esidrex, Uradipil, Morphine, Ketoprofene, Paracetamol, Pregabaline, Ondansetron Tramadol, Esomeprazole, Fer | Loco-regional + general | Sufentanyl, Midazolam, Ketamine, Propofol, Atracurium, Dexamethasone, Cefazoline, Exacyl, Ropivacaine Hypnovel, Ketamine, Propofol, Dexamethasone, Atracurium, Neosynephrine, Cefazoline, Ropivacaine, Sufentanyl Exacyl, Dexomethasone, Neosynephrine, Sufentanyl, Hypnovel, Ketamine, Propofol, Atracurium, Ephedrine, Ropivacaine Hypnovel, Ketamine, Sufentanyl, Propofol, Atracurium, Exacyl, Dexamethasone, Ephedrine, Ropivacaine |
| T15 | Control | W | Gibert's disease | Morphine, Droperidol, Ketoprofene, Esomeprazole Pregabaline, Vit C, Paracetamol, Tramadol | Loco-regional + general | |
| T16 | Control | W | None | Paracetamol, ketoprofene, Cefazoline, Ondansetron Vit C, Tramadol, Pregabaline | Loco-regional + general | Sufentanyl, Hypnovel, Ketamine, Propofol, Atracurium, Ephedrine, Ropivacaine Hypnovel, Ketamine, Sufentanyl, Propofol, Atracurium, Exacyl, Dexamethasone, Ephedrine, Ropivacaine |
| T17 | Control | W | Dyslipidemy | Rosuvastatine, Pregabaline, Paracetamol, Tramadol Vitamine C, Morphine, Droperidol, Ketoprofene, Ondansetron, Esomeprazole Bisoprolol, Omeprazole, Spiramycine, Metronidazole, Pregabaline, Alprazolam, Paracetamol, Cefazoline Ketoprofene, Hydrochlorothiazide, Morphine, Droperidol Ondansetron, Tramadol, Vitamine C Atenolol, Apranax, Pentoprazole, Lyrica, Simvastatine Clobetazol (shampoing), Uvedose, Flecaine, Valsartan, Ketoprofene, Morphine, Droperidol, Paracetamol, OndansetronEnoxaparine, Tramadol, Vitamine C, Infiltration radioguidée | Loco-regional + general | Hypnovel, Ketamine, Ropivacaine, Sufentanyl, Propofol, Atracurium, Exacyl, Dexamethasone Ropivacaine, Prostigmine, Atropine, Ephedrine, Sufentanil, Diprivan, Rocuronium, Ketamine, Midazolam, Phenylephrine |
| T18 | Control | M | Arterial hypertension | | Loco-regional + general | |
| T19 | Control | W | Arterial hypertension, Rhythm disturbances, gastroesophageal reflux, arthritis | Clobetazol (shampoing), Uvedose, Flecaine, Valsartan, Ketoprofene, Morphine, Droperidol, Paracetamol, OndansetronEnoxaparine, Tramadol, Vitamine C, Infiltration radioguidée | Loco-regional + general | Ropivacaine, Prostigmine, Atropine, Ephedrine, Sufentanil, Diprivan, Rocuronium, Ketamine, Midazolam, Phenylephrine |
| T20 | Control | W | Arterial hypertension, depression | Fosinopril, Seroplex, Atarax, Oestradiol, Vancomycine Morphine, Ondansetron, Esomeprazole, Venofer, Ketoprofene, Paracetamol, Pregabaline, naproxene, Vitamine C, Tramadol | Loco-regional + general | Sufentanil, propofol, Ketamine, Atracurium, Dexamethasone, Phenylephrine, Naropaine, Droleptan |

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Supplementary file S3. List of metabolites analyzed by LC-MSMS from serum and muscle of ALS patients and controls. Mean and standard deviation as well as raw p-values are provided.

| | Muscle samples | | | | | |
|-----------------------------------|----------------|----------------|-----------|----------------|-------------|--|
| | ALS | | Control | | | |
| | mean | standard error | mean | standard error | raw p-value | |
| 10-HYDROXYDECANOATE | 0,0002373 | 9,57E-05 | 0,0001558 | 3,37E-05 | 4,92E-05 | |
| 1-AMINOCYCLOPROPANE-1-CARBOXYLATE | 0,0003485 | 9,56E-05 | 0,0003095 | 4,09E-05 | 0,0001289 | |
| 1-OLEOYL-RAC-GLYCEROL | 1,97E-05 | 4,88E-06 | 1,707E-05 | 2,92E-06 | 0,0003645 | |
| 2-DEOXY-GLUCOSE | 0,0071521 | 0,00193817 | 0,005728 | 0,00145356 | 0,0017158 | |
| 2-HYDROXYPYRIDINE | 1,95E-05 | 7,82E-06 | 1,666E-05 | 4,72E-06 | 0,0017158 | |
| 3-AMINO-4-HYDROXYBENZOIC ACID | 7,08E-06 | 4,69E-06 | 7,62E-06 | 3,65E-06 | 0,0019192 | |
| 3-DEHYDROSHIKIMATE | 2,59E-05 | 1,80E-05 | 3,03E-05 | 1,99E-05 | 0,0023938 | |
| 3-HYDROXYBUTANOIC ACID | 0,0004373 | 0,0002467 | 0,0004502 | 0,00011871 | 0,0026694 | |
| 3-SULFINO-L-ALANINE | 0,0004349 | 0,00083938 | 0,0001348 | 8,71E-05 | 0,0033094 | |
| 3-UREIDOPROPIONATE | 1,643E-05 | 0,00001133 | 6,82E-06 | 6,09E-06 | 0,0050251 | |
| 4-AMINOBUTANOATE | 0,0002509 | 7,66E-05 | 0,0002417 | 5,46E-05 | 0,0055642 | |
| 4-GUANIDINOBUTANOATE | 3,25E-05 | 2,49E-05 | 2,68E-05 | 0,00001905 | 0,0055642 | |
| 4-HYDROXY-L-PROLINE | 0,0001849 | 7,59E-05 | 0,0001494 | 0,00006302 | 0,0080299 | |
| 4-METHYL-2-OXO-PENTANOIC ACID | 0,0022193 | 0,00147803 | 0,0009739 | 0,00083917 | 0,008281 | |
| 4-METHYL-2-OXOVALERIC ACID | 0,004383 | 0,00285358 | 0,0020611 | 0,00180899 | 0,008281 | |
| 5-HYDROXYINDOLEACETATE | 1,30E-05 | 7,99E-06 | 1,39E-05 | 7,55E-06 | 0,0110416 | |
| 5-HYDROXYLYSINE | 0,0002507 | 0,00010407 | 0,0002174 | 8,09E-05 | 0,0121288 | |
| 5'-METHYLTHIOADENOSINE | 1,93E-06 | 1,85E-06 | 1,23E-06 | 7,38E-07 | 0,0145914 | |
| 5-OXO-L-PROLINE | 0,0001684 | 5,71E-05 | 0,0001421 | 2,36E-05 | 0,0159806 | |
| ADENINE | 1,177E-05 | 2,14E-05 | 8,74E-06 | 0,00001375 | 0,0159806 | |
| ADENOSINE | 6,03E-06 | 5,45E-06 | 4,10E-06 | 2,40E-06 | 0,0159806 | |
| ADENOSINE 5'-MONOPHOSPHATE | 3,20E-05 | 9,32E-05 | 1,46E-06 | 2,48E-06 | 0,0174847 | |
| ALLANTOIN | 6,22E-06 | 4,25E-06 | 6,76E-06 | 0,00001275 | 0,0191115 | |
| ALPHA-AMINOADIPATE | 7,011E-05 | 4,55E-05 | 4,42E-05 | 0,00002347 | 0,0191115 | |
| ALPHA-GLUCOSE | 0,0022141 | 0,00063313 | 0,0019832 | 0,00048572 | 0,022766 | |
| ALPHA-GLUCOSE 1-PHOSPHATE | 1,112E-05 | 9,15E-06 | 1,55E-05 | 1,50E-05 | 0,022766 | |
| AZELAIC ACID | 0,0153696 | 0,00672659 | 0,0106358 | 0,00144963 | 0,022766 | |
| BETAINE | 0,0068781 | 0,0028287 | 0,0062028 | 0,00198387 | 0,022766 | |
| C10-CARNITINE | 4,71E-06 | 4,13E-06 | 1,02E-05 | 8,82E-06 | 0,027013 | |
| C14-CARNITINE | 2,69E-05 | 1,53E-05 | 4,76E-05 | 3,29E-05 | 0,027013 | |
| C16-CARNITINE | 5,67E-05 | 2,57E-05 | 8,06E-05 | 0,00004965 | 0,0319273 | |
| C18-CARNITINE | 7,37E-06 | 4,53E-06 | 1,14E-05 | 7,27E-06 | 0,0346595 | |
| C2-CARNITINE | 0,0039318 | 0,00170781 | 0,0041185 | 0,0013375 | 0,039891 | |
| C3-CARNITINE | 0,0004475 | 0,00022925 | 0,0004382 | 0,00013784 | 0,0407264 | |
| C4-CARNITINE | 7,98E-05 | 4,72E-05 | 0,0001099 | 8,35E-05 | 0,0440831 | |
| C5-CARNITINE | 6,10E-05 | 5,37E-05 | 4,612E-05 | 3,13E-05 | 0,0440831 | |
| C8-CARNITINE | 6,11E-06 | 4,40E-06 | 1,48E-05 | 1,23E-05 | 0,0476704 | |
| CARNOSINE | 0,0053794 | 0,00233373 | 0,0046249 | 0,00167656 | 0,0555837 | |
| CHOLINE | 1,24E-05 | 6,55E-06 | 1,05E-05 | 3,21E-06 | 0,0555837 | |
| CITRAMALATE | 2,69E-05 | 2,69E-05 | 6,97E-06 | 5,85E-06 | 0,0621942 | |
| CITRULLINE | 0,0003811 | 0,00027577 | 0,0003095 | 0,00018677 | 0,0645625 | |
| CREATINE | 0,0501782 | 0,01414327 | 0,0382527 | 0,00811664 | 0,0645625 | |
| CREATININE | 0,0145781 | 0,00460812 | 0,01504 | 0,00298713 | 0,0645625 | |
| Cyclic AMP | 7,14E-06 | 2,66E-06 | 7,49E-06 | 0,0000038 | 0,0747059 | |
| CYTIDINE | 4,46E-05 | 4,82E-05 | 5,322E-05 | 4,09E-05 | 0,080246 | |
| CYTOSINE | 4,42E-05 | 4,26E-05 | 5,37E-05 | 3,34E-05 | 0,080246 | |
| DEOXYCARNITINE | 0,0045976 | 0,00201981 | 0,0039689 | 0,00120071 | 0,0923266 | |

| | | | | | |
|------------------------------------|-----------|------------|-----------|------------|-----------|
| DIETHANOLAMINE | 9,69E-05 | 5,84E-05 | 5,97E-05 | 2,17E-05 | 0,0988927 |
| EPINEPHRINE | 0,0002012 | 0,00008257 | 0,0001461 | 3,94E-05 | 0,0988927 |
| ETHANOLAMINE | 3,93E-06 | 1,09E-06 | 3,55E-06 | 8,25E-07 | 0,1058263 |
| ETHANOLAMINE PHOSPHATE | 7,07E-06 | 3,51E-06 | 6,02E-06 | 3,52E-06 | 0,1208203 |
| ETHYLMALONIC ACID | 7,09E-06 | 4,87E-06 | 4,54E-06 | 1,54E-06 | 0,1374852 |
| FORMYL-L-METHIONYL PEPTIDE | 1,288E-05 | 8,42E-06 | 1,16E-05 | 2,17E-06 | 0,1374852 |
| FUMARATE | 0,0004625 | 0,00033108 | 0,0003603 | 0,00011744 | 0,1374852 |
| GALACTARATE | 1,19E-05 | 1,12E-05 | 1,44E-05 | 9,13E-06 | 0,1464415 |
| GAMMA-LINOLENIC ACID | 1,22E-05 | 4,82E-06 | 1,22E-05 | 5,74E-06 | 0,1558374 |
| GLUCONIC ACID | 0,0001674 | 7,51E-05 | 0,0001196 | 2,57E-05 | 0,1558374 |
| GLUCURONIC ACID | 1,26E-05 | 3,41E-06 | 1,25E-05 | 3,02E-06 | 0,1558374 |
| GLUCURONOLACTONE | 1,62E-07 | 1,15E-07 | 1,43E-07 | 8,83E-08 | 0,1656838 |
| GLUTATHIONE | 0,0008861 | 0,00045645 | 0,0011526 | 0,00034439 | 0,1656838 |
| GLYCERALDEHYDE 3-PHOSPHATE DIETHYL | 0,0030678 | 0,0018334 | 0,0025873 | 0,00083367 | 0,1759912 |
| GLYCERATE | 0,0007599 | 0,00059432 | 0,0004724 | 0,00023485 | 0,1759912 |
| GLYCINE | 0,0001267 | 3,96E-05 | 8,28E-05 | 2,28E-05 | 0,1867694 |
| GLYCOLALDEHYDE DIMER | 8,20E-06 | 2,78E-06 | 7,17E-06 | 2,17E-06 | 0,1867694 |
| GUANIDINOACETATE | 1,872E-05 | 0,00000736 | 2,017E-05 | 8,11E-06 | 0,1867694 |
| GUANINE | 7,791E-05 | 0,00003491 | 7,405E-05 | 0,00003409 | 0,1980274 |
| GUANOSINE | 2,45E-05 | 1,78E-05 | 2,22E-05 | 1,07E-05 | 0,1980274 |
| GUANOSINE 5'-MONOPHOSPHATE | 3,33E-05 | 1,29E-05 | 3,17E-05 | 2,37E-05 | 0,1980274 |
| HIPPURATE | 4,58E-06 | 1,77E-06 | 2,89E-06 | 1,98E-06 | 0,1980274 |
| HISTAMINE | 0,0001654 | 0,00011325 | 0,0001344 | 6,50E-05 | 0,2097741 |
| HOMOSERINE | 0,0003705 | 0,00013784 | 0,0002883 | 8,21E-05 | 0,214812 |
| HYPOXANTHINE | 0,0219341 | 0,00629716 | 0,017415 | 0,00371858 | 0,2220171 |
| INOSINE | 0,0019242 | 0,00058392 | 0,0014815 | 0,00025098 | 0,2220171 |
| INOSINE 5'-MONOPHOSPHATE | 0,0067994 | 0,00213629 | 0,006645 | 0,00156218 | 0,2255778 |
| LACTATE | 0,2542663 | 0,06991638 | 0,229564 | 0,01621965 | 0,2347637 |
| L-ALANINE | 0,002031 | 0,00052499 | 0,0014234 | 0,00022606 | 0,2347637 |
| L-ANSERINE | 5,06E-05 | 2,64E-05 | 4,25E-05 | 1,91E-05 | 0,2617914 |
| L-ARABITOL | 3,11E-05 | 2,48E-05 | 2,06E-05 | 5,54E-06 | 0,2617914 |
| L-ARGININE | 0,0009807 | 0,0003465 | 0,0008978 | 0,00037224 | 0,2617914 |
| L-ASPARAGINE | 0,0001027 | 3,98E-05 | 8,615E-05 | 1,87E-05 | 0,2760823 |
| L-ASPARTATE | 1,501E-05 | 2,88E-05 | 3,98E-05 | 4,98E-05 | 0,2760823 |
| LAUROYLCARNITINE | 1,50E-05 | 9,30E-06 | 2,99E-05 | 2,31E-05 | 0,2908963 |
| L-CARNITINE | 0,1587535 | 0,04466533 | 0,1290186 | 0,02301093 | 0,2908963 |
| LEUCINE | 0,0004732 | 0,00012694 | 0,0005227 | 0,00012958 | 0,2908963 |
| L-GLUTAMIC ACID | 0,0023692 | 0,0007236 | 0,0021081 | 0,00050886 | 0,3055574 |
| L-GLUTAMINE | 0,0146936 | 0,00437012 | 0,0116359 | 0,0022161 | 0,3062358 |
| L-HISTIDINE | 0,0019591 | 0,00077598 | 0,0016318 | 0,00046163 | 0,3062358 |
| L-ISOLEUCINE | 0,0001344 | 4,40E-05 | 0,0001525 | 4,61E-05 | 0,322102 |
| L-KYNURENINE | 1,508E-05 | 8,10E-06 | 2,54E-05 | 1,41E-05 | 0,322102 |
| L-LYSINE | 0,0004045 | 0,00017035 | 0,000369 | 0,00016839 | 0,3384953 |
| L-METHIONINE | 0,0001108 | 4,10E-05 | 0,0001105 | 5,01E-05 | 0,3384953 |
| L-ORNITHINE | 3,915E-05 | 1,60E-05 | 2,69E-05 | 1,34E-05 | 0,3384953 |
| L-PHENYLALANINE | 0,0005044 | 0,00018296 | 0,000517 | 0,00017197 | 0,3554146 |
| L-PIPECOLIC ACID | 0,0001492 | 7,81E-05 | 0,0001236 | 6,27E-05 | 0,3728579 |
| L-PROLINE | 0,0047555 | 0,00162714 | 0,0036908 | 0,00094889 | 0,3728579 |
| L-SERINE | 0,0003269 | 0,0001076 | 0,0002478 | 0,0000639 | 0,3728579 |
| L-THREONINE | 0,0006245 | 0,00017996 | 0,0005188 | 7,18E-05 | 0,3906891 |
| L-TRYPTOPHAN | 0,0002849 | 9,90E-05 | 0,000286 | 0,00010563 | 0,3908219 |
| L-TYROSINE | 0,0003369 | 9,83E-05 | 0,0003162 | 0,0001067 | 0,3908219 |
| LUMICHROME | 5,06E-06 | 6,85E-06 | 9,61E-06 | 4,69E-06 | 0,3908219 |
| L-VALINE | 0,000533 | 0,0001306 | 0,0004976 | 9,29E-05 | 0,3908219 |
| LYXOSE | 4,04E-05 | 0,00002028 | 4,40E-05 | 1,46E-05 | 0,4282919 |
| MALATE | 0,0041952 | 0,00218534 | 0,0036877 | 0,00115607 | 0,4282919 |
| MANNITOL | 0,0003071 | 9,41E-05 | 0,0002806 | 9,19E-05 | 0,4282919 |
| MANNOSE | 8,85E-05 | 7,86E-05 | 6,41E-05 | 0,00004995 | 0,4477849 |
| METHYL JASMONATE | 1,077E-05 | 4,33E-06 | 8,10E-06 | 1,53E-06 | 0,4477849 |
| METHYLGUANIDINE | 2,77E-05 | 2,06E-05 | 3,47E-05 | 1,98E-05 | 0,4677724 |

| | | | | | |
|---------------------------------|-----------|------------|-----------|------------|-----------|
| MYO-INOSITOL | 5,245E-05 | 0,00002806 | 5,16E-05 | 2,11E-05 | 0,4677724 |
| N(PAI)-METHYL-L-HISTIDINE | 9,108E-05 | 4,28E-05 | 8,24E-05 | 4,04E-05 | 0,4677724 |
| N6-(DELTA2-ISOPENTENYL)-ADENINE | 2,04E-05 | 1,38E-05 | 2,05E-05 | 1,28E-05 | 0,4882447 |
| N-ACETYL-L-ASPARTIC ACID | 1,37E-05 | 8,14E-06 | 1,12E-05 | 6,11E-06 | 0,4882447 |
| N-ACETYL-L-METHIONINE | 0,0001115 | 5,16E-05 | 0,000102 | 6,71E-05 | 0,4882447 |
| N-ACETYL-L-PHENYLALANINE | 3,55E-05 | 2,30E-05 | 3,067E-05 | 1,44E-05 | 0,4882447 |
| N-ACETYLNEURAMINATE | 1,78E-05 | 7,49E-06 | 2,10E-05 | 8,49E-06 | 0,4882447 |
| N,N,N-TRIMETHYL-LYSINE | 9,76E-06 | 2,93E-06 | 9,85E-06 | 4,65E-06 | 0,4882447 |
| NICOTINAMIDE | 0,0156191 | 0,00470442 | 0,0162493 | 0,00343638 | 0,4882447 |
| NICOTINATE | 5,22E-05 | 1,43E-05 | 4,21E-05 | 6,50E-06 | 0,5091907 |
| O-ACETYL-L-CARNITINE | 0,0356887 | 0,02214523 | 0,0330133 | 0,01857346 | 0,5091907 |
| OLEATE | 4,27E-06 | 1,38E-06 | 4,98E-06 | 1,80E-06 | 0,5091907 |
| OMEGA-HYDROXYDODECANOIC ACID | 0,0003059 | 8,77E-05 | 0,0002432 | 3,87E-05 | 0,5331531 |
| OPHTHALMIC ACID | 2,81E-05 | 1,43E-05 | 3,04E-05 | 0,0000138 | 0,541187 |
| PALMITATE | 1,85E-05 | 9,19E-06 | 1,61E-05 | 1,07E-05 | 0,5524529 |
| PANTOTHENIC ACID | 0,0029781 | 0,0023852 | 0,0021891 | 0,00099681 | 0,5524529 |
| PARAXANTHINE | 0,0003163 | 0,00036771 | 0,0001576 | 0,00016501 | 0,5524529 |
| PHOSPHOCHOLINE | 0,0001289 | 3,94E-05 | 9,311E-05 | 3,82E-05 | 0,5747405 |
| PICOLINIC ACID | 7,97E-06 | 5,07E-06 | 5,29E-06 | 1,45E-06 | 0,6203031 |
| PYRIDOXAMINE | 4,17E-06 | 3,02E-06 | 3,85E-06 | 3,27E-06 | 0,6205481 |
| QUINATE | 3,12E-05 | 3,38E-05 | 3,10E-05 | 2,83E-05 | 0,644032 |
| RAC-GLYCEROL 1-MYRISTATE | 3,502E-05 | 9,93E-06 | 2,96E-05 | 6,05E-06 | 0,6677892 |
| RETINOATE | 1,39E-06 | 7,81E-07 | 3,35E-06 | 4,73E-06 | 0,6678767 |
| RIBOSE 5-PHOSPHATE | 0,0005286 | 0,00023538 | 0,0003694 | 0,00012139 | 0,6678767 |
| S-(5'-ADENOSYL)-L-HOMOCYSTEINE | 1,48E-05 | 7,64E-06 | 1,20E-05 | 4,25E-06 | 0,6678767 |
| SARCOSINE | 0,0005266 | 0,00016003 | 0,0003942 | 9,05E-05 | 0,6678767 |
| SHIKIMATE | 3,67E-06 | 1,47E-06 | 2,37E-06 | 6,52E-07 | 0,6678767 |
| SN-GLYCERO-3-PHOSPHOCHOLINE | 0,0148173 | 0,00532371 | 0,0160334 | 0,00643818 | 0,7165645 |
| SN-GLYCEROL 3-PHOSPHATE | 0,0005759 | 0,00051698 | 0,0003302 | 0,000252 | 0,7165645 |
| STEARATE | 1,38E-05 | 7,62E-06 | 9,74E-06 | 3,00E-06 | 0,7413628 |
| SUBERIC ACID | 0,0061379 | 0,00282687 | 0,0041307 | 0,00059982 | 0,7664327 |
| SUCCINATE | 0,0010274 | 0,00053957 | 0,001049 | 0,00068644 | 0,7917496 |
| TAURINE | 0,0194249 | 0,00408937 | 0,0151794 | 0,00347114 | 0,7917496 |
| THEOPHYLLINE | 0,0002559 | 0,00029684 | 0,0002428 | 0,00028196 | 0,7917496 |
| Thiamine | 5,13E-07 | 7,16E-07 | 6,93E-07 | 1,23E-06 | 0,7917496 |
| TRANS-ACONITATE | 9,88E-06 | 3,43E-05 | 0,0001011 | 0,00042116 | 0,8125337 |
| TRANS-CINNAMALDEHYDE | 4,81E-06 | 2,31E-06 | 3,28E-06 | 1,08E-06 | 0,8172881 |
| TRANS-CINNAMATE | 0,0034775 | 0,0009822 | 0,0026841 | 0,00032495 | 0,8172881 |
| TRIGONELLINE | 0,0001259 | 0,00011601 | 9,248E-05 | 9,38E-05 | 0,8172881 |
| TRIMETHYLAMINE | 3,37E-05 | 1,32E-05 | 3,147E-05 | 9,30E-06 | 0,843022 |
| URACIL | 0,000127 | 0,00004522 | 9,956E-05 | 4,57E-05 | 0,8949687 |
| URATE | 0,000354 | 0,00015042 | 0,0003291 | 0,00014898 | 0,8949687 |
| URIDINE | 0,0013856 | 0,00053877 | 0,0011115 | 0,000627 | 0,8949687 |
| URIDINE-5-MONOPHOSPHATE | 0,000157 | 8,57E-05 | 0,000141 | 0,00003366 | 0,94737 |
| UROCANATE | 0,0001489 | 0,00011853 | 9,50E-05 | 8,29E-05 | 0,94737 |
| XANTHINE | 0,0003009 | 0,00017625 | 0,0002736 | 0,0001738 | 0,9735145 |
| XANTHOSINE | 1,20E-06 | 6,76E-07 | 9,55E-07 | 5,40E-07 | 0,9736707 |
| XANTHURENIC ACID | 2,39E-05 | 0,00002223 | 1,47E-05 | 7,63E-06 | 0,9736707 |

Serum samples

| | ALS | | Control | | |
|-------------------------------|-----------|----------------|-----------|----------------|-------------|
| | mean | standard error | mean | standard error | raw p-value |
| 10-HYDROXYDECANOATE | 0,0010336 | 0,00039457 | 0,0009184 | 0,00071497 | 0,0060909 |
| 1-METHYLADENOSINE | 3,153E-05 | 4,56E-06 | 3,028E-05 | 0,00000685 | 0,0135656 |
| 1-OLEOYL-RAC-GLYCEROL | 0,0025817 | 0,00095637 | 0,0023276 | 0,00083371 | 0,0241188 |
| 2-AMINO-2-METHYLPROPANOATE | 0,0002288 | 9,15E-05 | 0,0002227 | 8,04E-05 | 0,0241188 |
| 2-HYDROXYBUTYRIC ACID | 0,0442296 | 0,01045463 | 0,0535211 | 0,01765621 | 0,0304795 |
| 2-HYDROXYPYRIDINE | 7,37E-05 | 9,96E-05 | 3,449E-05 | 1,47E-05 | 0,0354788 |
| 3-(2-HYDROXYPHENYL)PROPANOATE | 0,0013851 | 0,00043531 | 0,0018882 | 0,00090982 | 0,0411616 |
| 3-AMINO-4-HYDROXYBENZOIC ACID | 0,0012551 | 0,00068451 | 0,0013245 | 0,00049327 | 0,0411616 |

| | | | | | |
|------------------------------------|-----------|------------|-----------|------------|-----------|
| 3-AMINOISOBUTANOATE | 0,0012308 | 0,00029288 | 0,0013718 | 0,00062375 | 0,0511201 |
| 3-DEHYDROSHIKIMATE | 0,0003955 | 0,0002391 | 0,0003807 | 0,000213 | 0,0511201 |
| 3-METHYGLUTARIC ACID | 0,001324 | 0,00031232 | 0,0028579 | 0,00650989 | 0,0511201 |
| 3-Methylhistidine | 0,0027384 | 0,00213251 | 0,0036443 | 0,00189194 | 0,0548586 |
| 4-GUANIDINOBUTANOATE | 6,93E-05 | 3,82E-05 | 6,25E-05 | 2,74E-05 | 0,0588224 |
| 4-HYDROXY-2-QUINOLINECARBOXYLIC AC | 0,0005474 | 0,00034279 | 0,0004383 | 0,00013622 | 0,0630212 |
| 4-METHYL-2-OXO-PENTANOIC ACID | 0,0773471 | 0,01135551 | 0,0780071 | 0,01745059 | 0,0674647 |
| 4-METHYL-2-OXO-VALERIC ACID | 0,1365614 | 0,02099466 | 0,1458267 | 0,03419622 | 0,0674647 |
| 5,6-DIHYDRO URACIL | 0,0006956 | 7,48E-05 | 0,0007139 | 0,0001421 | 0,0771255 |
| 5-OXO-L-PROLINE | 0,0001784 | 3,21E-05 | 0,0001744 | 5,07E-05 | 0,0823629 |
| ADENINE | 0,0005363 | 9,43E-05 | 0,0004906 | 0,00014626 | 0,087885 |
| ADIPIC ACID | 0,0002895 | 0,00005826 | 0,000336 | 7,96E-05 | 0,087885 |
| ALLANTOIN | 0,0002733 | 8,29E-05 | 0,0002165 | 5,02E-05 | 0,0998236 |
| ALPHA-TOCOPHEROL | 0,0003119 | 0,00010303 | 0,0002861 | 7,91E-05 | 0,1062602 |
| AZELAIC ACID | 0,0096081 | 0,00178034 | 0,0104866 | 0,00549149 | 0,1062602 |
| BENZYL ALCOHOL | 0,0091518 | 0,00655118 | 0,0080587 | 0,00603334 | 0,1130215 |
| BETA-ALANINE | 1,86E-05 | 5,93E-06 | 2,49E-05 | 1,17E-05 | 0,1130215 |
| BETAINE | 0,1501893 | 0,02935157 | 0,1524839 | 0,03887142 | 0,1201174 |
| BILIRUBIN | 0,0065791 | 0,00310278 | 0,0069696 | 0,00374026 | 0,1435077 |
| CAFFEATE | 0,0003414 | 4,46E-05 | 0,0003496 | 3,69E-05 | 0,1435077 |
| CATECHOL | 0,0004592 | 0,00052057 | 0,0002798 | 0,00020494 | 0,1799372 |
| CITRAMALATE | 0,0007209 | 0,00027261 | 0,0005437 | 0,00021064 | 0,1799372 |
| CITRULLINE | 0,0005596 | 0,00023804 | 0,000687 | 0,00070073 | 0,1900361 |
| CORTISOL | 0,0024404 | 0,00129256 | 0,0025322 | 0,00073049 | 0,2005465 |
| CREATINE | 0,017802 | 0,00677683 | 0,0157402 | 0,00665356 | 0,2114749 |
| CREATININE | 0,4844507 | 0,04663012 | 0,4861645 | 0,07678168 | 0,2228275 |
| CYTIDINE | 8,23E-05 | 2,20E-05 | 8,404E-05 | 4,12E-05 | 0,2468266 |
| CYTOSINE | 0,0004553 | 8,62E-05 | 0,000436 | 7,26E-05 | 0,2468266 |
| DEOXYCARNITINE | 0,0010053 | 0,00051427 | 0,0008853 | 0,00037795 | 0,2594823 |
| DIETHANOLAMINE | 0,0004164 | 0,00026174 | 0,0003189 | 0,00013422 | 0,2594823 |
| DOPAMINE | 0,0001746 | 3,49E-05 | 0,0002171 | 5,03E-05 | 0,2725804 |
| ELAIDIC ACID | 0,0016513 | 0,00115562 | 0,0011132 | 0,00051871 | 0,2725804 |
| EPINEPHRINE | 0,0071364 | 0,0021587 | 0,0064933 | 0,00164743 | 0,2725804 |
| ETHANOLAMINE | 7,24E-05 | 1,10E-05 | 7,10E-05 | 1,18E-05 | 0,2725804 |
| ETHYLMALONIC ACID | 8,38E-05 | 0,00002106 | 6,78E-05 | 2,07E-05 | 0,2861238 |
| FORMYL-METHIONYL PEPTIDE | 0,0003843 | 8,76E-05 | 0,0003782 | 6,68E-05 | 0,3001148 |
| FUMARATE | 0,0005294 | 0,00013045 | 0,0004494 | 0,00008428 | 0,3001148 |
| GALACTOSAMINE | 0,0008528 | 0,0002867 | 0,0006842 | 0,00016126 | 0,3145545 |
| GAMMA-LINOLENIC ACID | 0,0014754 | 0,0005647 | 0,0019755 | 0,00082389 | 0,3145545 |
| GLUCONIC ACID | 0,0016384 | 0,00049163 | 0,001358 | 0,00035428 | 0,3294436 |
| GLUCURONIC ACID | 0,0003325 | 0,00011144 | 0,0003441 | 0,00026158 | 0,3294436 |
| GLYCERATE | 0,0047406 | 0,00201929 | 0,0047189 | 0,00193504 | 0,3447819 |
| GLYCERO-3-PHOSPHOCHOLINE | 0,0098729 | 0,00458931 | 0,0170443 | 0,02650162 | 0,3447819 |
| GLYCINE | 7,29E-05 | 2,24E-05 | 9,79E-05 | 7,84E-05 | 0,3768005 |
| GUANIDINOACETATE | 0,0007738 | 0,00016346 | 0,000659 | 0,00017252 | 0,3934759 |
| GUANINE | 0,001248 | 0,00022431 | 0,0012598 | 0,00020316 | 0,3934759 |
| GUANOSINE | 0,0019067 | 0,00107959 | 0,0016032 | 0,00098642 | 0,3934759 |
| HIPPURATE | 0,0299184 | 0,02902913 | 0,0287961 | 0,02901914 | 0,4105907 |
| HYPOXANTHINE | 0,0091964 | 0,00404098 | 0,0085899 | 0,00280587 | 0,42814 |
| INDOLE-3-ACETIC ACID | 0,0078499 | 0,00483188 | 0,0059662 | 0,00264023 | 0,42814 |
| INDOXYL SULFATE | 0,0359751 | 0,02262754 | 0,0365553 | 0,02014305 | 0,4461182 |
| INOSINE | 0,021219 | 0,01414736 | 0,0117617 | 0,00924096 | 0,4461182 |
| ISOCITRIC ACID | 0,045421 | 0,01616773 | 0,0362928 | 0,01128755 | 0,4461182 |
| LACTATE | 0,4213917 | 0,03758585 | 0,42651 | 0,06465448 | 0,4461182 |
| L-ALANINE | 0,0017948 | 0,00049751 | 0,0019703 | 0,00084244 | 0,4644923 |
| L-ARGININE | 0,0038421 | 0,0014202 | 0,005264 | 0,00594235 | 0,4833341 |
| L-ASPARAGINE | 0,0009727 | 0,00044495 | 0,0007701 | 0,00021506 | 0,5025556 |
| L-ASPARTATE | 0,0004315 | 0,00019837 | 0,0004222 | 0,00012554 | 0,5221739 |
| LAUROYLCARNITINE | 0,000477 | 0,00049555 | 0,0004174 | 0,00021681 | 0,5221739 |
| L-CARNITINE | 0,0727098 | 0,01439749 | 0,0744165 | 0,03339634 | 0,5221739 |

| | | | | | |
|------------------------------------|-----------|------------|-----------|------------|-----------|
| LEUCINE | 0,0081901 | 0,00190335 | 0,0081791 | 0,00192571 | 0,5221739 |
| L-GLUTAMIC ACID | 0,0039593 | 0,00118489 | 0,0044389 | 0,00156879 | 0,5221739 |
| L-GLUTAMINE | 0,0405196 | 0,01459512 | 0,0329964 | 0,0096063 | 0,5421785 |
| L-HISTIDINE | 0,0052493 | 0,00091189 | 0,0066477 | 0,0039892 | 0,5421785 |
| LINOLEATE | 0,0006997 | 0,00035497 | 0,0005483 | 0,00026836 | 0,5421785 |
| L-KYNURENINE | 0,0002819 | 0,0001144 | 0,0002999 | 0,00012645 | 0,5421785 |
| L-LYSINE | 0,0008193 | 0,0003436 | 0,0016641 | 0,00306102 | 0,5421785 |
| L-METHIONINE | 0,0009616 | 0,00021875 | 0,0010046 | 0,00026697 | 0,5625582 |
| L-NORVALINE | 0,0003875 | 0,00024644 | 0,0004319 | 0,00036105 | 0,5625582 |
| L-ORNITHINE | 0,0001873 | 6,80E-05 | 0,0004227 | 0,00088311 | 0,5625582 |
| L-PHENYLALANINE | 0,0087045 | 0,00170519 | 0,0086299 | 0,00107723 | 0,5625582 |
| L-PIPECOLIC ACID | 0,0008307 | 0,00071652 | 0,0006123 | 0,00021375 | 0,5625582 |
| L-PROLINE | 0,0190599 | 0,00559921 | 0,019999 | 0,00662664 | 0,6043929 |
| L-SERINE | 0,0003307 | 0,00006836 | 0,0004214 | 0,00025701 | 0,6043929 |
| L-THREONINE | 0,0007415 | 0,00020694 | 0,0009016 | 0,00058155 | 0,6043929 |
| L-TRYPTOPHAN | 0,0249461 | 0,00533643 | 0,0257032 | 0,0058257 | 0,6043929 |
| L-TYROSINE | 0,011182 | 0,00305217 | 0,011148 | 0,00182869 | 0,6043929 |
| LUMICHROME | 0,0001971 | 0,00016652 | 0,000158 | 0,0001345 | 0,6043929 |
| L-VALINE | 0,0052863 | 0,00101146 | 0,0056029 | 0,00135197 | 0,6258208 |
| LYXOSE | 0,0016703 | 0,0002977 | 0,0017125 | 0,00028164 | 0,6258208 |
| MALATE | 0,0012963 | 0,00036207 | 0,0010562 | 0,00023452 | 0,6475694 |
| MANNOSE | 0,0091142 | 0,0067038 | 0,0110468 | 0,00771403 | 0,6475694 |
| METHYL INDOLE-3-ACETATE | 0,0056252 | 0,00426503 | 0,0049807 | 0,00409582 | 0,6475694 |
| METHYL-GUANIDINE | 4,88E-05 | 1,15E-05 | 5,34E-05 | 1,54E-05 | 0,6696231 |
| METHYL-JASMONATE | 0,0003283 | 0,00015166 | 0,0003183 | 0,00019196 | 0,6919654 |
| N,N,N-TRIMETHYL-LYSINE | 0,0009475 | 0,00020382 | 0,0008685 | 0,00024795 | 0,6919654 |
| N6-(DELTA2-ISOPENTENYL)-ADENINE | 0,0001989 | 0,00008174 | 0,0001518 | 0,00004329 | 0,6919654 |
| N-Acetyl-4-O-acetylneurameric acid | 0,0002943 | 9,54E-05 | 0,0002595 | 6,19E-05 | 0,6919654 |
| N-ACETYL-ALANINE | 0,0010114 | 0,00016646 | 0,0010915 | 0,00021259 | 0,6919654 |
| N-ACETYLGLYCINE | 0,0014782 | 0,0013675 | 0,0013468 | 0,00089021 | 0,7145144 |
| N-ACETYL-METHIONINE | 0,0002561 | 8,25E-05 | 0,0002323 | 6,16E-05 | 0,7145791 |
| N-ACETYL-SERINE | 0,0005792 | 0,00011663 | 0,0006394 | 0,000177 | 0,7145791 |
| N-ACETYL-TRYPTOPHAN | 0,0001591 | 0,00004502 | 0,0001499 | 5,12E-05 | 0,7145791 |
| NICOTINAMIDE | 0,0005049 | 0,00026604 | 0,0006988 | 0,00025828 | 0,7374463 |
| NICOTINATE | 0,0002942 | 0,00005535 | 0,0002895 | 4,66E-05 | 0,7374463 |
| NORLEUCINE | 0,0038241 | 0,00090075 | 0,0036592 | 0,00089918 | 0,7605482 |
| O-ACETYL-CARNITINE | 0,0162485 | 0,0082141 | 0,0139765 | 0,00447433 | 0,7605482 |
| OLEATE | 0,0006116 | 0,00028867 | 0,0005651 | 0,00022493 | 0,7838657 |
| PALMITATE | 0,0005367 | 0,00029417 | 0,00038 | 0,00012481 | 0,7838657 |
| PANTOTHENIC ACID | 0,0010121 | 0,00038371 | 0,0010061 | 0,00033841 | 0,7838657 |
| PARAXANTHINE | 0,0025616 | 0,00166642 | 0,0033562 | 0,00288434 | 0,8073789 |
| PHENYLETHANOLAMINE | 0,0001539 | 1,88E-05 | 0,0001579 | 3,42E-05 | 0,8073789 |
| PIPECOLATE | 0,0018699 | 0,00231978 | 0,003063 | 0,00393165 | 0,8073789 |
| PYRIDOXAMINE | 0,0036939 | 0,00070441 | 0,0038041 | 0,00056261 | 0,8310675 |
| QUINATE | 0,0012951 | 0,00143522 | 0,0006762 | 0,0005425 | 0,8310675 |
| RETINOATE | 0,0002293 | 0,00019832 | 0,0001666 | 0,00010456 | 0,8549105 |
| RETINOL | 0,0002994 | 8,93E-05 | 0,000335 | 9,94E-05 | 0,8549105 |
| RIBOFLAVIN | 4,01E-05 | 6,35E-05 | 4,78E-05 | 6,72E-05 | 0,8788868 |
| SARCOSINE | 0,0002898 | 0,00010977 | 0,0002603 | 0,00010764 | 0,8788868 |
| SEROTONINE | 0,0012833 | 0,00071677 | 0,0013366 | 0,00082996 | 0,8788868 |
| SPERMIDINE | 0,0003152 | 0,00015408 | 0,0002285 | 0,00013148 | 0,9029747 |
| SUBERIC ACID | 0,0052097 | 0,00084556 | 0,006784 | 0,00817032 | 0,9029747 |
| SUCCINATE | 0,0060813 | 0,00111311 | 0,0055893 | 0,00138837 | 0,9029747 |
| TAURINE | 0,0075309 | 0,00412892 | 0,0076318 | 0,00214303 | 0,9271523 |
| THEOBROMINE | 0,01454 | 0,01192705 | 0,0171095 | 0,01440866 | 0,9271523 |
| THEOPHYLLINE | 0,0221159 | 0,01934156 | 0,0193465 | 0,01841928 | 0,9271523 |
| TRANS-ACONITATE | 0,0003557 | 0,00013277 | 0,0002922 | 0,000094 | 0,9513973 |
| TRANS-CINNAMALDEHYDE | 0,0001428 | 3,41E-05 | 0,0001403 | 2,91E-05 | 0,9756874 |
| TYRAMINE | 0,0001539 | 1,88E-05 | 0,0001579 | 0,00003416 | 0,9756874 |
| URACIL | 0,0001143 | 3,47E-05 | 0,0001186 | 3,00E-05 | 0,9756874 |

| | | | | | |
|----------|-----------|------------|-----------|------------|-----------|
| URATE | 0,098234 | 0,01583568 | 0,0975781 | 0,02184737 | 0,9756874 |
| URIDINE | 0,0074105 | 0,00228636 | 0,0078206 | 0,00182818 | 1 |
| XANTHINE | 0,0055857 | 0,00148958 | 0,005904 | 0,00169298 | 1 |

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Supplementary file S4.Pathway analysis based on the metabolites identified in the muscle and the serum of ALS patients and controls. All the metabolic pathways generated by pathway analysis are listed with the fold enrichment and the p-value.

Serum

| Pathway | Total | Expected | Hits | Raw p | FDR p | Impact |
|--|-------|----------|------|--------|-------|--------|
| Biosynthesis of unsaturated fatty acids | 36 | 0.26 | 2 | 0.0253 | 0.981 | 0 |
| Tyrosine metabolism | 42 | 0.3 | 2 | 0.0338 | 0.981 | 0.15 |
| Linoleic acid metabolism | 5 | 0.04 | 1 | 0.035 | 0.981 | 1 |
| Arginine biosynthesis | 14 | 0.1 | 1 | 0.0953 | 1 | 0 |
| Nicotinate and nicotinamide metabolism | 15 | 0.11 | 1 | 0.102 | 1 | 0.19 |
| Citrate cycle (TCA cycle) | 20 | 0.14 | 1 | 0.134 | 1 | 0.03 |
| beta-Alanine metabolism* | 21 | 0.15 | 1 | 0.14 | 1 | 0 |
| Pyruvate metabolism | 22 | 0.16 | 1 | 0.146 | 1 | 0 |
| Propanoate metabolism | 23 | 0.16 | 1 | 0.152 | 1 | 0 |
| Alanine, aspartate and glutamate metabolism* | 28 | 0.2 | 1 | 0.182 | 1 | 0 |
| Glutathione metabolism* | 28 | 0.2 | 1 | 0.182 | 1 | 0.01 |
| Arginine and proline metabolism* | 38 | 0.27 | 1 | 0.24 | 1 | 0.03 |
| Fatty acid elongation | 39 | 0.28 | 1 | 0.245 | 1 | 0 |
| Fatty acid degradation | 39 | 0.28 | 1 | 0.245 | 1 | 0 |
| Fatty acid biosynthesis | 47 | 0.33 | 1 | 0.288 | 1 | 0.01 |

*commonly altered pathways

Muscle

| Pathway | Total | Expected | Hits | Raw p | FDR p | Impact |
|--|-------|----------|------|----------|--------|--------|
| Glycine, serine and threonine metabolism | 33 | 0.4 | 4 | 0.000528 | 0.0443 | 0.56 |
| Valine, leucine and isoleucine biosynthesis | 8 | 0.1 | 2 | 0.00382 | 0.16 | 0 |
| Aminoacyl-tRNA biosynthesis | 48 | 0.59 | 3 | 0.0191 | 0.534 | 0.17 |
| Glyoxylate and dicarboxylate metabolism | 32 | 0.39 | 2 | 0.0568 | 1 | 0.15 |
| Valine, leucine and isoleucine degradation | 40 | 0.49 | 2 | 0.0842 | 1 | 0.02 |
| Taurine and hypotaurine metabolism | 8 | 0.1 | 1 | 0.0942 | 1 | 0.43 |
| Primary bile acid biosynthesis | 46 | 0.56 | 2 | 0.107 | 1 | 0.02 |
| Phenylalanine metabolism | 10 | 0.12 | 1 | 0.116 | 1 | 0 |
| Pantothenate and CoA biosynthesis | 19 | 0.23 | 1 | 0.21 | 1 | 0.03 |
| Selenocompound metabolism | 20 | 0.25 | 1 | 0.22 | 1 | 0 |
| Sphingolipid metabolism | 21 | 0.26 | 1 | 0.229 | 1 | 0 |
| beta-Alanine metabolism* | 21 | 0.26 | 1 | 0.229 | 1 | 0.1 |
| Alanine, aspartate and glutamate metabolism* | 28 | 0.34 | 1 | 0.294 | 1 | 0 |
| Glutathione metabolism* | 28 | 0.34 | 1 | 0.294 | 1 | 0.09 |
| Porphyrin and chlorophyll metabolism | 30 | 0.37 | 1 | 0.312 | 1 | 0 |
| Cysteine and methionine metabolism | 33 | 0.4 | 1 | 0.337 | 1 | 0.02 |
| Arginine and proline metabolism* | 38 | 0.47 | 1 | 0.378 | 1 | 1 |
| Pyrimidine metabolism | 39 | 0.48 | 1 | 0.386 | 1 | 0.01 |
| Purine metabolism | 65 | 0.8 | 1 | 0.559 | 1 | 0 |

*commonly altered pathways

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Supplementary file S5. Pathway analysis based on the metabolites identified in the serum and the muscle of ALS patients regarding weight variation. All the metabolic pathways generated by pathway analysis are listed with the fold enrichment and the p-value.

Serum

| Pathway | Total | Expected | Hits | Raw p | FDR p | Impact |
|---|-------|----------|------|-----------|---------|--------|
| Citrate cycle (TCA cycle) | 20 | 0.19 | 4 | 0.0000252 | 0.00212 | 0.15 |
| Pyruvate metabolism | 22 | 0.21 | 3 | 0.00101 | 0.0425 | 0.03 |
| Alanine, aspartate and glutamate metabolism | 28 | 0.27 | 3 | 0.00208 | 0.0582 | 0.2 |
| Glyoxylate and dicarboxylate metabolism | 32 | 0.31 | 3 | 0.00308 | 0.0646 | 0,00 |
| Arginine biosynthesis | 14 | 0.14 | 2 | 0.00744 | 0.12 | 0.12 |
| Butanoate metabolism | 15 | 0.15 | 2 | 0.00854 | 0.12 | 0,00 |
| Lysine degradation | 25 | 0.24 | 2 | 0.0231 | 0.277 | 0,00 |
| Linoleic acid metabolism | 5 | 0.05 | 1 | 0.0475 | 0.477 | 1,00 |
| Nitrogen metabolism | 6 | 0.06 | 1 | 0.0568 | 0.477 | 0,00 |
| D-Glutamine and D-glutamate metabolism | 6 | 0.06 | 1 | 0.0568 | 0.477 | 0.5 |
| Aminoacyl-tRNA biosynthesis | 48 | 0.46 | 2 | 0.0763 | 0.583 | 0,00 |
| Nicotinate and nicotinamide metabolism | 15 | 0.15 | 1 | 0.136 | 0.935 | 0,00 |
| Histidine metabolism | 16 | 0.15 | 1 | 0.145 | 0.935 | 0,00 |
| Pantothenate and CoA biosynthesis | 19 | 0.18 | 1 | 0.17 | 1 | 0.02 |
| beta-Alanine metabolism | 21 | 0.2 | 1 | 0.186 | 1 | 0.06 |
| Propanoate metabolism | 23 | 0.22 | 1 | 0.202 | 1 | 0,00 |
| Glycolysis / Gluconeogenesis | 26 | 0.25 | 1 | 0.25 | 1 | 0,00 |
| Glutathione metabolism | 28 | 0.27 | 1 | 0.24 | 1 | 0.02 |
| Porphyrin and chlorophyll metabolism | 30 | 0.29 | 1 | 0.255 | 1 | 0,00 |
| Biosynthesis of unsaturated fatty acids | 36 | 0.35 | 1 | 0.298 | 1 | 0,00 |
| Arginine and proline metabolism | 38 | 0.37 | 1 | 0.312 | 1 | 0.09 |
| Pyrimidine metabolism | 39 | 0.38 | 1 | 0.319 | 1 | 0.03 |
| Tryptophan metabolism | 41 | 0.4 | 1 | 0.332 | 1 | 0.14 |
| Tyrosine metabolism | 42 | 0.41 | 1 | 0.339 | 1 | 0.02 |

Muscle

| Pathway | Total | Expected | Hits | Raw p | FDR p | Impact |
|---|-------|----------|------|----------|--------|--------|
| Arginine biosynthesis | 14 | 0.14 | 3 | 0.000251 | 0.0211 | 0.14 |
| Arginine and proline metabolism | 38 | 0.37 | 2 | 0.0503 | 0.916 | 0.17 |
| Pyrimidine metabolism | 39 | 0.38 | 2 | 0.0527 | 0.916 | 0.09 |
| D-Glutamine and D-glutamate metabolism | 6 | 0.06 | 1 | 0.0568 | 0.916 | 0,00 |
| Nitrogen metabolism | 6 | 0.06 | 1 | 0.0568 | 0.916 | 0,00 |
| Ascorbate and aldarate metabolism | 8 | 0.08 | 1 | 0.075 | 0.916 | 0,00 |
| Aminoacyl-tRNA biosynthesis | 48 | 0.46 | 2 | 0.0763 | 0.916 | 0,00 |
| Nicotinate and nicotinamide metabolism | 15 | 0.15 | 1 | 0.136 | 1 | 0,00 |
| Histidine metabolism | 16 | 0.15 | 1 | 0.145 | 1 | 0.12 |
| Galactose metabolism | 27 | 0.26 | 1 | 0.233 | 1 | 0,00 |
| Glutathione metabolism | 28 | 0.27 | 1 | 0.24 | 1 | 0,00 |
| Phosphatidylinositol signaling system | 28 | 0.27 | 1 | 0.24 | 1 | 0.04 |
| Alanine, aspartate and glutamate metabolism | 28 | 0.27 | 1 | 0.24 | 1 | 0.11 |
| Inositol phosphate metabolism | 0 | 0.29 | 1 | 0.255 | 1 | 0.13 |
| Glyoxylate and dicarboxylate metabolism | 32 | 0.31 | 1 | 0.27 | 1 | 0,00 |
| Tyrosine metabolism | 42 | 0.41 | 1 | 0.339 | 1 | 0.01 |
| Purine metabolism | 65 | 0.63 | 1 | 0.476 | 1 | 0,00 |

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Supplementary file S6. Pathway analysis based on the metabolites identified in the serum and in the muscle of ALS patients regarding disease duration. All the metabolic pathways generated by pathway analysis are listed with the fold enrichment and the p-value.

Serum

| Pathway | Total | Expected | Hits | Raw p | FDR p | Impact |
|---|-------|----------|------|--------|-------|--------|
| Riboflavin metabolism | 4 | 0.04 | 1 | 0.0382 | 1 | 0.50 |
| Pyrimidine metabolism | 39 | 0.38 | 2 | 0.0527 | 1 | 0.03 |
| Nitrogen metabolism | 6 | 0.06 | 1 | 0.0568 | 1 | 0 |
| D-Glutamine and D-glutamate metabolism | 6 | 0.06 | 1 | 0.0568 | 1 | 0.50 |
| Aminoacyl-tRNA biosynthesis | 48 | 0.46 | 2 | 0.0763 | 1 | 0 |
| Vitamin B6 metabolism | 9 | 0.09 | 1 | 0.084 | 1 | 0.08 |
| Caffeine metabolism | 10 | 0.1 | 1 | 0.0929 | 1 | 0 |
| Arginine biosynthesis | 14 | 0.14 | 1 | 0.128 | 1 | 0.12 |
| Butanoate metabolism | 15 | 0.15 | 1 | 0.136 | 1 | 0 |
| Histidine metabolism | 16 | 0.15 | 1 | 0.145 | 1 | 0 |
| Pantothenate and CoA biosynthesis | 19 | 0.18 | 1 | 0.17 | 1 | 0.02 |
| beta-Alanine metabolism | 21 | 0.2 | 1 | 0.186 | 1 | 0.06 |
| Glutathione metabolism | 28 | 0.27 | 1 | 0.24 | 1 | 0.02 |
| Alanine, aspartate and glutamate metabolism | 28 | 0.27 | 1 | 0.24 | 1 | 0.20 |
| Porphyrin and chlorophyll metabolism | 30 | 0.29 | 1 | 0.255 | 1 | 0 |
| Glyoxylate and dicarboxylate metabolism | 32 | 0.31 | 1 | 0.27 | 1 | 0 |
| Glycerophospholipid metabolism | 36 | 0.35 | 1 | 0.298 | 1 | 0.01 |
| Arginine and proline metabolism | 38 | 0.37 | 1 | 0.312 | 1 | 0.09 |
| Tryptophan metabolism | 41 | 0.4 | 1 | 0.332 | 1 | 0.14 |
| Tyrosine metabolism | 42 | 0.41 | 1 | 0.339 | 1 | 0.02 |
| Purine metabolism | 65 | 0.63 | 1 | 0.476 | 1 | 0.01 |

Muscle

| Pathway | Total | Expected | Hits | Raw p | FDR p | Impact |
|------------------------------------|-------|----------|------|--------|-------|--------|
| Taurine and hypotaurine metabolism | 8 | 0.06 | 2 | 0.0015 | 0.126 | 0.71 |
| Cysteine and methionine metabolism | 33 | 0.26 | 2 | 0.0254 | 1 | 0.04 |
| Vitamin B6 metabolism | 9 | 0.07 | 1 | 0.0677 | 1 | 0.08 |
| Biotin metabolism | 10 | 0.08 | 1 | 0.075 | 1 | 2.00 |
| Caffeine metabolism | 10 | 0.08 | 1 | 0.075 | 1 | 0.69 |
| Arginine biosynthesis | 14 | 0.11 | 1 | 0.104 | 1 | 0.06 |
| Ether lipid metabolism | 20 | 0.15 | 1 | 0.145 | 1 | 0 |
| Lysine degradation | 25 | 0.19 | 1 | 0.178 | 1 | 0 |
| Glutathione metabolism | 28 | 0.22 | 1 | 0.197 | 1 | 0 |
| Glycerophospholipid metabolism | 36 | 0.28 | 1 | 0.246 | 1 | 0.05 |
| Arginine and proline metabolism | 38 | 0.29 | 1 | 0.258 | 1 | 0.11 |
| Primary bile acid biosynthesis | 46 | 0.36 | 1 | 0.304 | 1 | 0.01 |
| Aminoacyl-tRNA biosynthesis | 48 | 0.37 | 1 | 0.315 | 1 | 0 |
| Purine metabolism | 65 | 0.50 | 1 | 0.403 | 1 | 0.06 |

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Supplementary file S7. Correlation between muscle metabolites with levels of *SOD3* and *GLRX2(1)* expression from ALS patients and controls. Significant correlated metabolites are listed together with the Spearman r and the p value.

Correlation of muscle metabolites and *GLRX2(1)* expression

| Controls | Pearson r | 95% confidence interval | P value | Number of XY Pairs |
|-------------------------------|-----------|-------------------------|---------|--------------------|
| 4-METHYL-2-OXO-PENTANOIC_ACID | 0,7062 | 0,3039 to 0,8948 | 0,0033 | 15 |
| 4-METHYL-2-OXOVALERIC_ACID | 0,662 | 0,2266 to 0,8769 | 0,0072 | 15 |
| 5-HYDROXYINDOLEACETATE | 0,7864 | 0,4591 to 0,9257 | 0,0005 | 15 |
| GUANINE | 0,7982 | 0,4838 to 0,9302 | 0,0004 | 15 |
| GUANOSINE | 0,6435 | 0,1957 to 0,8692 | 0,0096 | 15 |
| GUANOSINE_5'-MONOPHOSPHATE | 0,8697 | 0,6446 to 0,956 | <0,0001 | 15 |
| HISTAMINE | 0,6355 | 0,1826 to 0,8659 | 0,0109 | 15 |
| LACTATE | -0,7428 | -0,9091 to -0,3721 | 0,0015 | 15 |
| LEUCINE | 0,8626 | 0,6278 to 0,9535 | <0,0001 | 15 |
| L-ISOLEUCINE | 0,8389 | 0,5728 to 0,945 | <0,0001 | 15 |
| L-METHIONINE | 0,864 | 0,6311 to 0,954 | <0,0001 | 15 |
| L-PHENYLALANINE | 0,8602 | 0,622 to 0,9527 | <0,0001 | 15 |
| L-TRYPTOPHAN | 0,6086 | 0,1399 to 0,8545 | 0,0161 | 15 |
| L-TYROSINE | 0,8954 | 0,7078 to 0,965 | <0,0001 | 15 |
| L-VALINE | 0,661 | 0,2248 to 0,8765 | 0,0073 | 15 |
| LYXOSE | 0,831 | 0,555 to 0,9422 | 0,0001 | 15 |
| N-ACETYL-L-METHIONINE | 0,8869 | 0,6866 to 0,9621 | <0,0001 | 15 |
| N-ACETYLNEURAMINATE | 0,5225 | 0,01392 to 0,8163 | 0,0457 | 15 |
| RIBOSE_5-PHOSPHATE | 0,5999 | 0,1265 to 0,8507 | 0,0181 | 15 |
| TRIMETHYLAMINE | 0,6577 | 0,2193 to 0,8751 | 0,0077 | 15 |
| URIDINE | 0,703 | 0,2981 to 0,8935 | 0,0035 | 15 |
| XANTHINE | 0,8044 | 0,4968 to 0,9324 | 0,0003 | 15 |
| XANTHOSINE | 0,6096 | 0,1415 to 0,8549 | 0,0158 | 15 |
| ALS | | | | |
| GUANIDINOACETATE | Pearson r | 95% confidence interval | P value | Number of XY Pairs |
| | -0,648 | -0,9074 to -0,03099 | 0,0428 | 10 |

Correlation of muscle metabolites and *SOD3* expression

| Control | Pearson r | 95% confidence interval | P value | Number of XY Pair |
|----------------------------|-----------|-------------------------|---------|-------------------|
| 2-HYDROXYPYRIDINE | -0,5192 | -0,8148 to -0,009483 | 0,0473 | 15 |
| 5-HYDROXYLYSINE | 0,5742 | 0,08778 to 0,8395 | 0,0252 | 15 |
| ADENOSINE_5'-MONOPHOSPHATE | -0,6091 | -0,8547 to -0,1408 | 0,0159 | 15 |
| C10-CARNITINE | 0,6026 | 0,1307 to 0,8519 | 0,0174 | 15 |
| C4-CARNITINE | 0,6817 | 0,2604 to 0,8849 | 0,0051 | 15 |
| C8-CARNITINE | 0,6118 | 0,145 to 0,8559 | 0,0154 | 15 |
| CYTIDINE | -0,5149 | -0,8128 to -0,003601 | 0,0495 | 15 |
| EPINEPHRINE | 0,5441 | 0,04416 to 0,8261 | 0,036 | 15 |
| ETHANOLAMINE_PHOSPHATE | -0,5696 | -0,8375 to -0,08095 | 0,0267 | 15 |
| HIPPURATE | -0,6049 | -0,8529 to -0,1343 | 0,0169 | 15 |
| HYPOXANTHINE | -0,5709 | -0,8381 to -0,08289 | 0,0262 | 15 |
| INOSINE | -0,5149 | -0,8128 to -0,00362 | 0,0495 | 15 |
| L-CARNITINE | 0,6061 | 0,1361 to 0,8534 | 0,0166 | 15 |
| L-GLUTAMIC_ACID | 0,5457 | 0,04641 to 0,8268 | 0,0354 | 15 |
| SUCCINATE | 0,6995 | 0,2919 to 0,8921 | 0,0037 | 15 |
| URATE | -0,653 | -0,8732 to -0,2115 | 0,0083 | 15 |
| XANTHURENIC_ACID | -0,6771 | -0,8831 to -0,2524 | 0,0056 | 15 |
| ALS | | | | |
| | Pearson r | 95% confidence interval | P value | Number of XY Pair |

| | | | |
|----------------|----------------------------|--------|----|
| Cyclic_AMP | 0,7674 0,2666 to 0,9419 | 0,0096 | 10 |
| DEOXYCARNITINE | 0,6727 0,07473 to 0,9148 | 0,0331 | 10 |
| L-HISTIDINE | 0,6478 0,03073 to 0,9074 | 0,0428 | 10 |
| L-ISOLEUCINE | -0,7209 -0,9289 to -0,1672 | 0,0186 | 10 |
| MANNOSE | 0,7162 0,1577 to 0,9276 | 0,0198 | 10 |
| THEOPHYLLINE | 0,705 0,1354 to 0,9243 | 0,0228 | 10 |

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Supplementary file S8. Correlation between metabolites from muscle or serum with LDH activity or the ratio complex II/CS from ALS patients and controls. Significant correlated metabolites are listed together with the Spearman r and the p value.

| LDH x muscle metabolites | | | | |
|---------------------------------|------------|-------------------------|---------|--------------------|
| Controls | Spearman r | 95% confidence interval | P value | Number of XY Pairs |
| 3-UREIDOPROPIONATE | 0,4923 | 0,01802 to 0,7857 | 0,038 | 18 |
| CITRULLINE | 0,6223 | 0,2048 to 0,8482 | 0,0058 | 18 |
| DEOXYCARNITINE | 0,5955 | 0,1636 to 0,8358 | 0,0091 | 18 |
| ETHYLMALONIC_ACID | -0,6639 | -0,867 to -0,2718 | 0,0027 | 18 |
| L-KYNURENINE | -0,5046 | -0,7919 to -0,03448 | 0,0327 | 18 |
| L-PROLINE | 0,548 | 0,0942 to 0,8132 | 0,0186 | 18 |
| ALS | | | | |
| ALS | Spearman r | 95% confidence interval | P value | Number of XY Pairs |
| 2-DEOXY-GLUCOSE | 0,5417 | 0,0671 to 0,8164 | 0,0267 | 17 |
| 3-SULFINO-L-ALANINE | -0,5172 | -0,8047 to -0,03313 | 0,0355 | 17 |
| 4-AMINOBUTANOATE | 0,6609 | 0,2498 to 0,8702 | 0,0048 | 17 |
| ADENOSINE_5'-MONOPHOSPHATE | -0,5359 | -0,8136 to -0,05897 | 0,029 | 17 |
| ALPHA-GLUCOSE_1-PHOSPHATE | -0,5245 | -0,8082 to -0,04321 | 0,0327 | 17 |
| C16-CARNITINE | 0,488 | -0,005818 to 0,7905 | 0,0485 | 17 |
| C18-CARNITINE | 0,4902 | -0,00299 to 0,7916 | 0,0478 | 17 |
| C2-CARNITINE | 0,5515 | 0,08101 to 0,821 | 0,0237 | 17 |
| C3-CARNITINE | 0,7157 | 0,3447 to 0,8933 | 0,0017 | 17 |
| C4-CARNITINE | 0,7059 | 0,3271 to 0,8892 | 0,0021 | 17 |
| CHOLINE | 0,5763 | 0,1171 to 0,8325 | 0,0171 | 17 |
| CREATININE | 0,5123 | 0,02647 to 0,8023 | 0,0376 | 17 |
| GLUTATHIONE | 0,4902 | -0,00299 to 0,7916 | 0,0478 | 17 |
| HIPPURATE | 0,5025 | 0,01327 to 0,7976 | 0,0419 | 17 |
| LACTATE | 0,4975 | 0,006735 to 0,7952 | 0,0442 | 17 |
| L-PIPECOLIC_ACID | 0,5411 | 0,06631 to 0,8161 | 0,0267 | 17 |
| O-ACETYL-L-CARNITINE | 0,5074 | 0,01985 to 0,7999 | 0,0397 | 17 |
| PANTOTHENIC_ACID | 0,5637 | 0,09865 to 0,8267 | 0,0203 | 17 |
| S-(5'-ADENOSYL)-L-HOMOCYSTEINE | 0,6638 | 0,2546 to 0,8714 | 0,0046 | 17 |
| TRIMETHYLAMINE | 0,598 | 0,1496 to 0,8424 | 0,0128 | 17 |
| LDH x serum metabolites | | | | |
| Controls | Spearman r | 95% confidence interval | P value | Number of XY Pairs |
| 4-METHYL-2-OXOVALERIC_ACID | 0,5656 | 0,1505 to 0,8112 | 0,0093 | 20 |
| 5-HYDROXYINDOLEACETATE | 0,4883 | 0,04443 to 0,7712 | 0,0289 | 20 |
| DEOXYCARNITINE | 0,4528 | -0,001195 to 0,752 | 0,045 | 20 |
| L-ANSERINE | 0,6211 | 0,2329 to 0,8385 | 0,0035 | 20 |
| ALS | | | | |
| ALS | Spearman r | 95% confidence interval | P value | Number of XY Pairs |
| Methylhistidine | 0,5347 | 0,05737 to 0,8131 | 0,0288 | 17 |
| HIPPURATE | 0,5436 | 0,06977 to 0,8173 | 0,0259 | 17 |
| L-ORNITHINE | 0,4926 | 0,0002416 to 0,7928 | 0,0465 | 17 |
| MANNOSE | -0,5441 | -0,8175 to -0,07056 | 0,0259 | 17 |
| METHYL_INDOLE-3-ACETATE | 0,5858 | 0,1312 to 0,8368 | 0,0152 | 17 |
| N6-(DELTA2-ISOPENTENYL)-ADENINE | -0,5224 | -0,8072 to -0,04028 | 0,0332 | 17 |
| SUBERIC_ACID | -0,5383 | -0,8148 to -0,0624 | 0,0276 | 17 |

complex II/CS x muscle metabolites

Controls

| | Spearman r | 95% confidence interval | P value | Number of XY Pairs |
|--------------|------------|-------------------------|---------|--------------------|
| MANNOSE | 0,686 | 0,3089 to 0,8767 | 0,0017 | 18 |
| PARAXANTHINE | 0,6629 | 0,2701 to 0,8665 | 0,0027 | 18 |

ALS

| | Spearman r | 95% confidence interval | P value | Number of XY Pairs |
|-------------------------------|------------|-------------------------|---------|--------------------|
| Metabolites | 0,6066 | 0,1628 to 0,8463 | 0,0112 | 17 |
| 3-AMINO-4-HYDROXYBENZOIC_ACID | -0,4926 | -0,7928 to -0,0002197 | 0,0462 | 17 |
| 3-HYDROXYBUTANOIC_ACID | -0,5209 | -0,8065 to -0,03823 | 0,0338 | 17 |
| 4-METHYL-2-OXO-PENTANOIC_ACID | -0,5946 | -0,8408 to -0,1444 | 0,0133 | 17 |
| C5-CARNITINE | 0,5344 | 0,05691 to 0,8129 | 0,0289 | 17 |
| HISTAMINE | -0,4926 | -0,7928 to -0,0002197 | 0,0462 | 17 |
| SN-GLYCEROL_3-PHOSPHATE | | | | |

complex II/CS x serum metabolites**Controls**

| | Spearman r | 95% confidence interval | P value | Number of XY Pairs |
|----------------------------|------------|-------------------------|---------|--------------------|
| 4-HYDROXY-L-PROLINE | 0,6916 | 0,3021 to 0,8833 | 0,0028 | 17 |
| 5-HYDROXYINDOLEACETATE | 0,5605 | 0,09404 to 0,8252 | 0,0209 | 17 |
| Cyclic_AMP | 0,4853 | -0,009467 to 0,7891 | 0,05 | 17 |
| FORMYL-L-METHIONYL_PEPTIDE | 0,5221 | 0,03991 to 0,8071 | 0,0333 | 17 |
| GLYCERATE | -0,5126 | -0,8025 to -0,02694 | 0,037 | 17 |
| GLYCOLALDEHYDE_DIMER | 0,5 | 0,01 to 0,7964 | 0,0427 | 17 |
| INOSINE_5'-MONOPHOSPHATE | 0,5762 | 0,1169 to 0,8324 | 0,0171 | 17 |
| LACTATE | 0,5602 | 0,09354 to 0,825 | 0,0211 | 17 |
| L-ANSERINE | 0,6167 | 0,1784 to 0,8508 | 0,0097 | 17 |
| L-ARABITOL | 0,5528 | 0,08294 to 0,8216 | 0,0231 | 17 |
| L-GLUTAMINE | 0,6376 | 0,2116 to 0,86 | 0,007 | 17 |
| LYXOSE | -0,586 | -0,8369 to -0,1315 | 0,015 | 17 |

ALS

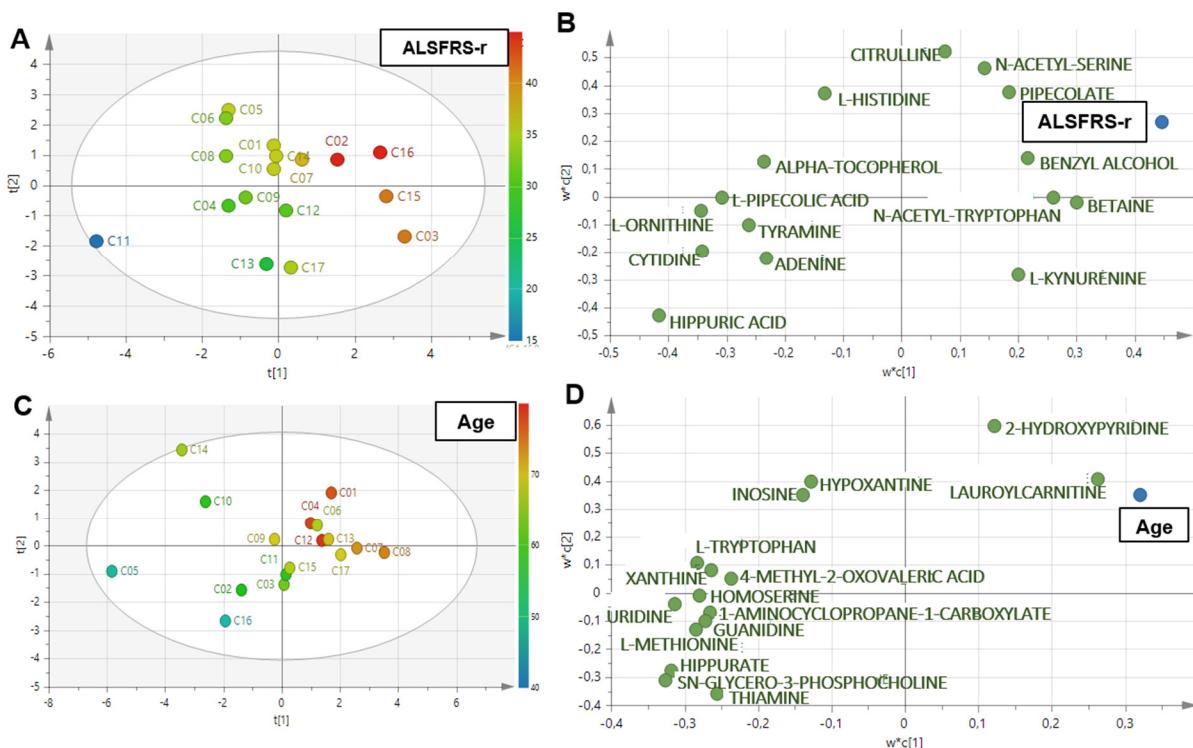
| | Spearman r | 95% confidence interval | P value | Number of XY Pairs |
|-------------------------------|------------|-------------------------|---------|--------------------|
| 1-METHYLADENOSINE | -0,6042 | -0,8452 to -0,159 | 0,0116 | 17 |
| 3-AMINO-4-HYDROXYBENZOIC_ACID | 0,4886 | -0,005049 to 0,7908 | 0,0481 | 17 |
| ALLANTOIN | 0,5921 | 0,1407 to 0,8397 | 0,0138 | 17 |
| CORTISOL | -0,5307 | -0,8112 to -0,05178 | 0,0302 | 17 |
| EPINEPHRINE | -0,527 | -0,8094 to -0,04668 | 0,0315 | 17 |
| FUMARATE | -0,5504 | -0,8205 to -0,07944 | 0,0238 | 17 |
| GALACTOSAMINE | -0,5516 | -0,821 to -0,08119 | 0,0234 | 17 |
| GLYCERATE | 0,5925 | 0,1413 to 0,8399 | 0,0136 | 17 |
| L-ALANINE | -0,6177 | -0,8512 to -0,18 | 0,0095 | 17 |
| LAUROYLCARNITINE | -0,5074 | -0,8 to -0,01987 | 0,0394 | 17 |
| L-PROLINE | -0,5577 | -0,8239 to -0,09 | 0,0217 | 17 |
| MALATE | -0,5012 | -0,797 to -0,01164 | 0,0421 | 17 |
| METHYL-GUANIDINE | 0,5467 | 0,0742 to 0,8187 | 0,0249 | 17 |
| OLEATE | -0,5823 | -0,8353 to -0,126 | 0,0158 | 17 |
| RIBOFLAVIN | 0,5 | 0,01 to 0,7964 | 0,0427 | 17 |
| TRANS-CINNAMALDEHYDE | -0,6847 | -0,8803 to -0,29 | 0,0031 | 17 |

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Supplementary figure S1. Metabolomics findings from serum and clinical parameters of ALS at diagnosis. **A)** Score scatter plot based on the PLS-DA models from serum to explain ALSFRS-r ($p\text{-CV-ANOVA}=0.004$). **B)** Loading scatter plot presenting the top 15 metabolites identified in serum by the PLS-DA model to explain ALSFRS-r at diagnosis. **C)** Score scatter plot based on the PLS-DA models from serum to explain the age at onset ($p\text{-CV-ANOVA}=0.05$). **D)** Loading scatter plot presenting the top 15 metabolites identified in serum by the PLS-DA model to explain age at onset.

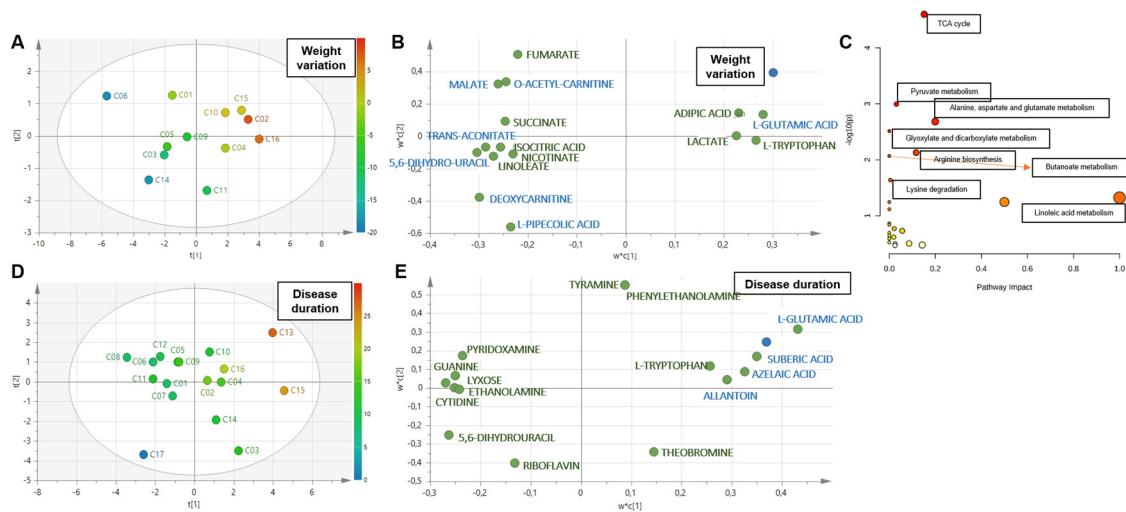


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Supplementary Figure S2. Multivariate analysis of the metabolome profile from serum of ALS patients and the variation of weight over 9 months (A-C) and disease duration (D-E). A) Score scatter plot based on the PLS-DA models from serum to explain the weight variation, with $R^2X=0.66$, $R^2Y=0.917$, $Q^2=0.695$ and $p=0.07$ for the CV ANOVA; the color scale represents the weight variation (gain towards red, loss towards blue) over 9 months. B) Loading scatter plot presenting the top 15 metabolites identified by the PLS-DA. The seven metabolites that had VIP score higher than 1 are written in blue. C) Pathway analysis with the 15 VIP metabolites highlighted alterations in the TCA cycle ($p<0.001$); pyruvate metabolism ($p=0.001$); alanine, aspartate and glutamate metabolism ($p=0.002$); glyoxylate and dicarboxylate metabolism ($p=0.003$); arginine biosynthesis ($p=0.007$); butanoate metabolism ($p=0.008$); lysine degradation ($p=0.0231$) and linoleic acid metabolism ($p=0.0475$). Each node represents a metabolite set with its color based on its p -value and its size is based on the pathway impact. The complete list of metabolic pathways is described in Supplementary file 5. D) Score scatter plot based on the PLS-DA models from serum to explain the weight variation, with $R^2X=0.599$, $R^2Y=0.882$, $Q^2=0.757$ and $p=0.0008$ for the CV-ANOVA test; the color scale represents the disease duration (long towards red, short towards blue). E) Loading scatter plot presenting the top 15 metabolites identified by the PLS-DA. The four metabolites that had VIP score higher than 1 are written in blue.

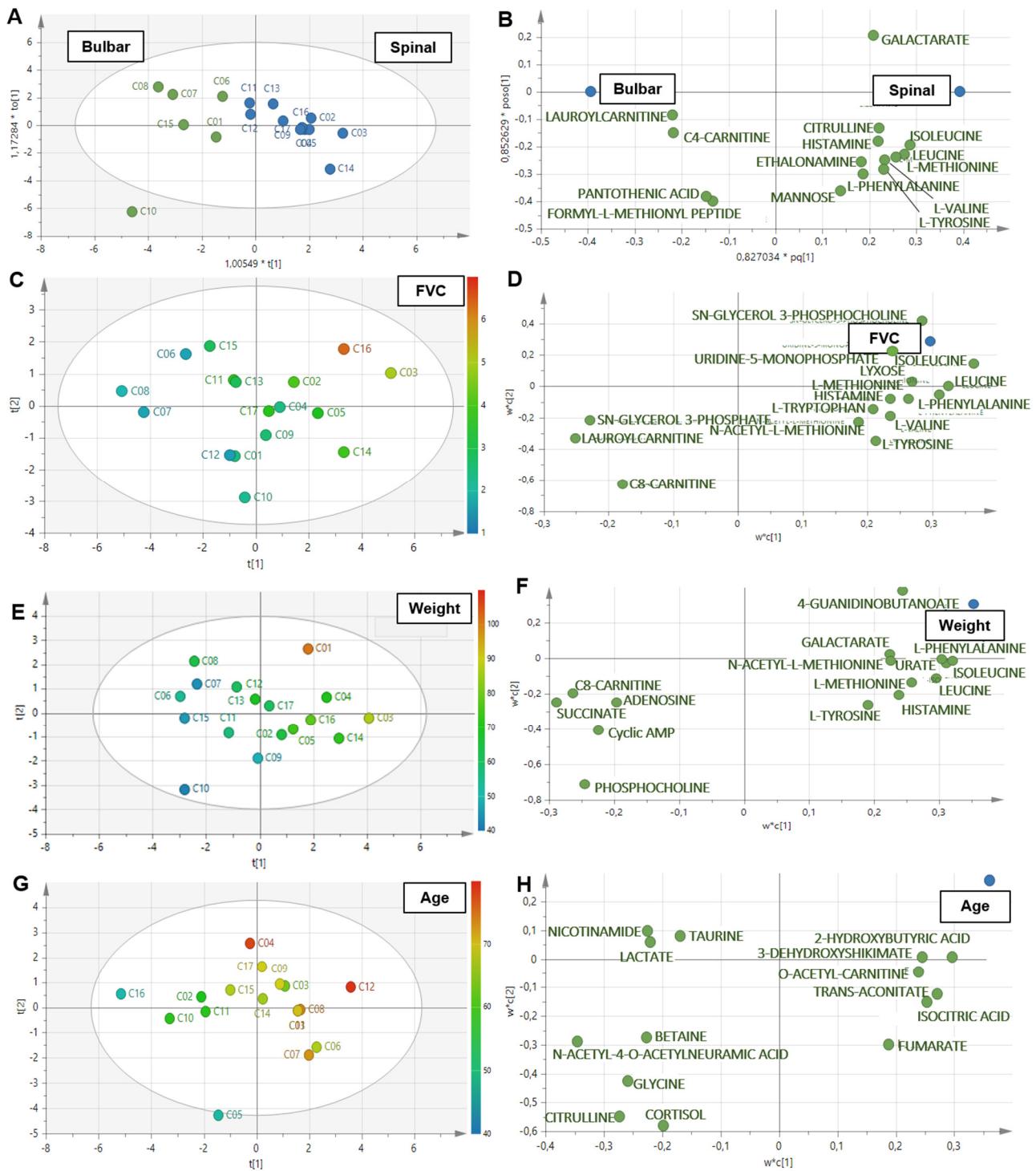


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Supplementary figure S3. Metabolomics findings in muscle and clinical parameters of ALS at diagnosis. **A)** Score scatter plot based on the OPLS-DA models from muscle to explain the site at onset ($p\text{-CV-ANOVA}=0.017$) (blue: spinal onset; green: bulbar onset). **B)** Loading scatter plot presenting the top 15 metabolites identified by the OPLS-DA to explain the site of onset. **C)** Score scatter plot based on the PLS-DA models from muscle to explain the FVC ($p\text{-CV-ANOVA}=0.036$). The color scale represents the FVC (high towards red, low towards blue). **D)** Loading scatter plot presenting the top 15 metabolites identified by the PLS-DA model to explain FVC at diagnosis. **E)** Score scatter plot based on the PLS-DA models from muscle to explain the weight ($p\text{-CV-ANOVA}=0.04$). The color scale represents the weight at diagnosis (high towards red, low towards blue). **F)** Loading scatter plot presenting the top 15 metabolites identified by the PLS-DA model to explain weight at diagnosis. **G)** Score scatter plot based on the PLS-DA models from muscle to explain the age at onset ($p\text{-CV-ANOVA}=0.004$). The color scale represents the age at onset (old towards red, young towards blue). **H)** Loading scatter plot presenting the top 15 metabolites identified by the PLS-DA model to explain age at onset.

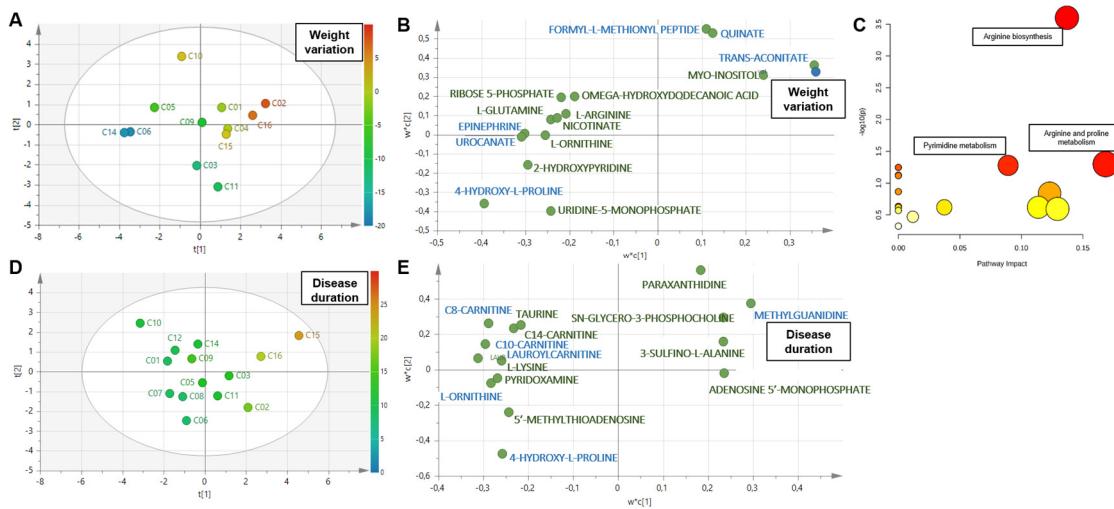


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Supplementary Figure S4. Multivariate analysis of the metabolome profile muscle of ALS patients and the variation of weight over 9 months (A-C) and disease duration (D-E). A) Score scatter plot based on the PLS-DA models from muscle to explain the weight variation, with $R^2X=0.601$, $R^2Y=0.924$, $Q^2=0.766$ and $p=0.04$ for the CV-ANOVA test; the color scale represents the weight variation (gain towards red, loss towards blue) over 9 months. B) Loading scatter plot presenting the top 15 metabolites identified by the PLS-DA. The six metabolites that had VIP score higher than 1 are written in blue. C) Pathway analysis with the 15 VIP metabolites highlighted alterations in arginine biosynthesis ($p<0.001$); arginine and proline metabolism ($p=0.0503$); and pyrimidine metabolism ($p=0.0527$). The complete list of metabolic pathways is described in Supplementary file 5. D) Score scatter plot based on the PLS-DA models from muscle to explain the disease duration, with $R^2X=0.472$, $R^2Y=0.9$, $Q^2=0.492$ and $p=12$ for the CV-ANOVA test; the color scale represents the disease duration (long towards red, short towards blue). E) Loading scatter plot presenting the top 15 metabolites identified by the PLS-DA. The six metabolites that had VIP score higher than 1 are written in blue.



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Supplementary Figure S5. Analysis of the most discriminant metabolites from both serum and muscle of ALS patients. Venn diagrams drawn between serum and muscle revealed that lauroylcarnitine was common to models explaining site at onset (A) and FVC (B). Correlation of lauroylcarnitine levels with FVC at diagnosis revealed that high levels of this metabolite correlated with low FVC in serum (C; Pearson r: -0.4291, 95%CI: -0.76;0.08; R2: 0.18; p=0.0972) and specially in muscle (D; Pearson r: -0.5311, 95%CI: -0.81; -0.07; R2: 0.28; p=0.0283). Dotted lines represent the 95%CI. F-G) Venn diagram analysis performed with the 15 metabolites with the highest VIP used to explain disease progression (variation of weight, ALSFRS-r over 9 months and disease duration) from serum (E) or muscle (F) metabolome. Venn diagrams build with Venny 2.1.0 (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>).

