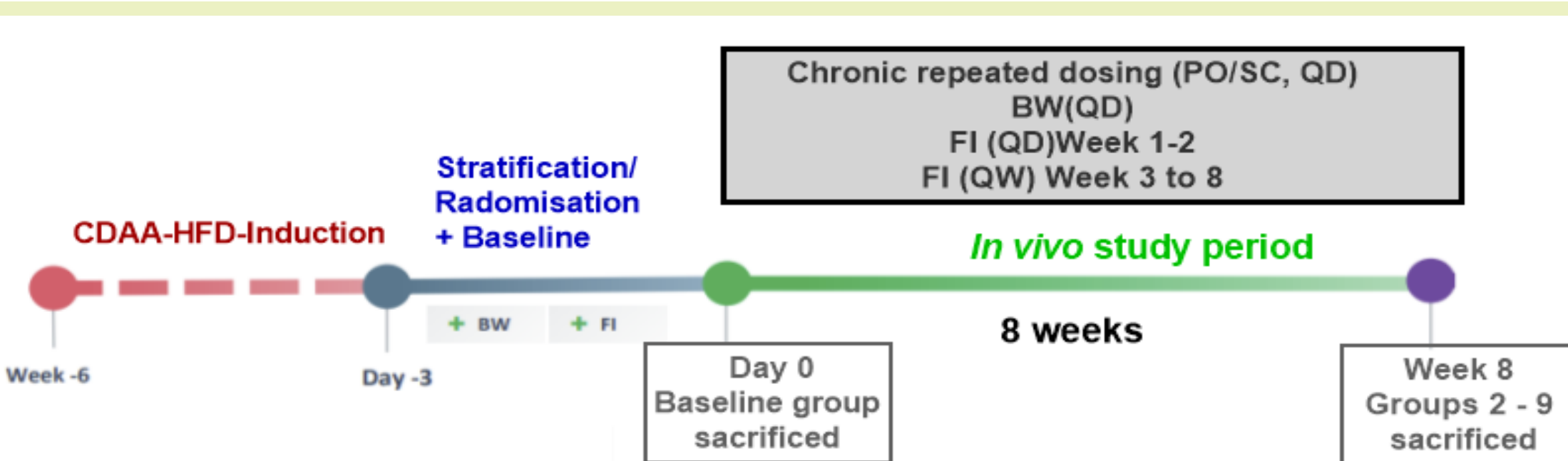


Introduction

- Icosabutate (ICOSA), a semi-synthetic eicosapentaenoic acid derivative targeting FFAR4 and the arachidonic acid cascade, is currently in P2b clinical development for the treatment of NASH (NCT04052516)
- To assess if additional anti-inflammatory and/or anti-fibrotic effects could be achieved via combination therapy, a comparison of ICOSA, firsocostat [FIR, a liver-targeted acetyl-coenzyme A carboxylase (ACC 1/2) inhibitor], semaglutide [SEMA, an injectable glucagon-like 1 (GLP-1) receptor agonist] or obeticholic acid [OCA, a farnesoid-X receptor agonist] as monotherapies was performed in a choline-deficient, L-amino acid defined high-fat (46%) diet fed (CDAA-HFD) mouse model
- The effects of combining ICOSA with either FIR, SEMA or OCA were simultaneously assessed, in addition to hepatic eicosanoid concentrations by LC-MS/MS in the ICOSA and SEMA groups only

Methods

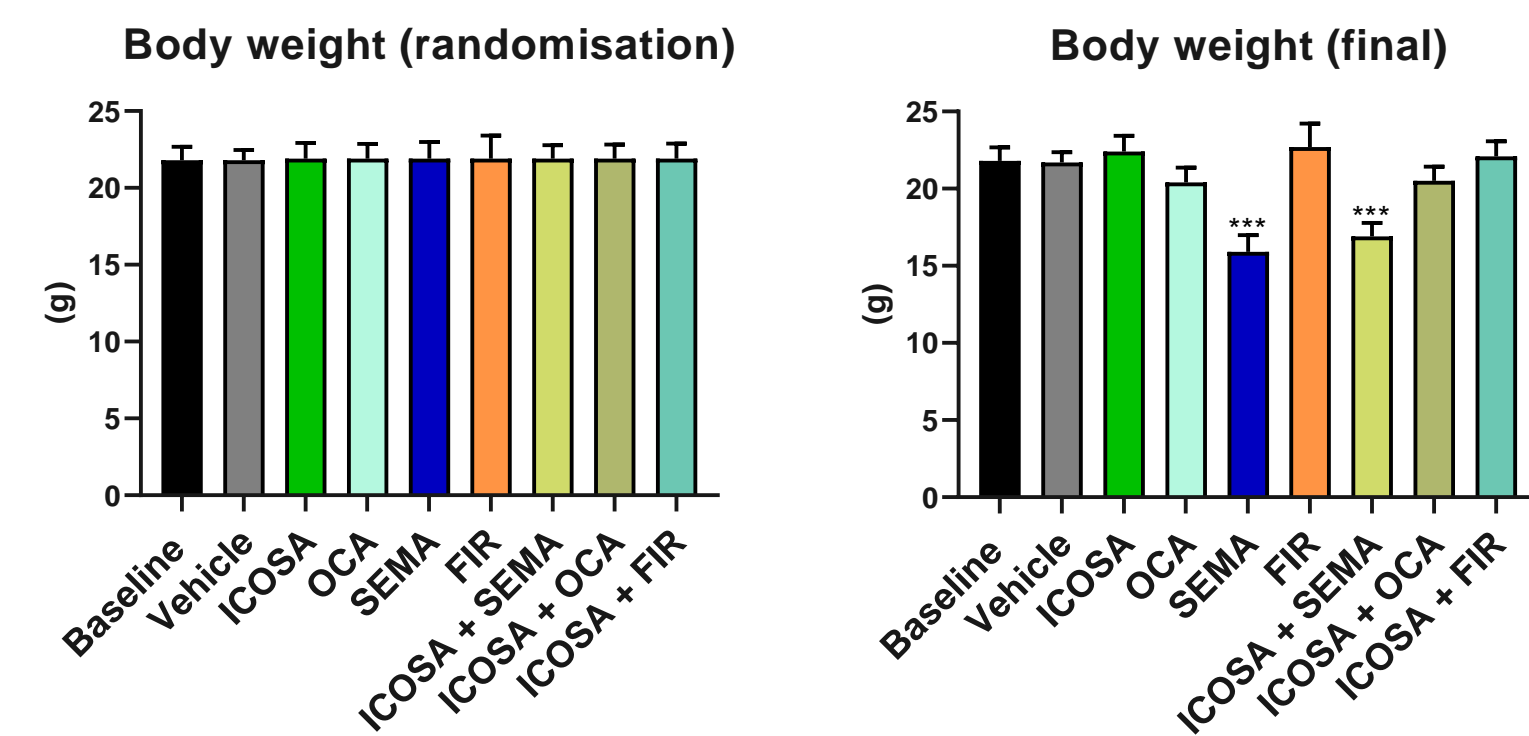
- Male C57BL/6JRj mice were fed CDAA-HFD (A16092001, Research Diets) for 6 weeks before treatment start. A baseline control group (n=12) was terminated at study start
- CDAA-HFD fed mice [n=10-12 per group] received daily per oral (PO) treatment with vehicle (corn oil), ICOSA (112mg/kg), OCA (30mg/kg), SEMA (30nmol/kg SC), FIR (5mg/kg) as monotherapy or combinations of ICOSA + either SEMA, OCA or FIR (all dosing as for monotherapy) for 8 weeks. Inflammation and fibrosis was assessed in terminal liver biopsy by IHC, biochemical and morphometric assays. Values expressed as mean + SEM, n = 9-12 per group.



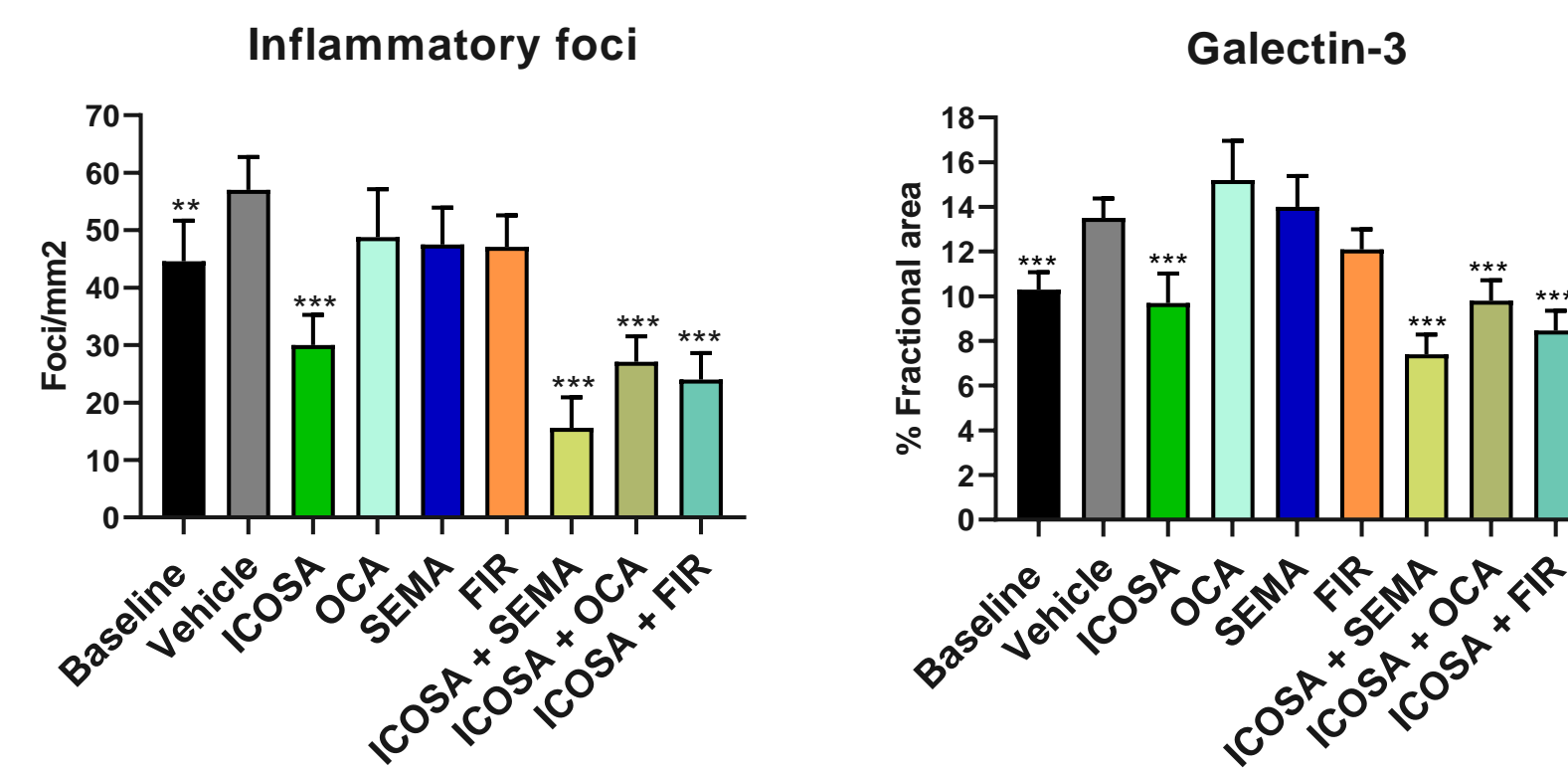
Group	po administration	sc administration
Baseline control		
Vehicle	Vehicle	Vehicle
ICOSA	ICOSA 112 mg/kg	
OCA	OCA 30 mg/kg	
SEMA		SEMA 30nmol/kg
FIR	FIR 5 mg/kg	
ICOSA + SEMA	ICOSA 112 mg/kg	SEMA 30nmol/kg
ICOSA + OCA	ICOSA 112 mg/kg + OCA 30 mg/kg	
ICOSA + FIR	ICOSA 112 mg/kg + FIR 5 mg/kg	

Results

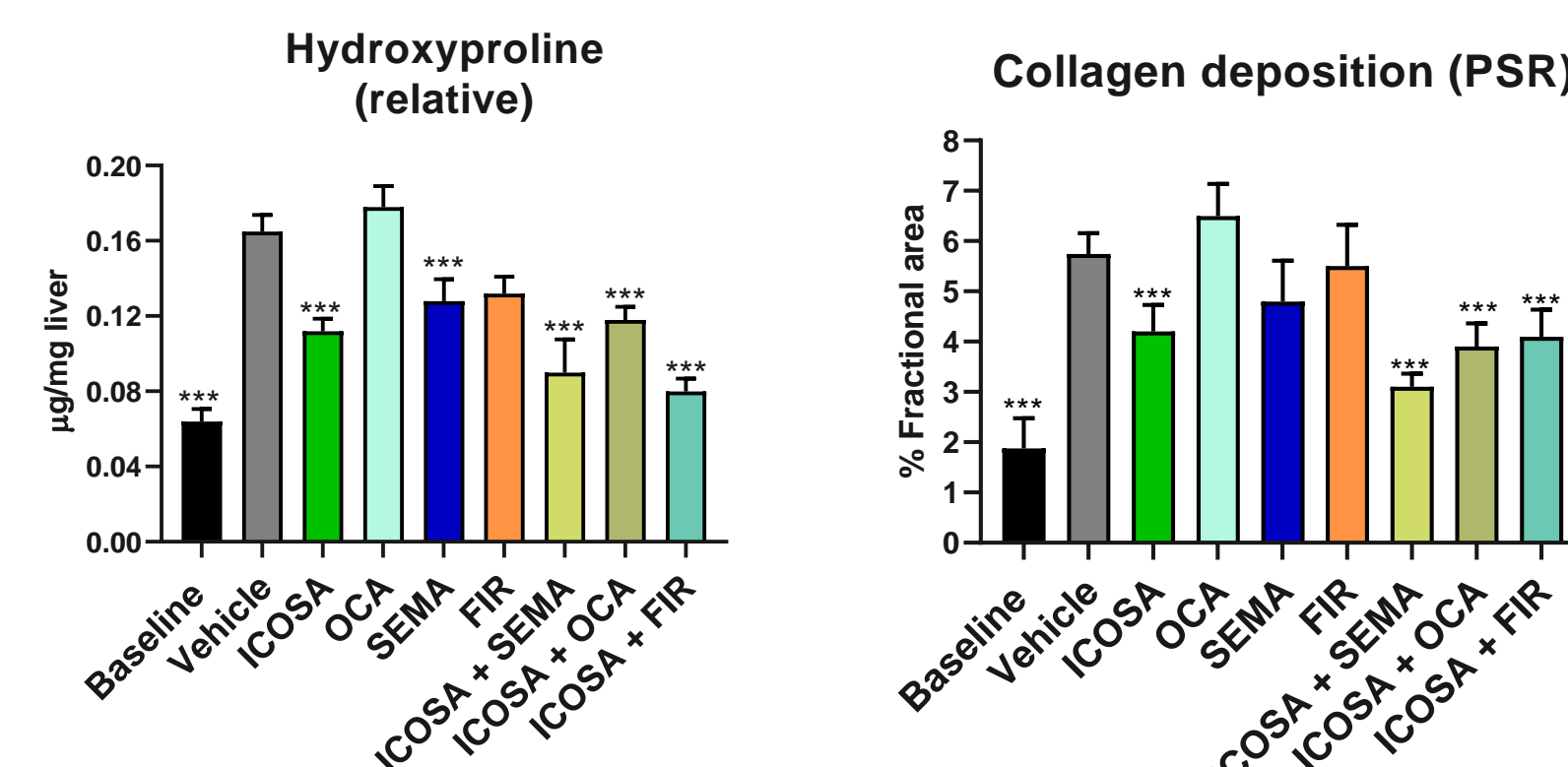
Body weight. Equivalent body weight at randomisation (left) but SEMA treated mice have 26% lower bodyweight (p<0.001 vs. vehicle) at study end (right) as both mono- and combination therapy secondary to decreased food consumption (not shown).



Hepatic inflammation. As monotherapy, ICOSA reduced inflammatory foci by 47% (below left) and galectin-3 (a macrophage marker) by -28% (right). All combinations achieved significant reductions of both foci and galectin-3, the most pronounced effect achieved by ICOSA + SEMA (-73% and -45% for inflammatory foci and galectin-3 respectively). ***p<0.001 vs vehicle

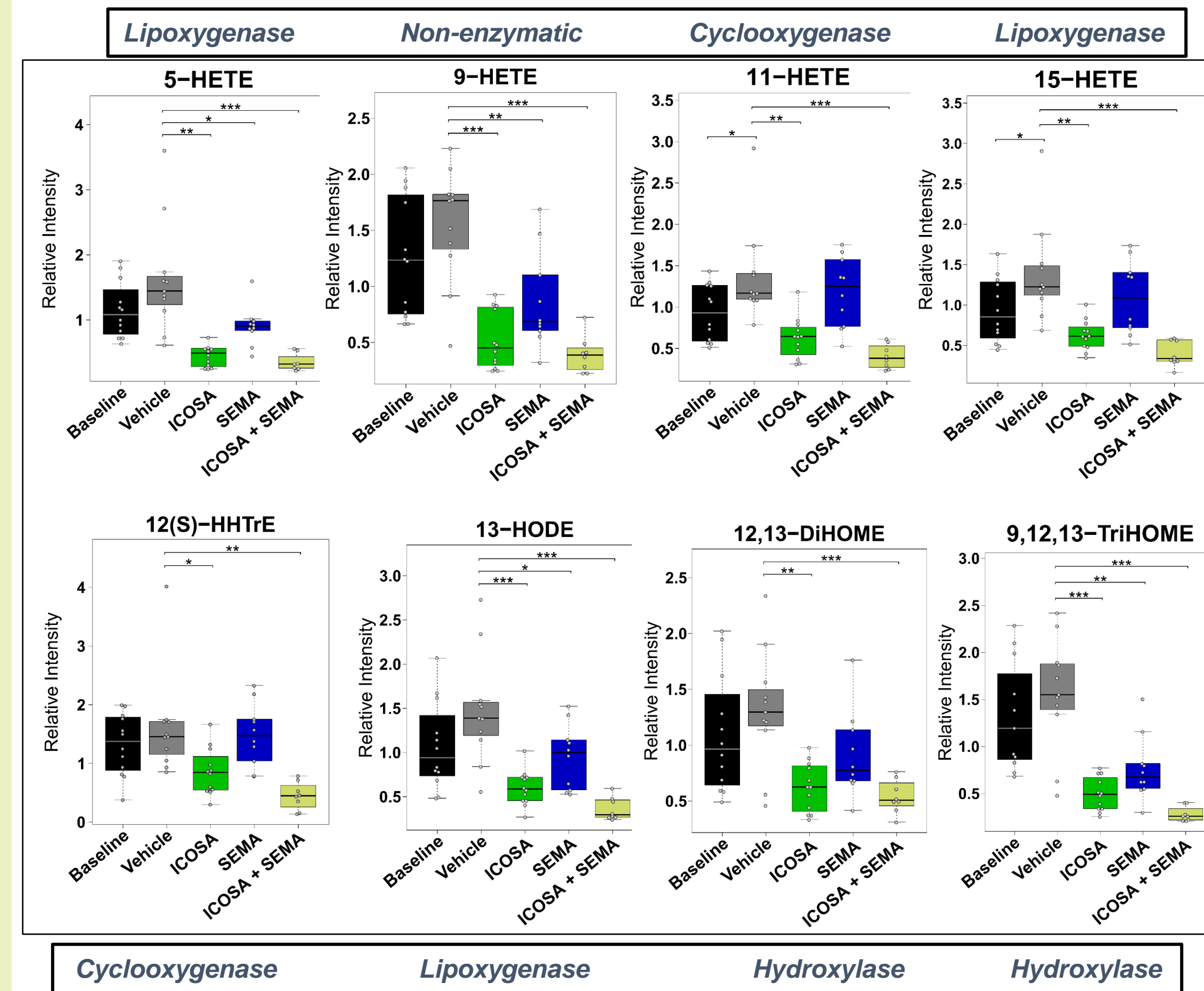


Hepatic fibrosis. ICOSA and SEMA significantly reduced hydroxyproline (HYP) content (by 33 and 22% respectively). ICOSA was also the only monotherapy that induced a significant anti-fibrotic effect as measured by picrosirius red (PSR)-morphometry (right). Optimal combination effects were observed in the ICOSA + SEMA group (-46% and -45% for PSR and HYP respectively). **p<0.01, ***p<0.001 vs vehicle



Comparative effects of ICOSA and SEMA on hepatic oxygenated arachidonic (AA) and linoleic acid (LA) derived metabolites

ICOSA potently reduced both enzymatic (inflammation) and non-enzymatically (oxidative stress) formed eicosanoids with additional effects seen in combination with SEMA. *p<0.05, **p<0.01, ***p<0.001



With direct pro-inflammatory effects and as precursors to potent chemotactic leukotrienes (e.g., LTB4 from 5-HETE), the reductions in enzymatically formed hepatic HETEs may underlie the decreased inflammatory cells numbers in ICOSA treated mice.

Conclusions

- As monotherapy, ICOSA is a more potent anti-inflammatory/anti-fibrotic compound than either SEMA, OCA or FIR in a delayed treatment CDAA-HFD NASH mouse model
- ICOSA significantly enhanced the anti-inflammatory and anti-fibrotic effects of SEMA and offered the optimal combination therapy
- Mechanistically, ICOSA potently inhibits the hepatic arachidonic acid, a highly relevant target for the treatment of human NASH (1) and NASH driven fibrosis (2)

(1) Hall Z, et al. Lipid zonation and phospholipid remodelling in non-alcoholic fatty liver disease. *Hepatology*. 2017;65(4):1165
 (2) Simon TG, et al. Daily aspirin use associated with reduced risk for fibrosis progression in patients with non-alcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2019;17(13):2776