HelmholtzZentrum münchen
German Research Center for Environmental Health

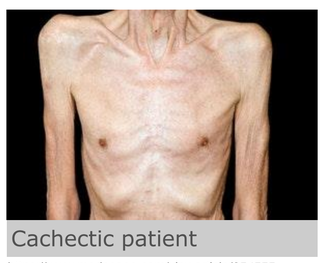
Derangements of amino acids in cachectic skeletal muscle are caused by mitochondrial dysfunction

Thomas Kunzke[1], Achim Buck[1], Verena M. Prade[1], Annette Feuchtinger[1], Olga Prokopchuk[2], Marc E. Martignoni[2], Simone Heisz[3,4], Hans Hauner[3,4], Klaus-Peter Janssen[2], Axel Walch[1*] & Michaela Aichler[1]

Introduction & Aim

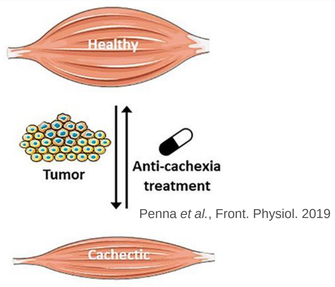
Cachexia is the direct cause of at least 20% of cancer-associated deaths. Muscle wasting in skeletal muscle results in weakness, immobility, and death secondary to impaired respiratory muscle function. Muscle proteins are massively degraded in cachexia; nevertheless, the molecular mechanisms related to this process are poorly understood. Previous studies have reported conflicting results regarding the amino acid abundances in cachectic skeletal muscle tissues. There is a clear need to identify the molecular processes of muscle metabolism in the context of cachexia, especially how different types of molecules are involved in the muscle-wasting process.

Material and methods

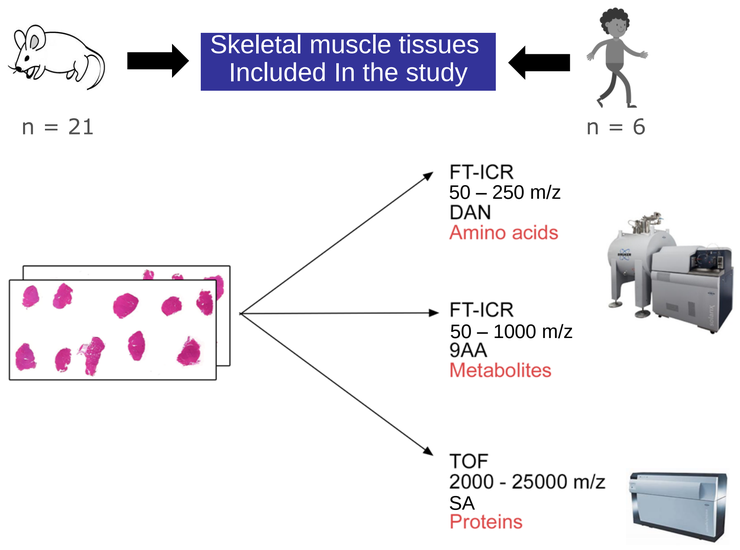


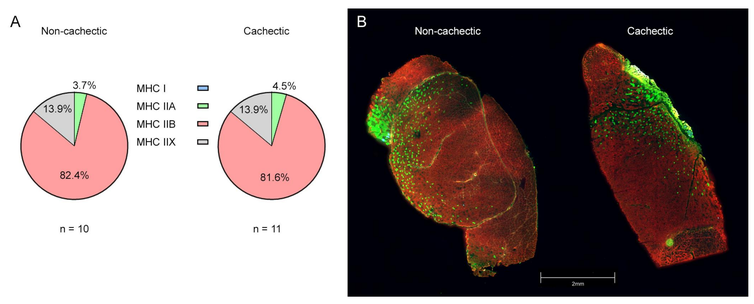
Cachectic patient

https://www.medscape.com/viewarticle/854555



Penna *et al.*, Front. Physiol. 2019

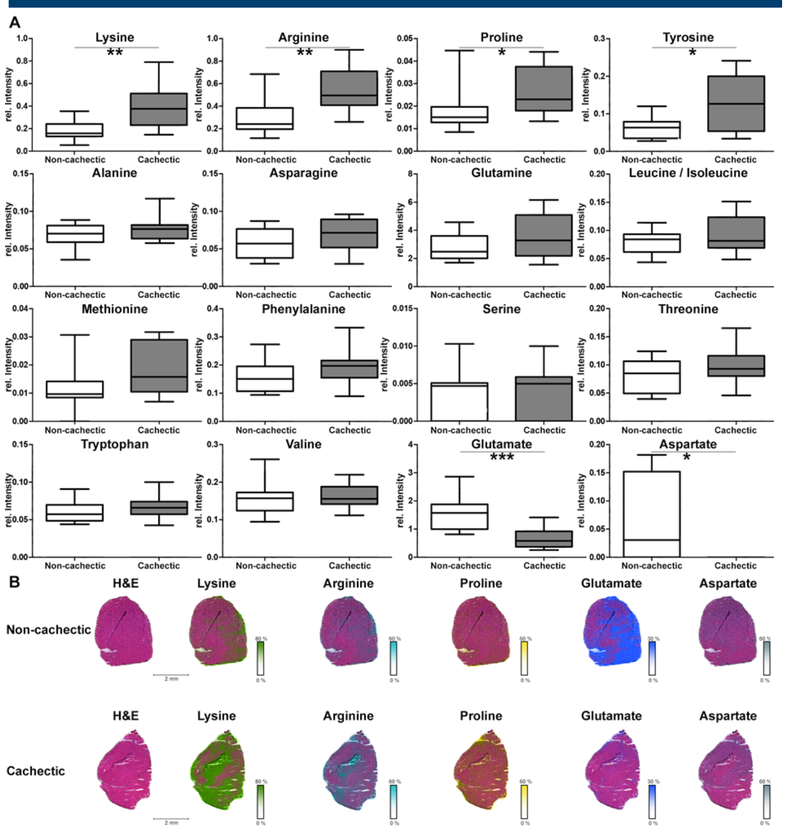




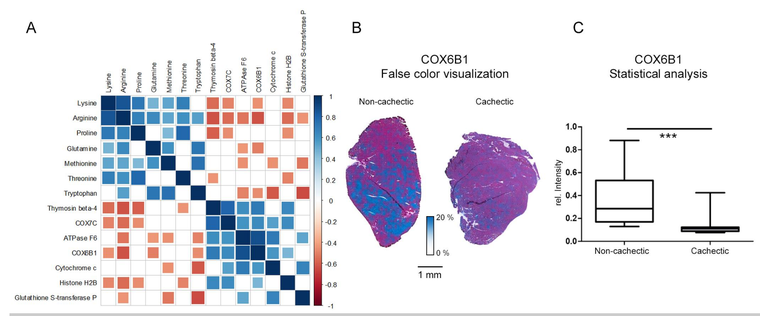
Applied methods:

- Metabolomics: MALDI FT-ICR mass spectrometry imaging
- Proteomics: MALDI TOF mass spectrometry imaging
- Targeted protein analysis: Immunohistochemical staining
- Analysis of the ultrastructure: Transmission electron microscopy
- Quantification of immunohistochemistry: Digital image analysis

Molecular analysis in mice

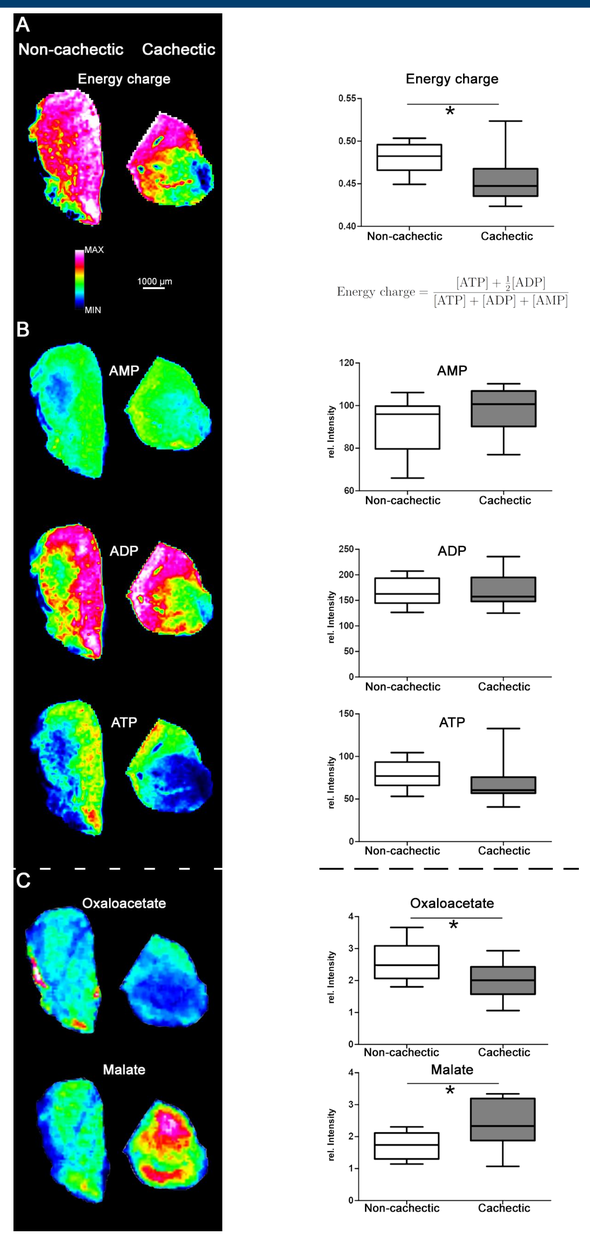


In situ analysis of amino acids in cachectic mouse skeletal muscle tissues. **(A)** Quantities of amino acids in skeletal muscles from cachectic and non-cachectic mice. **(B)** False colour visualisation of amino acids in mouse skeletal muscle tissues.



Evaluation of potential protein breakdown targets. **(A)** Visualisation of Spearman's rank correlation analysis results examining the relationships between amino acids and proteins. **(B)** False colour visualisation of COX6B1. **(C)** Statistical analysis of COX6B1.

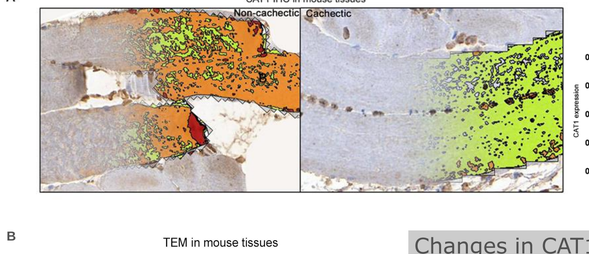
Changed energy charge in cachexia



$$\text{Energy charge} = \frac{[\text{ATP}] + \frac{1}{2}[\text{ADP}]}{[\text{ATP}] + [\text{ADP}] + [\text{AMP}]}$$

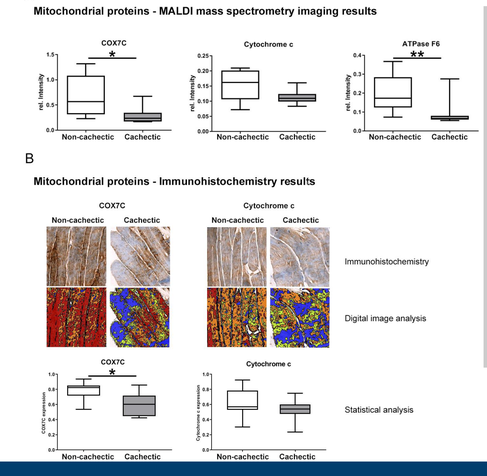
Energy changes in cancer cachexia. **(A)** Heatmap visualisation and statistical analysis of the calculated energy charge. **(B)** AMP, ADP, and ATP distribution in cachectic and non-cachectic mouse skeletal muscle tissues. **(C)** Heatmap visualisation and statistical analysis of changes in molecules of the tricarboxylic acid cycle.

Focusing mitochondria for cancer cachexia



Changes in CAT1 expression in cachexia **(A)** Digital image analysis of the CAT1 immunohistochemistry results. **(B)** Number of mitochondria in mouse skeletal muscle tissues determined by transmission electron microscopy at 1600x magnification. The red colour represents the mitochondria.

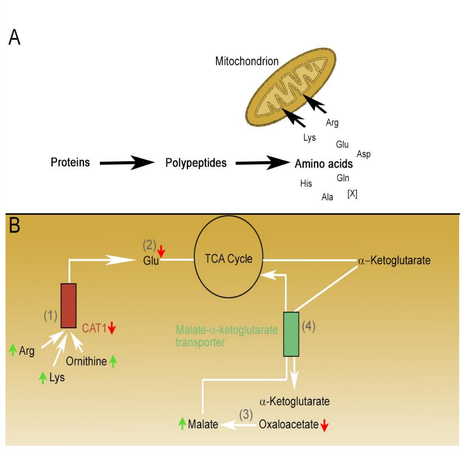
Validation experiments for MALDI imaging results



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.
(B) Immunohistochemistry (IHC) results confirmed changes of mitochondrial proteins detected by MALDI mass spectrometry imaging.

A hypothesis regarding molecular changes

(A) Proteins in muscle tissues of cachectic mice are degraded and subsequently processed
(B) (1) Lys, Arg, and ornithine are transported via CAT1 into the mitochondria.
(2) Transaminase proteins metabolise Lys, Arg, ornithine, and other amino acids and produce Glu. Glu is decreased in cachexia because of reduced CAT1 expression.
(3) Increased NADH concentration could be associated with increased malate and decreased oxaloacetate abundances.



Summary

Metabolic derangements in cachectic mouse muscle tissues were detected, with significantly increased quantities of lysine, arginine, proline, and tyrosine and significantly reduced quantities of glutamate and aspartate. A majority of altered amino acids was released by the breakdown of proteins involved in oxidative phosphorylation. Additionally, expression of the cationic amino acid transporter CAT1 was significantly decreased in the mitochondria of cachectic mouse muscles; this decrease may play an important role in the alterations of cationic amino acid metabolism and decreased quantity of glutamate observed in cachexia. Our results suggest that mitochondrial dysfunction has a substantial influence on amino acid metabolism in cachectic skeletal muscles, which appears to be triggered by diminished CAT1 expression, as well as the degradation of mitochondrial proteins. These findings provide new insights into the pathobiochemistry of muscle wasting.

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[1] Research Unit Analytical Pathology, Helmholtz Zentrum München, Oberschleißheim, Germany
[2] Department of Surgery, Klinikum rechts der Isar, TUM, Munich, Germany
[3] Else Kroener-Fresenius-Center for Nutritional Medicine, Klinikum rechts der Isar, TUM, Munich, Germany
[4] ZIEL-Institute for Food and Health, Nutritional Medicine Unit, TUM, Freising, Germany

Bundesministerium für Bildung und Forschung
SFB 824
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