Urinary proteomic analysis in Brazilian marines exposed to exercise-induced rhabdomyolysis with acute kidney injury

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Exertional rhabdomyolysis (ERM) is defined as the process of injury and rupture of muscle cell membranes resulting in the release of their contents into the bloodstream after intense training. Acute kidney injury (AKI) is a life-threatening complication of ERM. Evidence suggests that healthy and fit individuals may develop ERM and associated AKI, as observed among military subjects. Typical manifestations include muscle pain and myoglobinuria causing dark urine. Creatine kinase (CK) has been used for ERM diagnosis, although sizable discrepancies between CK levels and clinical signs or symptoms have been reported. To address these limitations, we evaluated the urinary proteome as a tool for predicting ERM and associated AKI. An integrative analysis using MALDI-TOF and nLC-MS/MS was performed to investigate the differential urinary proteomic signatures of military subjects submitted to intense training. Nineteen marine soldiers enrolled in a special training program were recruited for this study. Urine samples were collected in four periods as follows: before training (T1), after exercise (T2), pre-escape (T3), 7 days recovery (T4), and classified into two groups: AKI (Delta-Creatinine stage 1, sCr > 0.3 mg/dL over index) and no-AKI (sCr < 0.3 mg/dL). A total of 20 ug of urinary protein were purified using C18 column, and spotted directly in the MALDI plate using HCCA matrix solution. Samples were analyzed in a MALDI-TOF Autoflex (Bruker Daltonics). For 1-DE, 20 ug protein was resolved by SDS-PAGE. Bands of T2 were excised in the MW range of 8–15 kDa, corresponding to the “m/z” of discriminant peaks and subjected to in-gel trypsin digestion followed by nLC-MS/MS analysis using an Easy nano LC1000 (Thermo Fisher Scientific) HPLC coupled with an LTQ Orbitrap Velos (Thermo Fisher Scientific). MALDI-TOF data preprocessing were performed using the ClinProTools and R-packages, with spectra range between 2.5–15 kDa. nLC-MS/MS raw data were searched using MaxQuant and processed using Perseus. The combination of 1-DE and nLC-MS/MS in T2 identified a total of 589 proteins, with 25 differentially regulated. Of these, seven proteins were upregulated and 18 were downregulated in the AKI group compared to the no-AKI group. Urine proteomic analysis revealed the overexpression of proteins associated with muscle and renal damage, including Fatty acid-binding protein, heart (FABP3); Acylphosphatase-2 (ACYP2); Insulin-

like growth factor-binding protein 1 (IGFBP1); Myoglobin (MB); Protein deglycase DJ-1 (PARK7); Superoxide dismutase [Cu-Zn] (SOD1); and Thymosin beta-4 (TMSB4X). These proteins represent potential urinary biomarkers for ERM and AKI resulting from intense exercise. In conclusion, the urinary proteome allows monitoring pathophysiological changes in a non-invasive manner, providing a better understand about the mechanism of ERM and associated AKI during military training.

Agradecimentos: The authors would like to thank to Brazilian Navy - Marinha do Brasil (Diretoria Geral de Desenvolvimento Nuclear e Tecnológico da Marinha, Comando Geral do Corpo de Fuzileiros Navais, Hospital Naval Marcilio Dias, Instituto de Pesquisas Biomédicas da Marinha, Centro de Instrução Almirante Sylvio de Camargo) for the research support and providing the facilities. We thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP - 2021/00140-3, 2018/18257-1, 2018/ 15549-1, 2018/13283-4, 2020/04923-0 and 2014/27198-8), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) CNPQ 425025/2021-7, 381841/2023-5, Fundação Carlos Chagas de Amparo à Pesquisa do Rio de Janeiro (FAPERJ) APQ1, E-26/010001697/2019, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for providing financial support for this study.