A Leucine, Metformin, and Sildenafil Combination Regresses Nonalcoholic Steatohepatitis (NASH) in Mice

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MICHAEL B. ZEMEL, ANTJE BRUCKBAUER, OMAR FLORES, *Knoxville*, *TN*, *Nashville*, *TN*

Non-alcoholic fatty liver disease (NAFLD) and NASH occur predominantly in diabetes and obesity and are characterized by suppression of the Sirt1/AMPK axis, while Sirt1 stimulation or overexpression relieves both the steatosis and inflammation of NASH. We have shown leucine to allosterically activate Sirt1 and synergize with other Sirt/AMPK pathway activators, and that leucine-metformin reverses NAFLD in mice. PDE5 inhibition also activates Sirt1 secondary to eNOS activation, and leucine-PDE5 inhibitor combinations similarly reverse NAFLD in mice; however, we have found the triple combination of leucine, metformin and sildenafil to generate greater synergy and reverse NASH in mice. To optimize doses of metformin and sildenafil in the combination, we induced NASH in mice via high fat (60%) supplemented with cholesterol (1.25%) and cholate (0.5%)(HF/ATH) diet and then randomized to combinations of leucine (24 g/kg diet), metformin (0.5 – 1 g/kg diet) and sildenafil (6.25-100 mg/kg diet). The HF/ATH diet caused significant steatosis, inflammation, Kupfer cell activation and fibrosis. The leucine-metformin-sildenafil combinations significantly reduced steatosis (43%), inflammation (55%), Kupfer cell activation (40%) and fibrosis (50%). Since our previous work demonstrated dose-response up to 0.5 metformin/kg diet (~40% of therapeutic dose), this was utilized as the lowest dose here, and no further benefit accrued from a higher dose in the combination. Sildenafil in the combination exhibited dose-response improvements in each variable up to a maximum effect at 25 mg/kg diet (<10% therapeutic dose). Consistent with our *in vitro* work, there were corresponding effects on downstream Sirt1 targets, as well as on PPARα and PPARδ (~70% stimulation, $p<0.01$). These data support the clinical potential of a combination of L-leucine with low doses of metformin (calculated as 0.5-1 g/d human equivalent) and sildenafil (1-2 mg/d calculated human equivalent) in treatment of NASH.

Keywords Fatty liver disease, NASH

American Diabetes Association
1701 North Beauregard Street Alexandria, VA 22311