Background
Nonalcoholic fatty liver disease (NAFLD) is a chronic condition that originates as lipid accumulation within hepatocytes (steatosis) and progresses into nonalcoholic steatohepatitis (NASH), characterized by lipid accumulation, inflammation, oxidative stress, and fibrosis. NAFLD is now recognized as the most common cause of chronic liver disease, with a prevalence of 25% worldwide, and is projected to become the leading indication for liver transplant by 2035. Despite decades of research, the mechanisms of NAFLD progression and non-invasive diagnoses are still resoundingly absent. The study of steatosis and NASH has traditionally utilized rodent models, which are time consuming to generate and do not fully recapitulate the complex phenotypes associated with the human disease. Furthermore, current 2D cell culture models lack relevant liver cell types and have limited utility due to rapid loss of cell viability and function. Thus, there is a significant need for a more predictive human multicellular 3D in vitro model to study the progression of steatosis into NASH.

Methods
ExVive® Human Liver Tissue, a human in vitro 3D bioprinted liver model comprising primary human hepatocytes, hepatic stellate cells, and endothelial cells, exhibits a complex multicellular architecture similar to that of native liver and retains metabolic competence and liver-specific functions for at least 4 weeks in culture. To mimic the proposed pathogenesis of NASH via a “Two-Hit Hypothesis”, base ExVive Human Liver Tissue was supplemented with Kupffer cells to achieve immune competency then exposed to steatogenic cues via a nutrient overload approach of simple sugars and fatty acids, followed by inflammatory stimulation using prototypical inducers.

Technological Overview
Using the proprietary NovoGen Bioprinter Platform, Organovo builds 3D tissues through automated, spatially-controlled cellular deposits to deliver reproducible native tissue structure and function.

Figure 1: Schematic of bioprinted human liver tissue, comprised of primary human hepatocytes (HC), hepatic stellate cells (HSC), and endothelial cells (EC) bioprinted into a computer-aided geometric scaffold onto the membranes of skewed transwell culture inserts. 3D representative reconstruction image of 3D human liver tissue. [A] ExVive human liver tissue retains structural integrity and hepatocyte viability and function as evidenced by alamarBlue® live/dead activity and CYP3A4 activity. 3D x matched hepatocytes, grown on collagen I coated plates.

Figure 2: A) Schematic of ExVive Human Liver Tissue, comprised of primary human hepatocytes (HC), hepatic stellate cells (HSC), and endothelial cells (EC) bioprinted into a computer-aided geometric scaffold onto the membranes of skewed transwell culture inserts. [B] Representative reconstruction image of 3D human liver tissue. ExVive human liver tissue retains structural integrity and hepatocyte viability and function as evidenced by alamarBlue® live/dead activity and CYP3A4 activity. 3D x matched hepatocytes, grown on collagen I coated plates.

Figure 3: A) ExVive Liver Tissue exhibits sustained hepatocyte function as indicated by alamarBlue® levels versus standard 2D hepatocyte culture, as well as [B] sustained CYP3A4 activity. 3D x matched hepatocytes, grown on collagen I coated plates.

NASH Induction in ExVive Human Liver Tissue

Figure 4: Progression of steatosis to NASH-like inflammation can be observed following co-incubation of hepatocytes with high levels of glucose and fatty acids. See Figure 4 to visualize the histological changes associated with inflammation. [A] Chronic induction occurs in a dose-dependent manner, similar to native tissue samples. [B] Chronic induction reveals increased fibrosis (arrow) regions of increased collagen staining in ExVive liver tissue, typical of changes seen in native liver tissues. [C] Chronic induction reveals increased fibrosis (F = regions of increased collagen staining) in ExVive liver tissue, typical of changes seen in native liver tissues.

Figure 5: Tissues treated with high sugars resulted in increased presence of putative lipid vesicles. H&E staining of PA treated tissues shows both macro- (arrows) and microvesicular steatosis (arrowhead) similar to native tissue samples. [A] Chronic induction reveals increased fibrosis (F = regions of increased collagen staining) in ExVive liver tissue, typical of changes seen in native liver tissues. [B] Chronic induction reveals increased fibrosis (F = regions of increased collagen staining) in ExVive liver tissue, typical of changes seen in native liver tissues.

Figure 6: To mimic the proposed pathogenesis of NASH via a “Two-Hit Hypothesis”, base ExVive Human Liver Tissue was supplemented with Kupffer cells to achieve immune competency then exposed to steatogenic cues via a nutrient overload approach of simple sugars and fatty acids, followed by inflammatory stimulation using prototypical inducers.