

Inflammation and Fibrosis in Nonalcoholic Steatohepatitis (NASH) Characterized by a Mathematical Model

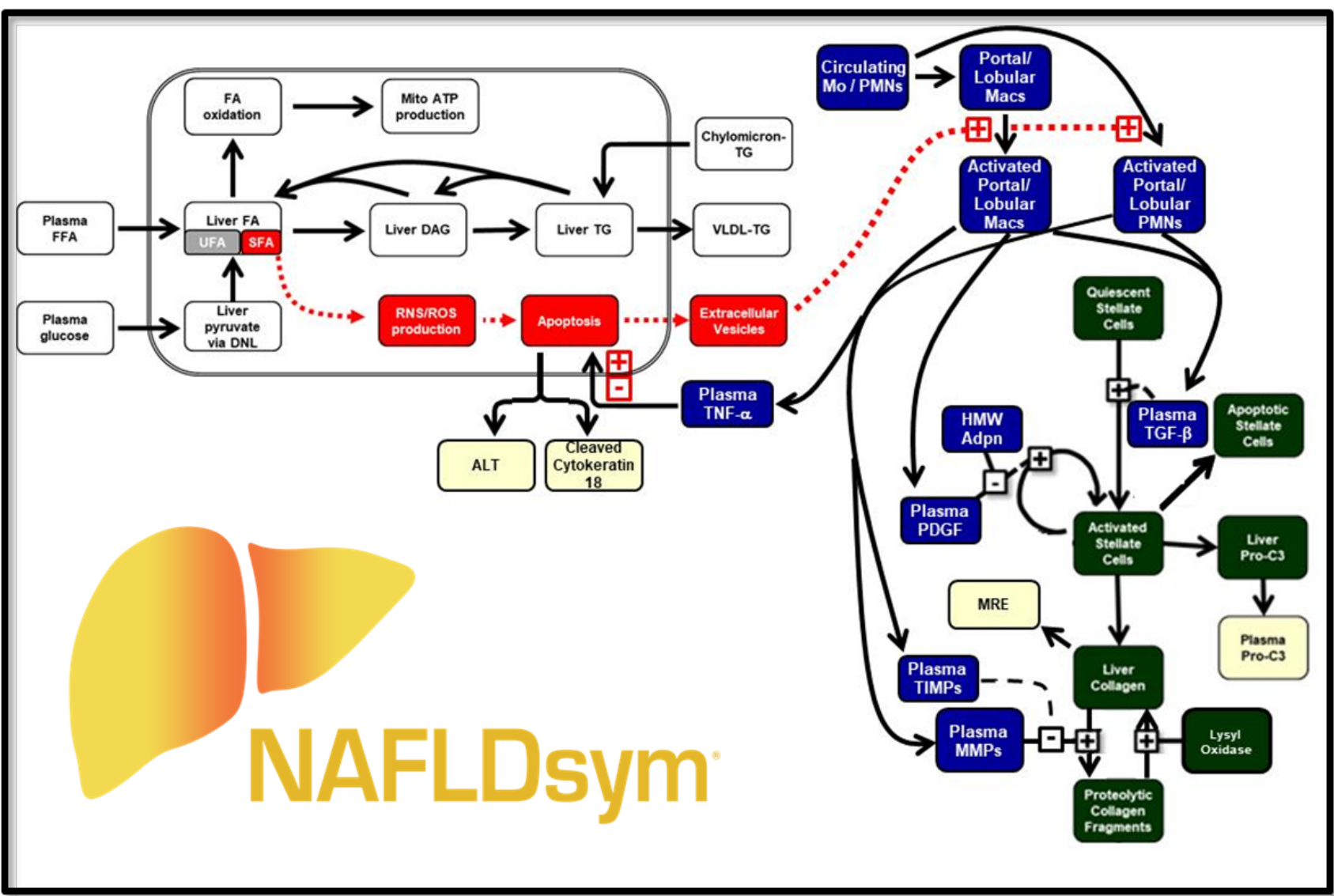
Zackary R Kenz¹, Grant T Generaux¹, Diane M Longo¹, Christina Battista¹, Paul B Watkins²,
Lisl Shoda¹, Brett A Howell¹, Scott Q Siler¹

¹DILIsym Services, Inc., Research Triangle Park, NC; ²UNC School of Medicine & UNC Eshelman School of Pharmacy,
The University of North Carolina at Chapel Hill, Chapel Hill, NC

INTRODUCTION

- Non-alcoholic fatty liver disease (NAFLD) is of growing concern within developed countries, with recent estimates suggesting up to 30% of the US population may be affected [1]. NAFLD represents a spectrum of pathophysiology, ranging from hepatic steatosis, through non-alcoholic steatohepatitis (NASH) and hepatic fibrosis, and may result in cirrhosis and liver failure.
- Inflammation in NASH can be driven by the release of extracellular vesicles (EVs) from steatotic hepatocytes, intensifying and modulating disease progression. Hepatic fibrosis in NASH is caused by excessive accumulation of extracellular matrix (ECM) proteins. Fibrosis progresses over time due to an increased number of activated hepatic stellate cells (HSCs) and subsequently increased production of hepatic ECM proteins. The relay race of steatosis, inflammation, and subsequent zonal ECM accumulation has not been captured in mathematical models of NASH fibrosis prior to this work.
- The objective of this work is to build a mechanistic model for NAFLD/NASH inflammation and fibrosis where patient variability can be overlaid, a model which can be utilized going forward to guide development of therapeutics aimed at treating NASH.

METHODS



NAFLDsym
QSP Model

Components of
the inflammation
sub-model shown in blue
boxes.

Components of
the fibrosis sub-
model shown in the green boxes.

- Mathematical models of inflammation and fibrosis were developed as sub-models within the NAFLDsym quantitative systems pharmacology (QSP) model to represent dynamics of disease enhancement, collagen turnover, as well as zonal accumulation of collagen during the development of fibrosis in NASH. The model consists of a system of ordinary differential equations (ODEs) which describe the following processes in three discrete acinar zones of the liver: inflammatory cell responses and regulation due to steatosis; regulation of inflammatory response; activated hepatic stellate cell-driven collagen I and III synthesis; MMP/TIMP modulation of collagen degradation; and collagen crosslinking by lysyl oxidase.
- Inflammatory macrophage and neutrophil populations as described in clinical data [2] were represented as a function of cellular activation due to EV release from lipid-laden hepatocytes [3] and cellular recruitment rates. Related changes in pro- and anti-inflammatory mediators as well as pro-fibrotic mediators consistent with disease progression were calibrated using clinical studies, for example [5,6]. Collagen I and III synthesis rates and amounts were estimated across the stages of fibrosis using a combination of clinical studies measuring turnover of ²H₂O-labeled collagen [7] as well as collagen content from Elastica van Gieson-stained liver biopsy tissues in NASH patients [7,8]. The amount of collagen present across the stages of fibrosis was optimized by balancing the enzymatic activity of MMP/TIMP and lysyl oxidase. The spatio-temporal pattern of zonal collagen accumulation observed in histological scoring of fibrosis patients was captured by varying the K_m of TGF-β driven activation of HSCs, with the K_m increasing from the centrilobular (CL) to periportal (PP) to midlobular (ML) zones of the hepatic acinus.
- Variability in the inflammatory response, collagen synthesis rates, and hepatic collagen content across stages of fibrosis was accounted for by the creation of a simulated population (SimPops®) which incorporated variability in parameters related to stellate cell activation and collagen synthesis/degradation, as well as other mechanisms related to the pathophysiology of NAFLD/NASH.

REFERENCES

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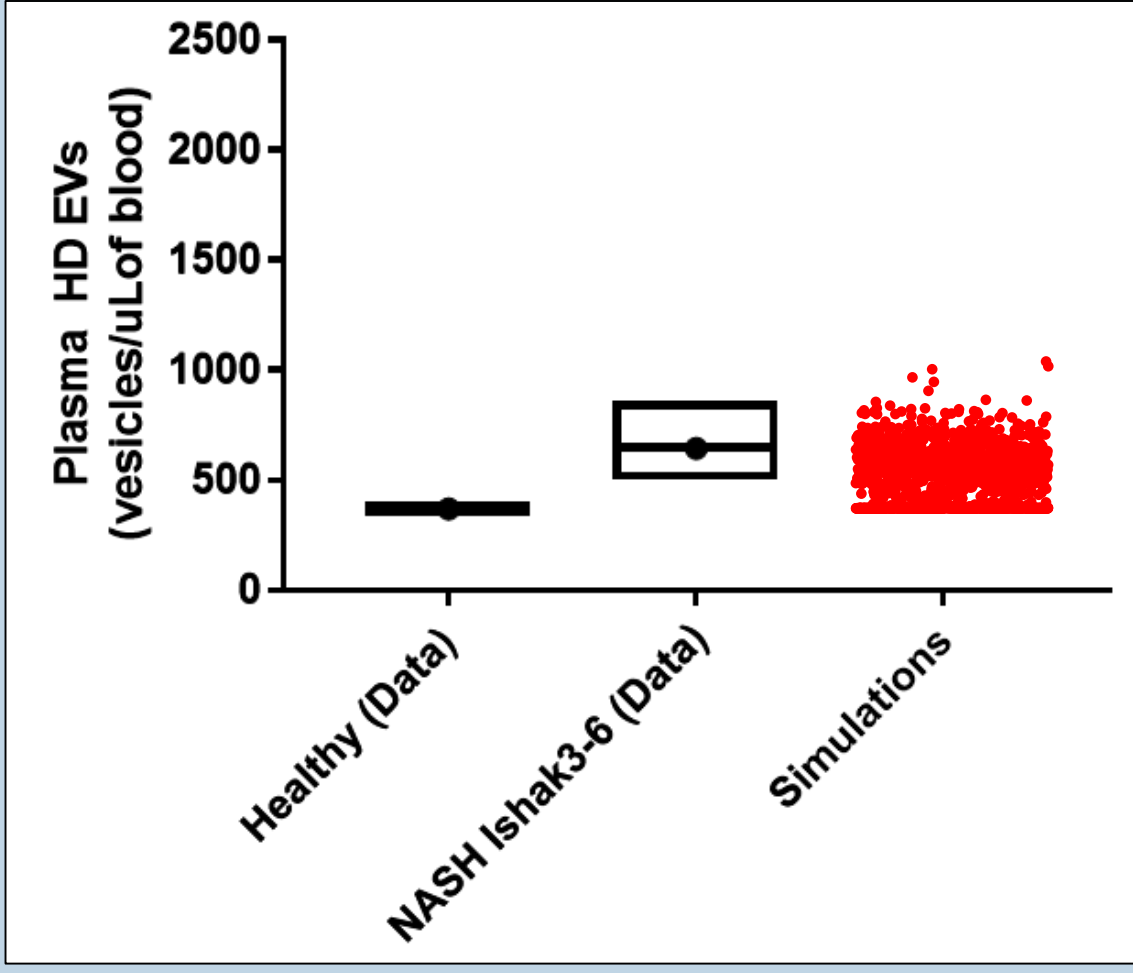
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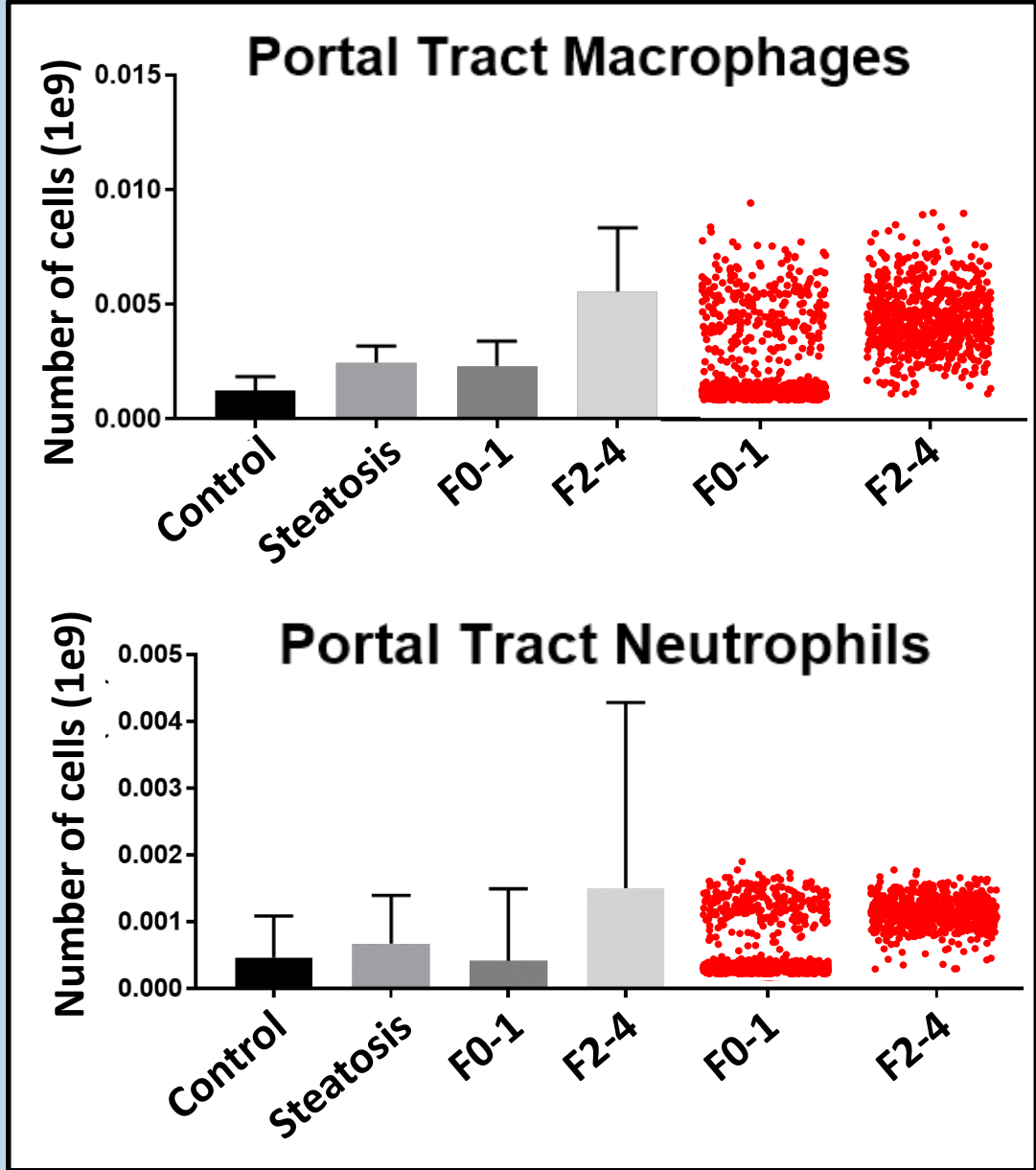
RESULTS

NAFLDsym Simulations Demonstrate Inflammation Contribution Consistent with Clinical Data

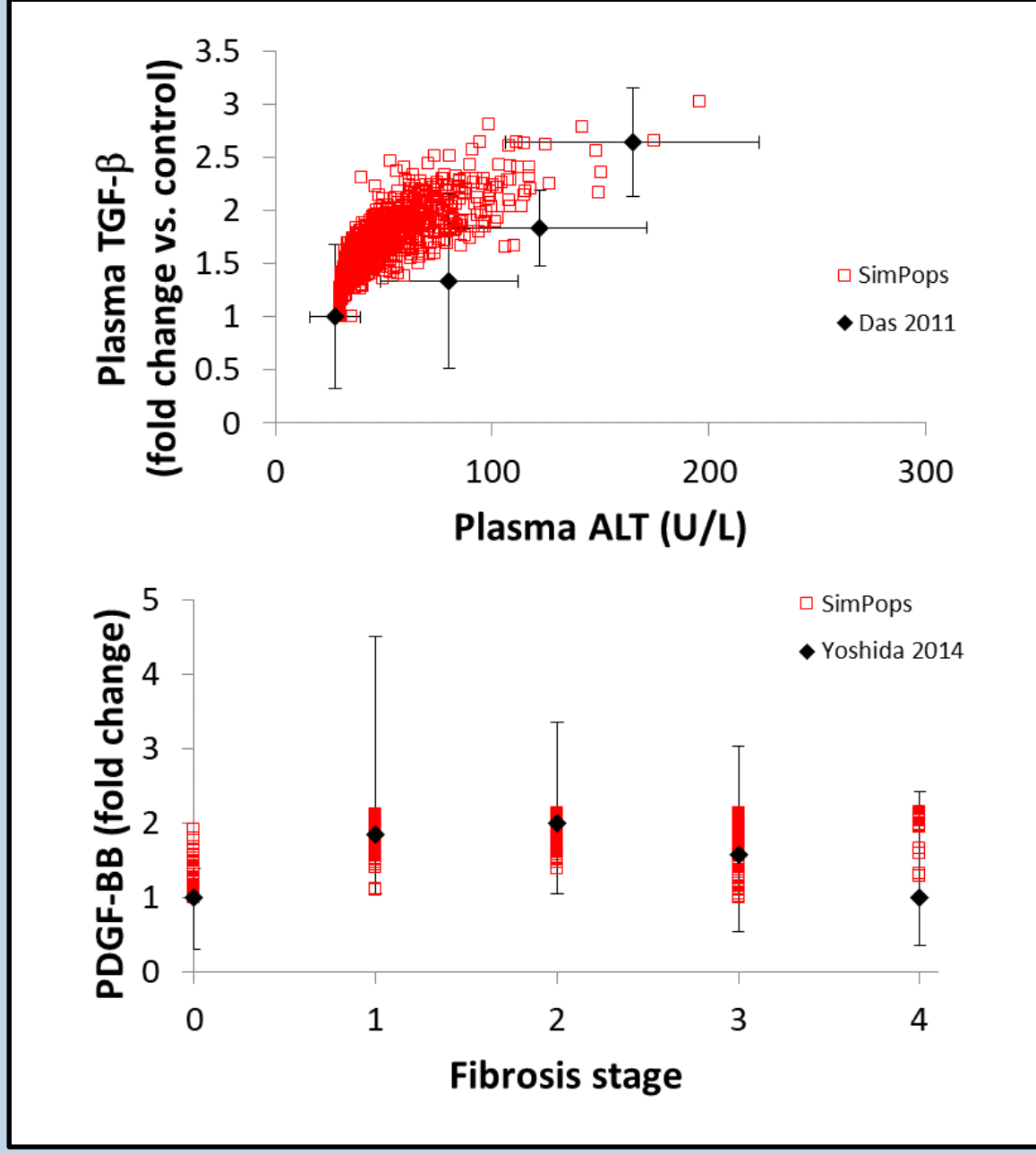
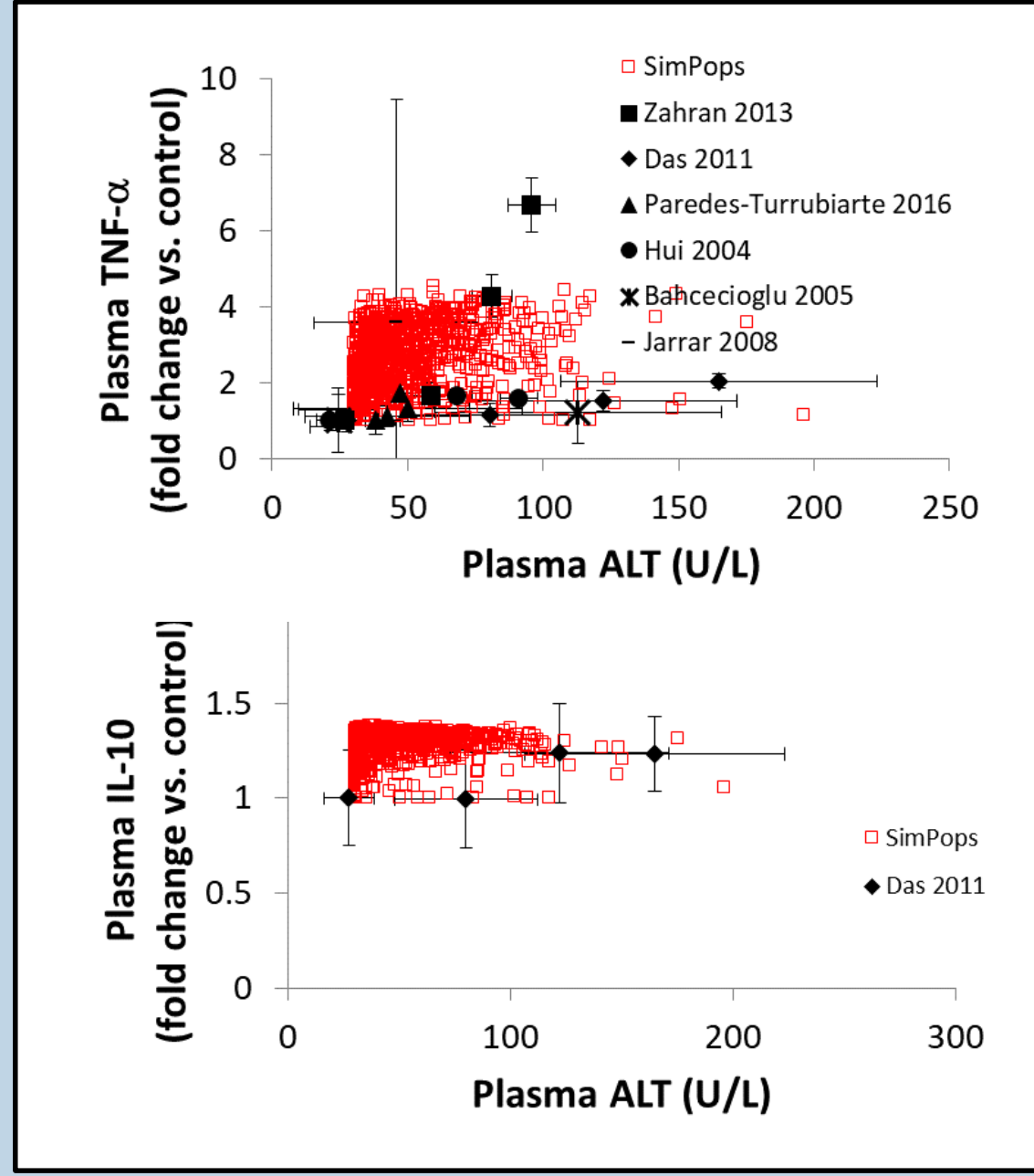
- Lipid-laden apoptotic hepatocytes release EVs, at levels consistent with measured data [3] by design.



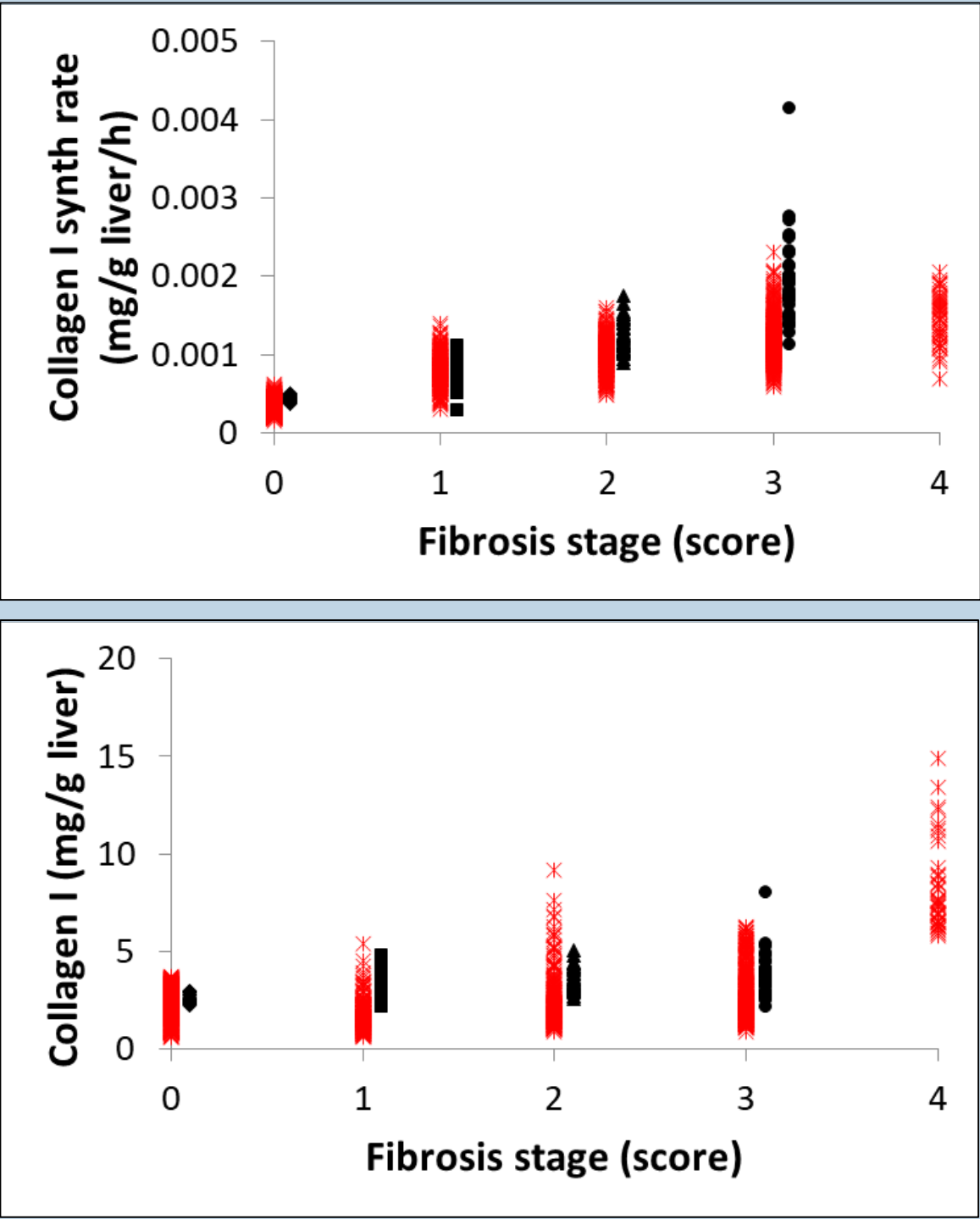
- Macrophages and neutrophils are recruited to the portal tract in response to EV release, at levels consistent with measured data [4] by design.



- Due to changes in macrophage and neutrophil levels and production, there are different simulated levels of pro- (TNF-α) and anti- (IL-10) inflammatory cytokines and as well as cytokines such as TGF-β and PDGF which modulate fibrosis components. These levels are consistent with reported data (shown in figure) by design.

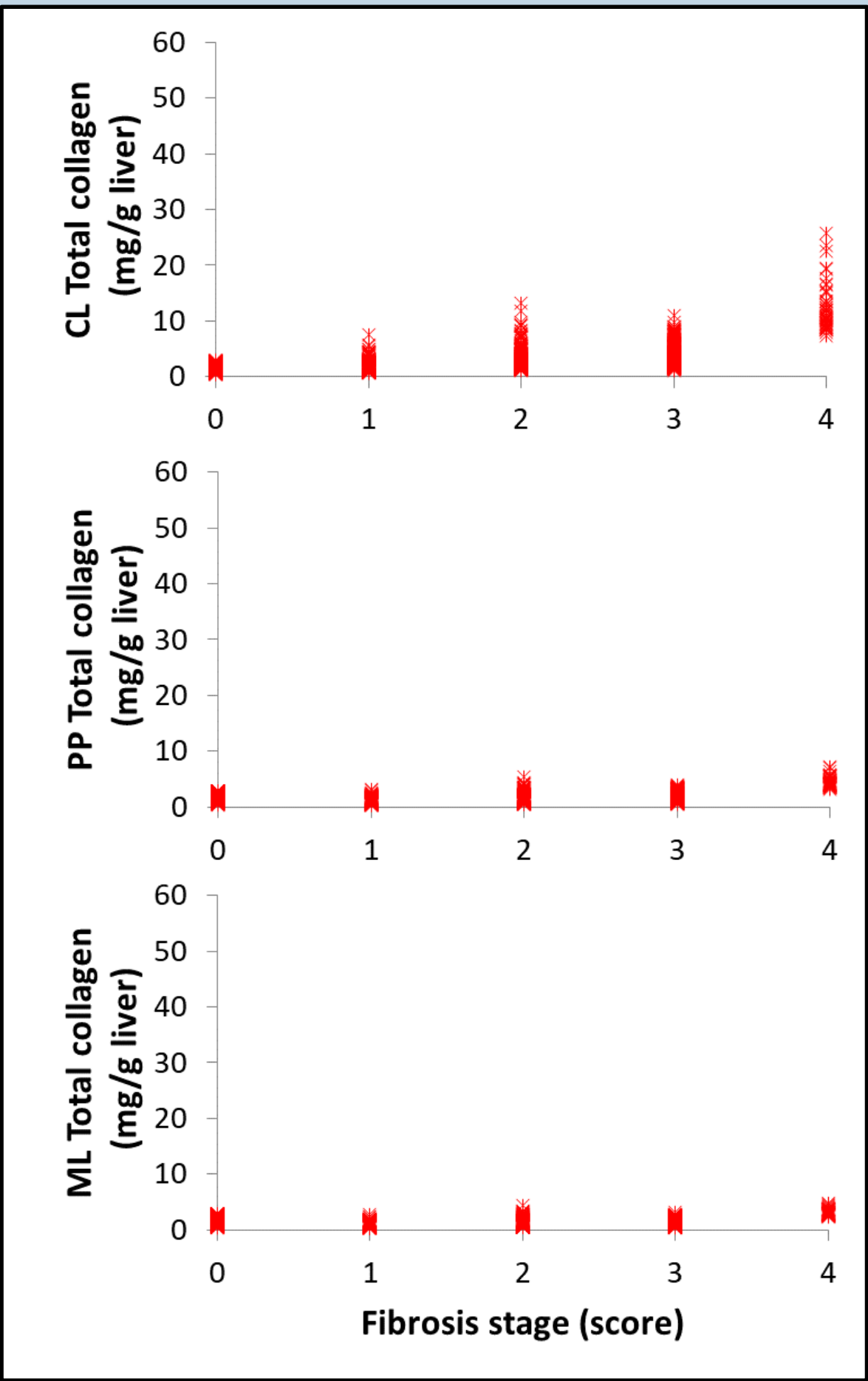


NAFLDsym Simulations Demonstrate Collagen Synthesis Rates and Levels Which are Consistent with Clinical Data



- Consistent with clinical data showing increased collagen synthesis rates in NASH patients, simulated rates of collagen I synthesis are greater in higher fibrosis stages [7].
- Rates of collagen III synthesis (not shown) are also predicted to be greater with higher fibrosis stages. However, clinical data on collagen III synthesis in NASH patients is not yet available for comparison.
- Simulated hepatic collagen I (shown) and III (not shown) levels are comparable in fibrosis stages 0, 1, and 2, consistent with clinical data showing collagen levels in NASH patients [8].
- F3 and F4 collagen levels can dramatically exceed collagen levels seen in stages 0 – 2.

Zonal HSC Activation Results in Different Collagen Levels Across Liver Acinus



Increasing K_m of
HSC activation

CL

PP

ML

- Histological scoring of hepatic fibrosis is based on the spatio-temporal pattern of collagen accumulation, with collagen first appearing in the centrilobular region, followed by accumulation in the periportal region of the acinus, and finally with the collagen bridging the centrilobular and periportal region as it accumulates in the midlobular region.
- NAFLDsym simulations accurately re-capitulate the zonal accumulation of collagen during progression of fibrosis.
- Simulated differences in zonal collagen accumulation observed between the centrilobular, periportal, and midlobular regions are driven by different K_m values for TGF-β induced activation of hepatic stellate cells across the different zones.

CONCLUSION

The integration of inflammation and fibrosis sub-models into the NAFLDsym QSP model accurately captures disease progression and zonal patterns of collagen accumulation in NASH-driven hepatic fibrosis and shows simulation results consistent with non-linear increases in hepatic collagen observed across fibrosis stages in NASH patients. This mechanistic mathematical model is serving as a useful tool to guide development of therapeutics aimed at treating NASH.