LOW ERYTHOCYTE LEVELS OF PROTEASOME AND ACYL PEPTIDE HYDROLASE (APEH) ACTIVITIES IN ALZHEIMER'S DISEASE: A SIGN OF DEFECTIVE PROTEOSTASIS?

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Introduction

Neurodegenerative diseases, such as Alzheimer's disease (AD), are characterized by the presence of protein aggregates due to alterations in protein-quality control systems. The Proteasome complex is a major regulator of intracellular protein quality control. Besides the proteasome-system, APEH was also reported to play a key role in protein degradation machinery and antioxidant processes. APEH is a ubiquitous bifunctional enzyme, exhibiting exopeptidase activity (**EPA**) towards N-acyl-peptides and endoprotease activity (**OPA**) towards oxidized proteins. The aim of this study was to investigate the blood levels of APEH-proteasome system in AD, to further validate their cooperation in protein and redox homeostasis.

Materials and methods

Fifty-two participants were recruited from the Centre for Research and Training in Medicine for Aging (CeRMA), University of Molise. They were divided in two groups based on their clinical profiles: 26 participants with probable AD and 26 cognitively healthy controls (HC). Venous blood samples were taken from HC and AD donors. In the hemolysates and purified samples, the proteasome chimotripsin-like (CT-like) activity, EPA and OPA were evaluated by mass chromatography / spectrometry. The collected data were analyzed using the SPSS statistical analysis software package (SPSS Inc., Chicago, III, v. 17.0).

		AD (N. 26)	HC (N. 26)
Age (mean±SD, y)		77±8.8	66.7±12
Gender (N, %)	Male	10 (38.5%)	9 (32.0%)
	Female	16 (61.5%)	17 (68.0%)
Education level (mean±SD, y)		7.1±4.7	12.9±4.4
BMI (mean±SD, kg/m^2)		24.7±4.2	25.4±3.7
MMSE		7.6±7.9	29.5+1.1

Table1. Demographic and clinical characteristics of study groups *(AD, Alzheimer disease; HC, cognitively healthy controls; BMI, Body mass index; MMSE, Mini Mental State Examination* **).**

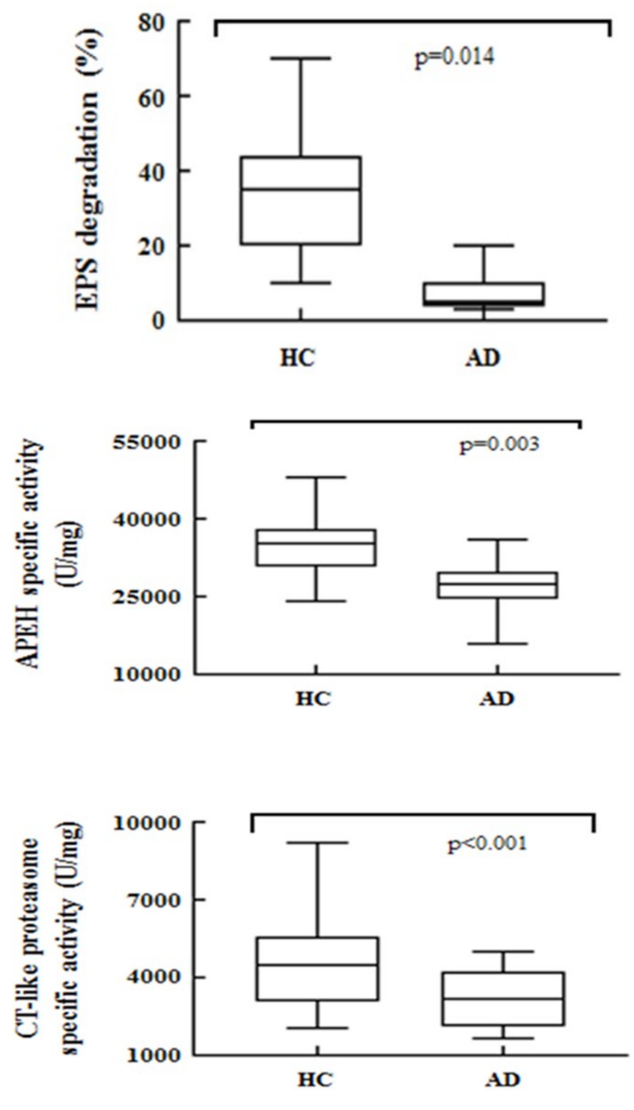

Figure 1. Endopeptidase and exopeptidase APEH, and chymotrypsin-like (CT-like) proteasome specific activities measured in the partially purified AD and HC enzyme samples (EPS, Endopeptidase Peptide Substrate).

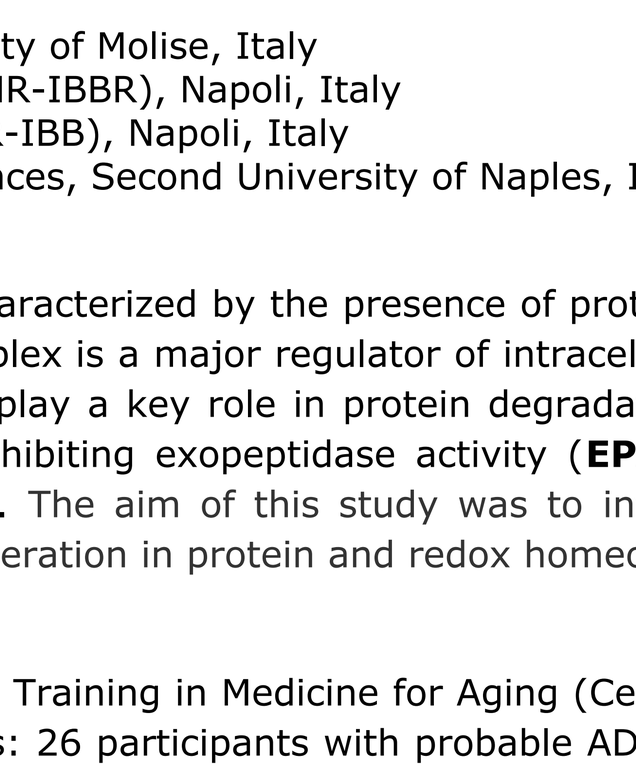

Figure 2. RT-PCR assessment of transcript expression levels of *apeh* and *β5* proteasome subunit genes in AD and HC blood samples.

Results

Results obtained from hemolysates correlated well with those from purified samples carried out to confirm the enzyme activity data in the crude extracts. Proteasome, EPA and OPA activities, as well as proteasome gene expression, were significantly reduced in AD patients respect to HCs, whereas no significant differences were observed in APEH gene expression.

Moreover, EPA and proteasome activity displayed a significant correlation only in the HC group ($r = 0.458$; $p = 0.019$), suggesting that AD also affects the functional cooperation of the two enzymes involved in the degradation machinery.

Conclusion

This study shows that EPA, OPA and proteasome activity levels, as well as proteasome gene expression, significantly decrease from HC to AD. Conversely, reduced APEH activity is not associated with a decrease of its expression, suggesting that post-translational changes might cause this reduced activity. Based on these results, a blood sample could allow early AD diagnosis. Moreover, finding a possible link between APEH-proteasome levels and AD might be helpful in finding targeted therapeutic strategies.