Metabolic consequences of the inhibition of the mammalian target of rapamycin complex in mice fed a ketogenic diet and ethanol

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Introduction: A ketogenic diet, with a high fat and low carbohydrate content, inhibits the mammalian target of rapamycin (mTOR) complex[1]. Since mTOR plays a pivotal role in controlling critical cellular growth and survival pathways, several diseases are associated with its aberrant induction, including age-related diseases like cancer, neurodegeneration and diabetes[2,3]. A growing list of evidence however suggests that mTOR signaling influences longevity and aging, and that the inhibition of mTOR with rapamycin increases lifespan in disease models[3,4]. However, the effect of ethanol on the efficacy of the ketogenic diet to inhibitmTOR remains unclear.

Objectives: To this end, the effect of ethanol and a ketogenic diet on the phosphorylation of mTORC1, metabolism and the redox state was assessed.

Methods: Liver and urine were collected from C57BL/6 male mice after being fed a normal diet (ND), ketogenic diet (KD), normal diet with alcohol (ND + Alc) and a ketogenic diet with alcohol (KD + Alc) respectively for 3 weeks. Liver samples were subjected to western blot analysis to evaluate p70S6K1 phosphorylation to assess mTOR activity. Urine samples were subjected to multi-platform metabolomics, including LC-MS/MS, GC-TOF-MS and ^{1}H-NMR toevaluate the metabolic perturbations as well as fluctuations of the cellular redox state.

Results: Mice fed a ketogenic diet were significantly lighter than the ND group after 3 weeks, while ethanol had no significant effect on body weight. mTOR inhibition was observed in the dietary groups fed with a ketogenic diet. Moreover, it seemed as if alcohol consumption was also correlated with some inhibition of mTOR, where the greatest inhibition of mTOR was seen in the groups fed with the KD + Alc. In addition, metabolic profiling showed metabolic alterations linked to branched chain amino acid metabolism as well as pathways linked with the one carbon cycle. Moreover, known metabolic indicators of an altered redox state (the ratios of alanine, leucine and isoleucine relative to glutamic acid) indicated a reduced redox state in the KD as well as the KD + Alc groups, relative to the ND group.

Conclusion: This study investigated the influence of a ketogenic diet alongside ethanol intake on mTOR and elucidated the associated effect on metabolic reprogramming and a shift in cellular redox state. Ultimately understanding these processes might provide novel therapeutic approaches to influence longevity and aging-related diseases.

Keywords*:* Metabolomics, mTOR, redox, western blot, ketogenic diet

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