Dietary supplementation with an essential amino acid mixture prevents mitochondrial damage and fat accumulation in liver of rats chronically consuming ethanol

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Chronic ethanol (EtOH) consumption induces liver disease, with mitochondrial dysfunction (e.g. mitochondrial DNA alterations, impairement of respiratory chain flux, reduction of respiratory protein synthesis), inflammation, and fat accumulation (hepatic steatosis). Moreover, complications of EtOH-induced liver disease is worsened by malnutrition, which normally affects EtOH abusers. Hepatic steatosis patients present a severe damage of amino acid metabolism, correlated to low circulating levels of branched-chain amino acids (BCAAs). At present specific therapeutic approaches to preserve mitochondrial function and to prevent fatty liver do not exist. Thus, in the present study we analysed the ability of an amino acid mixture enriched in essential amino acids (EAAem) to improve the mitochondrial biogenesis and function in liver of chronically EtOH-consuming rats. Twenty-four male Wistar rats (3-month-old) normally housed and fed ad libitum, were separated into four groups and treated for 2 months with: 1) drinking water (Ctrl group); 2) 1.5 mg/g body weight EAAem in drinking water (EAAem group); 3) 20 % EtOH in drinking water (EtOH group); and 4) 20 % EtOH and EAAem supplementation (EtOH+EAAem group). Firstly we investigated morphological and morphometrical features of mitochondria in hepatocytes, through optic and electron microscopy. EtOH consumption increased fat accumulation in hepatocytes, while the concomitant EAAem supplementation prevented this fat accumulation. Electron microscopy analysis showed a marked reduction of mitochondrial number, as well as of mitochondrial cristae length and density in hepatocytes of EtOH consuming rats, while EAAem supplementation preserved mitochondrial number and morphology. Then, we studied the mitochondrial biogenesis by measuring PGC-1α, NRF1, and Tfam mRNA levels, mtDNA amount, eNOS, cytochrome c oxidase IV (COX IV) and cytochrome c (Cyt c) protein levels, and citrate synthase activity. We found that the PGC-1α (-48 %), NRF1 (-41 %), Tfam (-30 %) and eNOS (-38 %) mRNA levels, as well as the mtDNA amount (-35 %) were statistically lower in liver of EtOH consuming rats when compared to Ctrl animals. On the contrary, the dietary EAAem supplementation prevented the EtOH effects, by increasing the expression of mitochondrial biogenesis markers (PGC-1α, +54 %; NRF1, +146 %; Tfam, +200 %, eNOS, +111 % and mtDNA, +55 %) in respect to the levels leasured in rats consuming EtOH alone. We also observed a relevant reduction of COXIV (-39 %) and Cyt c (-40 %) in EtOH group compared to Ctrl group, and a recovery of these parameters with EAAem suppementation. Finally, the cytrate synthase activity was decreased (-30 %) in liver of EtOH-consuming rats as compared to Ctrl rats, while EAAem mediated a recovery by +178 % of the enzyme activity compared to EtOH group. Taken together, our results demonstrate that EAAem supplementation is able to preserve liver mitochondra from the EtOH damage, by normalizing their morphology/morphometry and recovering impaired mitochondrial biogenesis and function.