

Leucine synergizes with metformin and sildenafil to treat Non-Alcoholic Fatty Liver Disease (NAFLD) and Steatohepatitis (NASH)



Disease (NAFLD) and Steatohepatitis (NASH)

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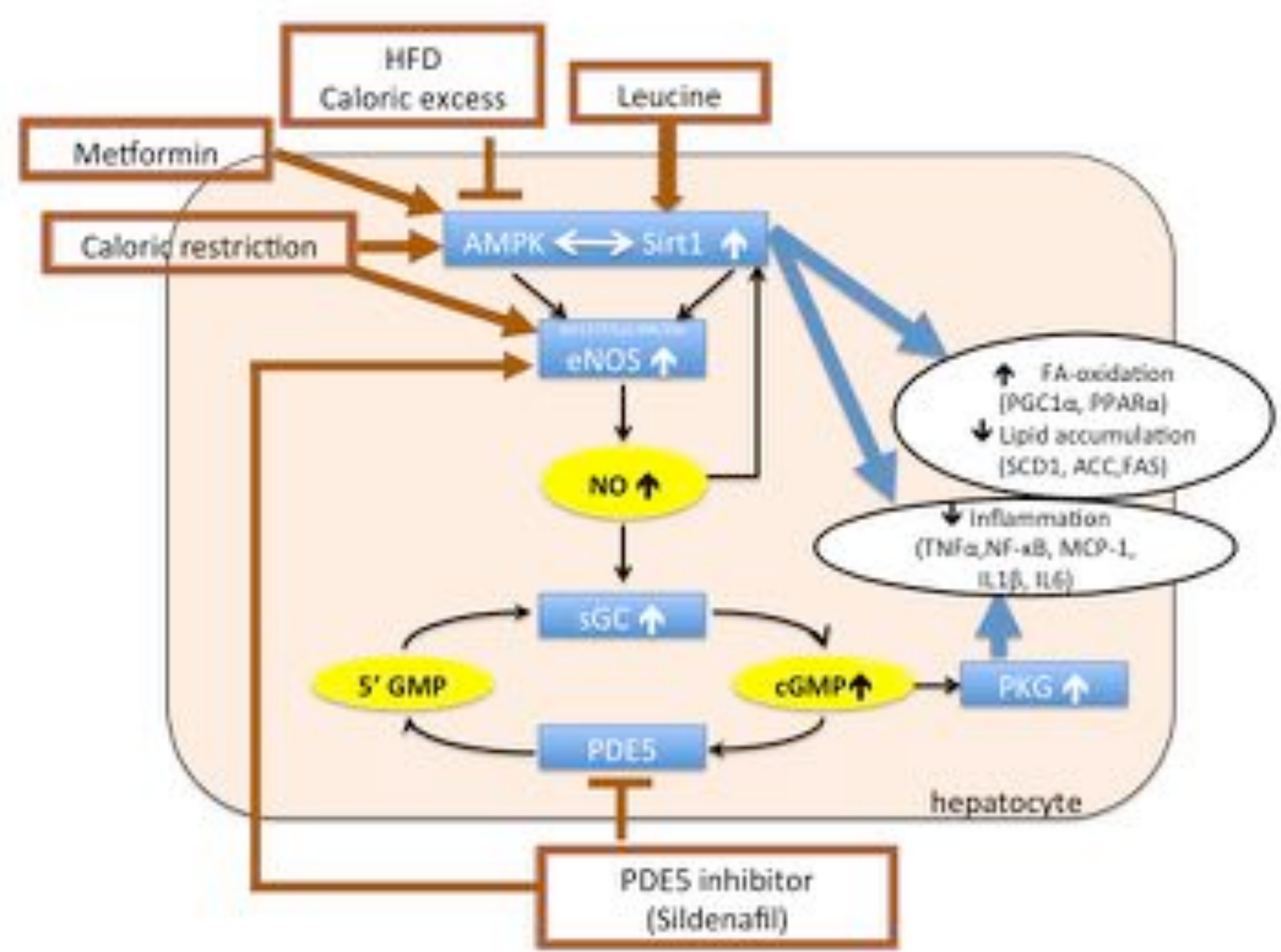
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Introduction

High fat-diets and diabetes downregulate the Sirt1/AMPK axis, resulting in hepatic steatosis and inflammation, while Sirt1 overexpression or activation protects against NASH (1-3) and represents a therapeutic target for NASH (3).

We have found leucine to allosterically activate Sirt1 (4), and to synergize with metformin. This enables a 65-80% dose reduction with no loss of antidiabetic efficacy and reverses NAFLD in mice despite the lack of efficacy of full-dose metformin (5-7). PDE5 inhibitors also activate the Sirt1/AMPK axis via eNOS/NO signaling, and leucine also synergizes with sildenafil via eNOS to activate Sirt1 and inhibit steatosis and inflammation (8). **Accordingly, we tested the efficacy of a multi-component activation of this pathway in a more severe preclinical model of NASH.**



Materials & Methods

animals: C57/BL6 mice (n=10/group) fed either a low fat (LF) or a high fat/atherogenic diet (HF/HC) containing 1.25% cholesterol (w/w) and 60% calories as saturated fat (lard) for 8 weeks to induce the development of NASH and insulin resistance. Then they were randomized to one of the treatment groups of study 1 or study 2 for additional 8 weeks.

Treatment groups (Study 1): Control LF = 10% calories from fat, Control HF/HC = 1.25% cholesterol, 0.5% cholate, 60% calories from fat, HF/HC + leucine (24 g/kg diet; a two-fold increase over control levels) + metformin (0.25 g/kg diet, <20% of therapeutic dose), HF/HC + sildenafil (25 mg/kg/diet, <10% of therapeutic dose), HF/HC + leucine + sildenafil, HF/HC + metformin + sildenafil, HF/HC + leucine + metformin + sildenafil

Results: Interactive effects of leucine, metformin and sildenafil

Figure 1: Liver weight, triglycerides (TG) and Alanine-Amino-Transferase (ALT)

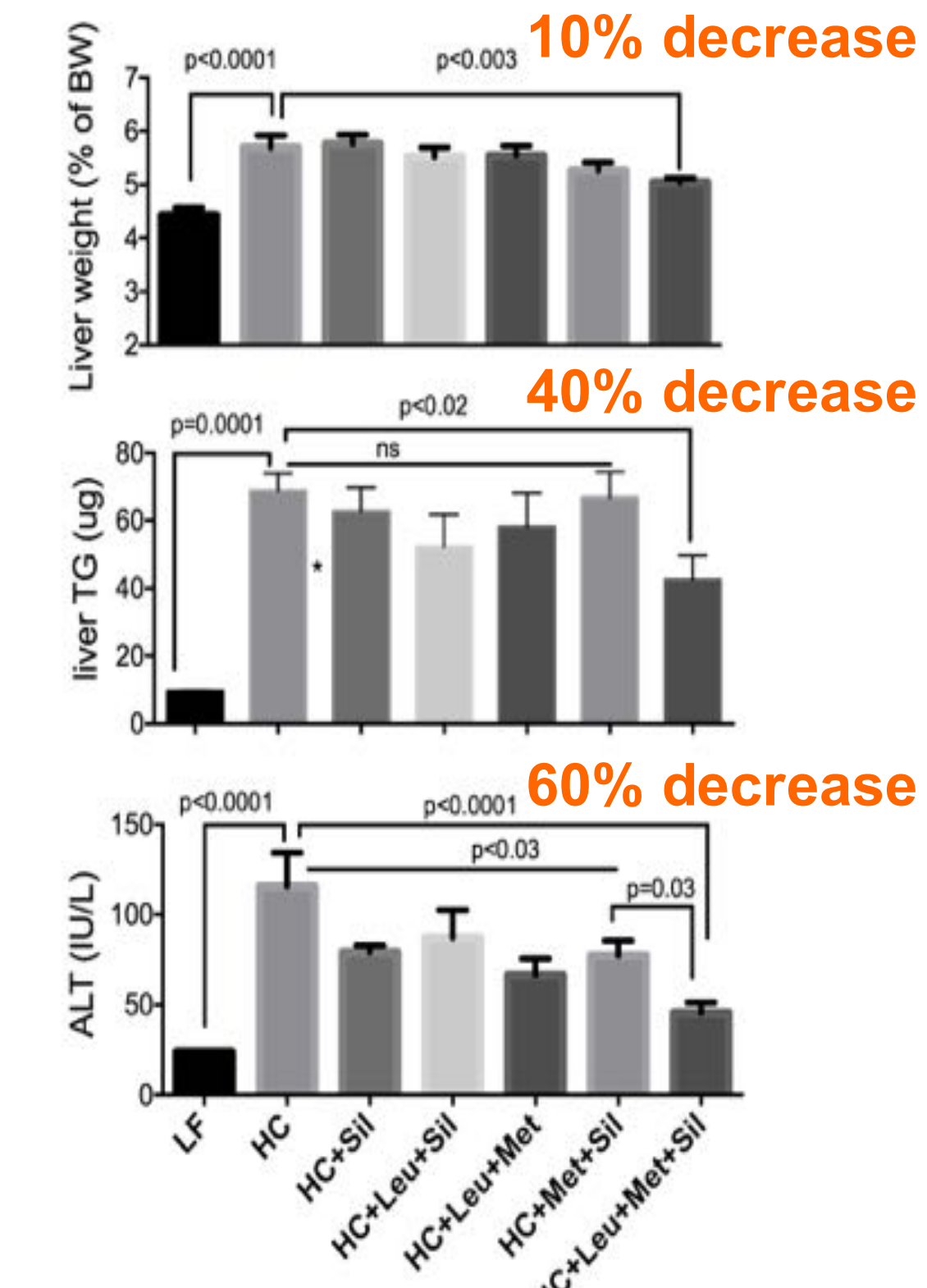


Figure 3: Liver Histology

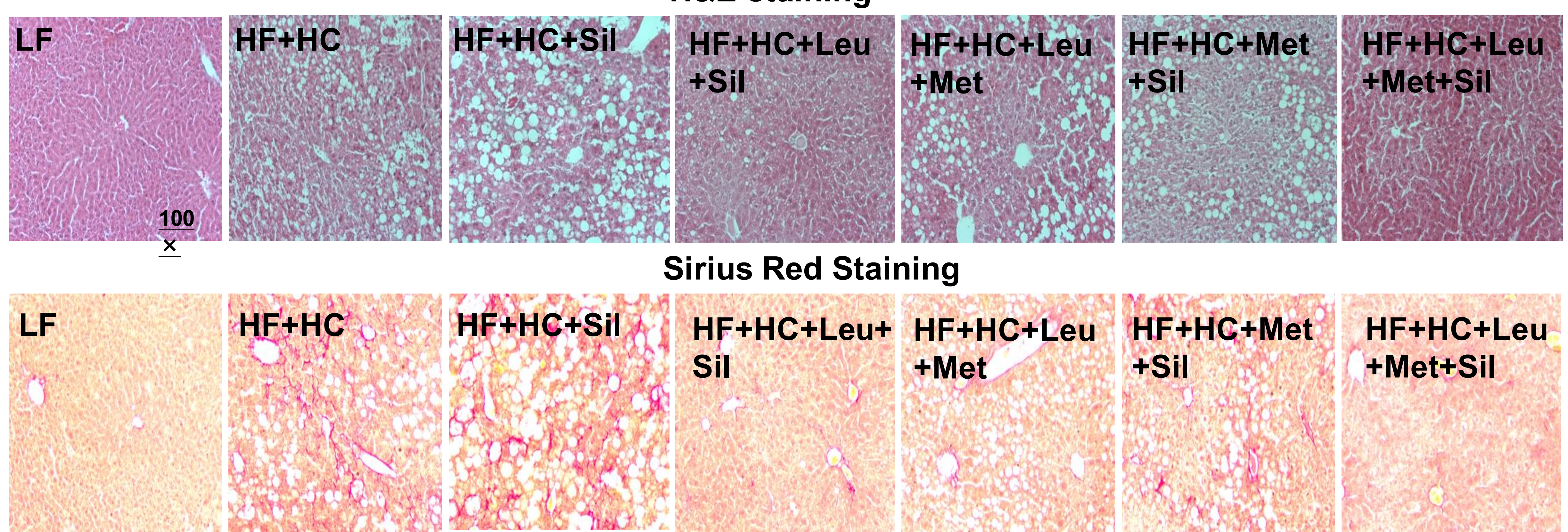


Figure 2: Sirius Red (Collagen) and CD68 staining

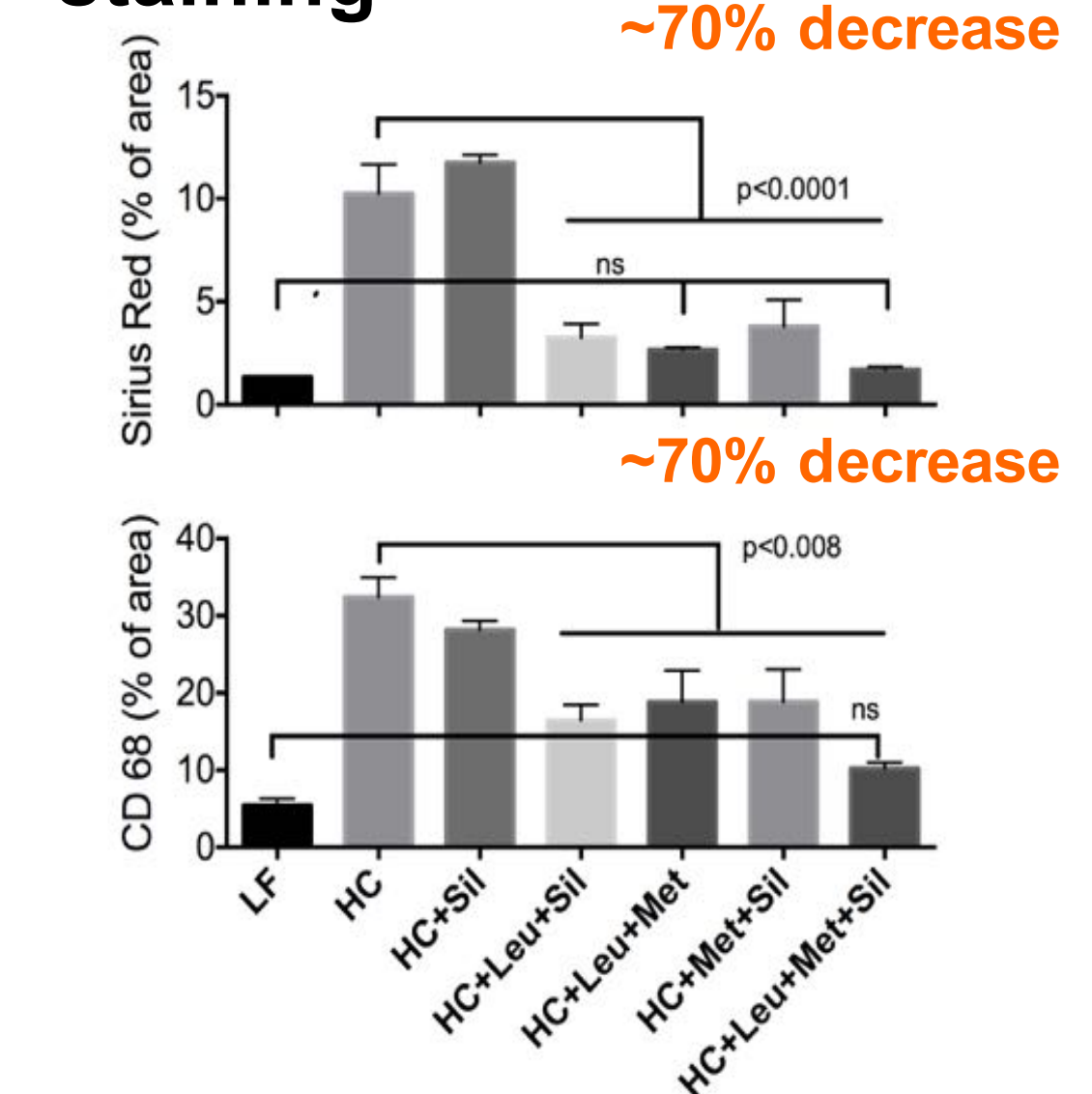


Figure 4: Gene expression of fibrogenetic, inflammatory, and oxidative genes in liver

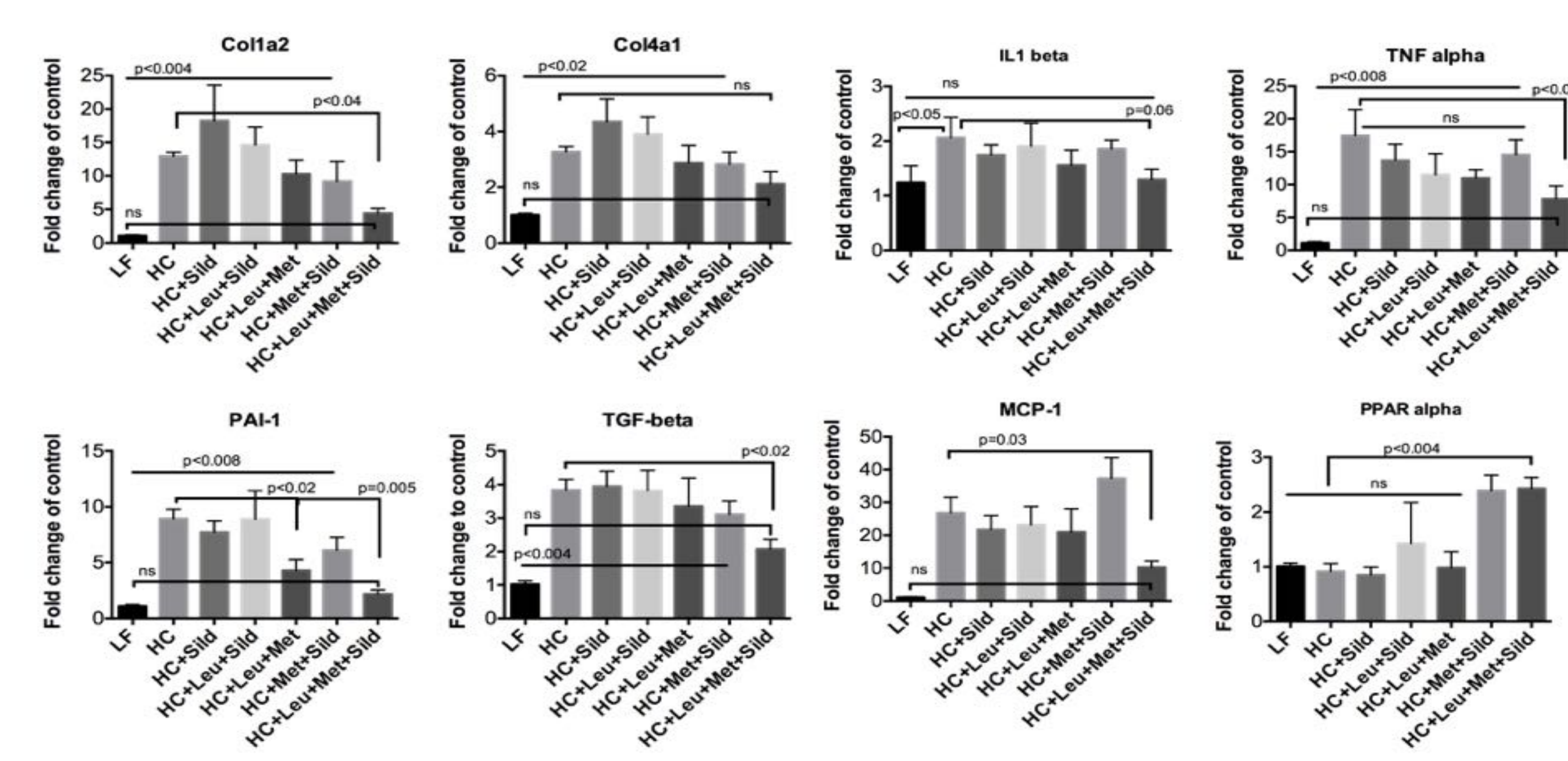
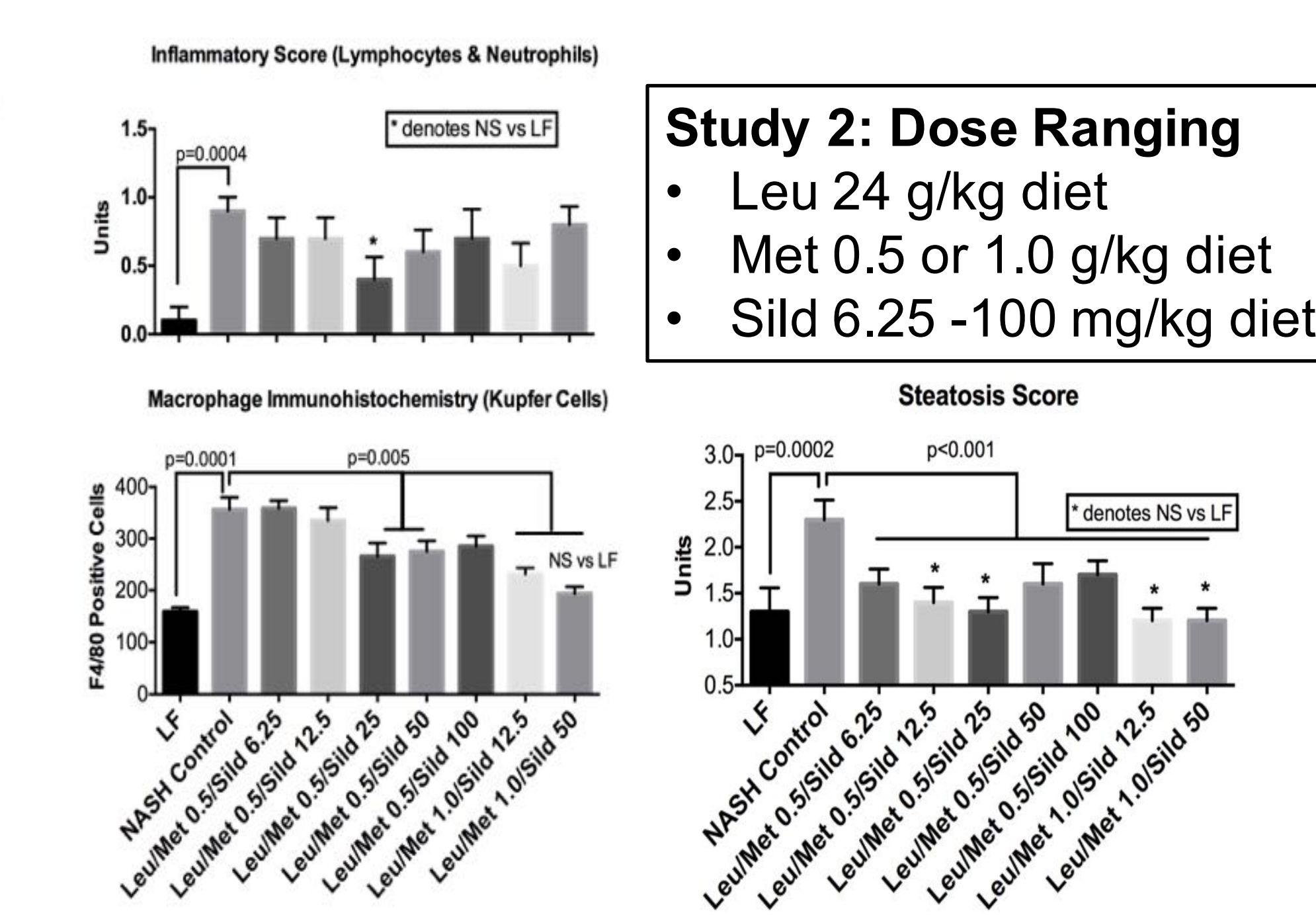


Figure 5: Inflammation and steatosis score in dosing efficacy study (Study 2)



Conclusions

- Leu-Met-Sil feeding resulted in significant reductions of liver weight, liver triglycerides and ALT, and reversed the diet-induced steatohepatitis
- Leu-Met-Sil combination markedly reduced fibrogenesis and inflammation
- Leu-Met-Sil shows promise for the treatment of NAFLD and NASH

References

- Pfluger PT, Herranz D, Velasco-miguel S, Serrano M, Tscho MH. Sirt1 protects against high-fat diet-induced. PNAS 2008;105:9793-8.
- Liu Y, Wan Q, Guan Q, Gao L, Zhao J. High-fat diet feeding impairs both the expression and activity of AMPKα in rats' skeletal muscle. Biochem Biophys Res Commun 2006;339:701-7
- Hou X, Xu S, Maitland-Toolan K a, Sato K, Jiang B, Ido Y, et al. SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. J Biol Chem 2008;283:20015-26.
- Bruckbauer A, Zemel MB. Synergistic effects of polyphenols and methylxanthines with leucine on AMPK/Sirtuin-mediated metabolism in muscle cells and adipocytes. PLoS One 2014;9:e89166.
- Bruckbauer A, Zemel MB. Synergistic effects of metformin, resveratrol, and hydroxymethylbutyrate on insulin sensitivity. Diabetes Metab Syndr Obes 2013;6:93-102.
- Fu L, Bruckbauer A, Li F, Cao Q, Cui X, Wu R, et al. Leucine amplifies the effects of metformin on insulin sensitivity and glycemic control in diet-induced obese mice. Metabolism 2015;7:845-56
- Shi H, Fu L, Li F, Bruckbauer A, Cao Q, Cui X, et al. Synergy between Metformin and Leucine in Sirtuin Signaling and Fat Oxidation in vitro, and in Reducing Lipid Accumulation in Diet-induced Obese Mice. Diabetes 2014;63:A463
- Fu L, Li F, Bruckbauer A, Cao Q, Cui X, Wu R, et al. Interaction between leucine and phosphodiesterase 5 inhibition in modulating insulin sensitivity and lipid metabolism. Diabetes Metab Syndr Obes 2015;8:227-39

Disclosure information:
Authors Zemel and Bruckbauer are employees & Stockholders of NuSirt Biopharma.
Authors Xue and Shi have no conflicts to disclose.

Study 2: Dose Ranging
 • Leu 24 g/kg diet
 • Met 0.5 or 1.0 g/kg diet
 • Sild 6.25 - 100 mg/kg diet