**Supporting Information Appendix S1. Advanced baseline characteristics for patients, which is separating patient material in individual fixation methods. FFPE tissues were used for immunohistochemical staining, and fresh frozen tissues were used for the determination of amino acids.**

|  |  |  |
| --- | --- | --- |
| **Characteristic** | **Non-cachectic** | **Cachectic** |
|  |  |  |
| **Total** | **23** | **13** |
|  |  |  |
| **FFPE tissues** |  |  |
|  |  |  |
| **Number** | **20** | **10** |
|  |  |  |
| **Gender [n]** |  |  |
| **Male** | **13** | **3** |
| **Female** | **7** | **7** |
|  |  |  |
| **Age [years]** |  |  |
| **Mean** | **66** | **67** |
| **Range** | **45–79** | **50–83** |
|  |  |  |
| **Weight [kg]** |  |  |
| **Mean** | **78** | **63** |
| **Range** | **40–106** | **41–87** |
|  |  |  |
| **Body mass index [kg/m2]** |  |  |
| **Mean** | **25.9** | **22.3** |
| **Range** | **16.2–34.2** | **15.8–28.4** |
|  |  |  |
| **Weight loss [%]** |  |  |
| **Mean** | **1.1** | **14.4** |
| **Range** | **0–4.8** | **9.1–25.5** |
|  |  |  |
| **Fresh frozen tissues** |  |  |
|  |  |  |
| **Number** | **3** | **3** |
|  |  |  |
| **Gender [n]** |  |  |
| **Male** | **2** | **1** |
| **Female** | **1** | **2** |
|  |  |  |
| **Age [years]** |  |  |
| **Mean** | **71** | **58** |
| **Range** | **55-88** | **44-74** |
|  |  |  |
| **Weight [kg]** |  |  |
| **Mean** | **72.3** | **68.0** |
| **Range** | **55-90** | **51-98** |
|  |  |  |
| **Body mass index [kg/m2]** |  |  |
| **Mean** | **23.5** | **23.1** |
| **Range** | **20.5-26.9** | **20.4-28.3** |
|  |  |  |
| **Weight loss [%]** |  |  |
| **Mean** | **0.5** | **9.8** |
| **Range** | **0-1.5** | **7.7-12** |

**Supporting Information Appendix S2. Intensities of amino acids in skeletal muscle tissues of cachectic and non-cachectic mice. *P* values were calculated by Mann–Whitney *U* test.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Non-cachectic** | **Cachectic** | **Non-cachectic** | **Cachectic** | **Mean change** | **Median change** |  |
| **Amino acid** | **Mean rel. intensity** | **Mean rel. intensity** | **Median rel. intensity** | **Median rel. intensity** | **[%]** | **[%]** | ***P* value** |
| **Ala** | 0.0685 | 0.0767 | 0.0703 | 0.0764 | 12.04 | 8.68 | 0.3071 |
| **Arg** | 0.3041 | 0.5304 | 0.2415 | 0.4957 | 74.40 | 105.30 | **0.0048** |
| **Asn** | 0.0570 | 0.0677 | 0.0570 | 0.0714 | 18.76 | 25.26 | 0.2816 |
| **Asp** | 0.0611 | 0.0000 | 0.0307 | 0.0000 | -100.00 | -100.00 | **0.0124** |
| **Gln** | 2.7845 | 3.5141 | 2.4817 | 3.2855 | 26.20 | 32.39 | 0.2230 |
| **Glu** | 1.5454 | 0.6578 | 1.5746 | 0.5822 | -57.43 | -63.02 | **0.0008** |
| **Leu/Ile** | 0.0795 | 0.0933 | 0.0843 | 0.0815 | 17.38 | -3.26 | 0.4262 |
| **Lys** | 0.1776 | 0.4012 | 0.1576 | 0.3764 | 125.93 | 138.83 | **0.0037** |
| **Met** | 0.0118 | 0.0192 | 0.0097 | 0.0158 | 63.02 | 62.89 | 0.0669 |
| **Phe** | 0.1580 | 0.1962 | 0.1510 | 0.1973 | 24.22 | 30.71 | 0.1734 |
| **Pro** | 0.0179 | 0.0264 | 0.0151 | 0.0230 | 47.51 | 52.32 | **0.0430** |
| **Ser** | 0.0038 | 0.0036 | 0.0047 | 0.0050 | -4.82 | 6.38 | 0.8278 |
| **Thr** | 0.0823 | 0.0993 | 0.0852 | 0.0931 | 20.56 | 9.34 | 0.3494 |
| **Trp** | 0.0602 | 0.0673 | 0.0573 | 0.0659 | 11.78 | 15.11 | 0.2512 |
| **Tyr** | 0.0622 | 0.1271 | 0.0634 | 0.1266 | 104.32 | 99.84 | **0.0357** |
| **Val** | 0.1562 | 0.1620 | 0.1571 | 0.1555 | 3.71 | -1.02 | 0.7513 |

**N:\Path2\Daten\Microimage\Thomas\Paper_2_Cachexia\submitted\Revision\Figures\Appendix_S3_amino_acids_human.tif****Supporting Information Appendix S3.** *In situ* amino acid quantities in skeletal muscle tissues from cachectic patients with cancer. Changes in lysine, arginine, proline, glutamate, and aspartate with cachexia in humans **revealed similarities** to the changes observed in cachectic mouse skeletal muscle tissues. The horizontal lines represent the mean intensity of each group.

**Supporting Information Appendix S4. *P* values (Spearman’s rank correlation analysis) of significant negative correlations between proteins and amino acids. Included proteins were may degraded in skeletal muscle tissues of cachectic mice.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Protein** | **Ala** | **Arg** | **Asn** | **Asp** | **Gln** | **Glu** | **Leu/Ile** | **Lys** | **Met** | **Phe** | **Pro** | **Ser** | **Thr** | **Trp** | **Tyr** | **Val** |
| **Thymosin beta 10** | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.0357 | - | - |
| **Thymosin beta-4** | - | 0.0026 | - | - | - | - | - | 0.0090 | - | - | 0.0049 | - | 0.0431 | - | - | - |
| **Cytochrome c oxidase subunit (COX) 7C** | - | 0.0090 | - | - | - | - | - | 0.0244 | - | - | 0.0368 | - | - | - | - | - |
| **Cytochrome c oxidase copper chaperone** | - | 0.0244 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Purkinje cell protein 4** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.0186 |
| **Cysteine-rich protein 1 (CRP-1)** | - | - | 0.0472 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Ubiquitin (Gly-Gly)** | - | - | - | - | - | 0.0212 | - | - | - | - | - | - | - | - | - | - |
| **ATPase F6** | - | 0.0145 | - | - | 0.0493 | - | - | - | 0.0367 | - | - | - | - | 0.0244 | - | - |
| **Acyl-CoA-binding protein (ACBP)** | - | - | - | - | - | - | - | - | 0.0376 | - | - | - | - | 0.0340 | - | - |
| **Cytochrome c oxidase subunit (COX) 6B1** | - | 0.0027 | - | - | 0.0209 | - | - | 0.0405 | - | - | - | - | - | 0.0368 | - | - |
| **Cytochrome c** | - | - | - | - | - | - | - | - | 0.0385 | - | - | - | - | 0.0038 | - | - |
| **Prothymosin alpha** | - | - | - | - | - | - | - | - | - | 0.0129 | - | - | - | 0.0198 | - | - |
| **Histone H2B** | - | 0.0086 | - | - | - | - | 0.0340 | 0.0270 | - | - | 0.0368 | - | 0.0236 | - | - | - |
| **Plasma serine protease inhibitor** | - | 0.0198 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Glutathione S-transferase P** | - | 0.0465 | - | - | - | - | - | - | 0.0139 | 0.0071 | - | - | - | 0.0016 | 0.0124 | - |

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**Supporting Information Appendix S5. Analysis of mitochondrial proteins in mouse skeletal muscle tissues (A)** Statistical analysis for the OXPHOS-related proteins COX7C, **cytochrome c, and ATPase F6 determined by MALDI mass spectrometry imaging.** COX7C (*P* = 0.0127) and ATPase F6 (*P* = 0.0048) expression was significantly decreased in cachexia, compared with **non-cachectic skeletal muscle tissues.** Cytochrome c was also decreased in cachectic mouse skeletal muscle tissues, but the differences did not reach statistical significance **(*P* = 0.1145)**. **(B) Immunohistochemistry (IHC) results confirmed changes of mitochondrial proteins detected by MALDI mass spectrometry imaging. Quantification of the IHC, performed by digital image analysis, revealed a lower expression of COX7C in cachectic mouse skeletal muscle tissues (*P* = 0.0159) and a similar change of cytochrome c without reaching significance level (*P* = 0.2512)**. \* *P* < 0.05, \*\* *P* < 0.01.

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**Supporting Information Appendix S6. Immunofluorescence analysis of myosin heavy chain (MHC) expressions. (A) Statistical analysis of the expression of myosin heavy chains in cachectic and non-cachectic mice skeletal muscle tissues. MHC1 expressions were in both comparison groups 0.02%. The Mann–Whitney *U* test was performed for all fibre types and revealed no significant change between non-cachectic and cachectic mouse comparison groups. (B) Exemplary pictures for immunofluorescence stained cross-sectioned mouse skeletal muscle tissues. The left tissue section is representing a non-cachectic mouse, whereby the right tissue is belonging to a cachectic mouse. Shown are type I (blue), type IIA (green), type IIB (red), and type IIX (unstained) fibres.**

**N:\Path2\Daten\Microimage\Thomas\Paper_2_Cachexia\submitted\Revision\Figures\Appendix_S7_TCA.tifSupporting Information Appendix S7.** Statistical analysis regarding changes of molecules in the tricarboxylic acid (TCA) cycle. Peak intensity was signiﬁcantly higher for malate (*P* = 0.0295) and lower for oxaloacetate (*P* = 0.0448) in tissues of cachectic mice, compared with **non-cachectic ones**. Boxplot whiskers represent the lowest and highest peak intensities in each group. \* *P* < 0.05.