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RAPID COMMUNICATION

Hepatology

Parenteral nutrition emulsion inhibits CYP3A4 in an iPSC derived liver organoids testing platform

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Abstract

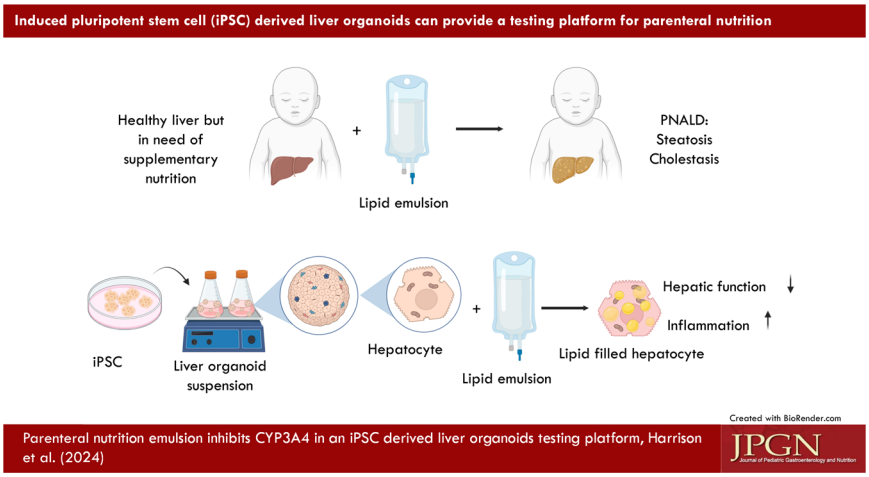
Objectives: Parenteral nutrition (PN) is used for patients of varying ages with intestinal failure to supplement calories. Premature newborns with low birth weight are at a high risk for developing PN associated liver disease (PNALD) including steatosis, cholestasis, and gallbladder sludge/stones. To optimize nutrition regimens, models are required to predict PNALD.

Methods: We have exploited induced pluripotent stem cell derived liver organoids to provide a testing platform for PNALD. Liver organoids mimic the developing liver and contain the different hepatic cell types. The organoids have an early postnatal maturity making them a suitable model for premature newborns. To mimic PN treatment we used medium supplemented with either clinoleic (80% olive oil/20% soybean oil) or intralipid (100% soybean oil) for 7 days.

Results: Homogenous HNF4a staining was found in all organoids and PN treatments caused accumulation of lipids in hepatocytes. Organoids exhibited a dose dependent decrease in CYP3A4 activity and expression of hepatocyte functional genes. The lipid emulsions did not affect overall organoid viability and glucose levels had no contributory effect to the observed results.

Conclusions: Liver organoids could be utilized as a potential screening platform for the development of new, less hepatotoxic PN solutions. Both lipid treatments caused hepatic lipid accumulation, a significant decrease in CYP3A4 activity and a decrease in the RNA levels of both *CYP3A4* and *CYP1A2* in a dose dependent manner. The presence of high glucose had no additive effect, while Clinoleic at high dose, caused significant upregulation of interleukin 6 and TLR4 expression.

Sean P. Harrison and Saphira F. Baumgarten contributed equally to this study.



KEYWORDS
cholestasis, induced pluripotent stem cells, lipogenesis, PNALD

1 | INTRODUCTION

Patients of varying ages with intestinal failure can present with malabsorption, leading to micronutrient deficiencies, to combat these calories are delivered intravenously through parenteral nutrition (PN).[1,2] Unfortunately some patients develop PN associated liver disease (PNALD), a liver dysfunction caused by PN use.[3] There are three types of PNALD, including steatosis, cholestasis, and gallbladder sludge/stones. Premature new-borns with low birth weight are at a special risk for PNALD.[1] Necrotizing enterocolitis, believed to be driven by both dietary and bacterial factors, is a serious gastrointestinal disease in neonates.[4,5] Infants who experience this often have to undergo surgery for small-bowel resection or proximal jejunostomy, and depend longer on PN,[6] increasing the chances of developing PNALD. In addition to PN treatment time, its composition has been related to complications,[7] in regard to the source of fatty acids and the delivery.[2] Mouse models are often used to study PN effects,[8–10] even though there are differences between mice and humans which have to be taken into account when testing PN, for example, humans express four, and mice express six cytochrome P450 3 A (CYP3A) isoforms.[11] This is very relevant since CYPs are involved in the metabolism of 70–80% of all clinically used drugs.[12,13] At the same time, access to human primary hepatocytes is limited, combined with the inability to culture human primary hepatocytes long term,[14] resulting in loss of metabolic function over time.[15,16] Even though it is possible to expand liver progenitor cells in vitro, these hepatocytes do not recapitulate the complexity of an organ.[17]

Our group has successfully established a protocol to differentiate human induced pluripotent stem cells (iPSCs) to liver organoids (hLOs).[18] A strength of iPSC technology is the ability to propagate the stem cells almost indefinitely[19] and they do not have the ethical baggage associated with embryonic stem cells.[20] Our protocol is scalable, is growth factor and extracellular matrix independent thus providing a low cost solution for the production of complex liver organoids that exhibit liver like functions.[18] These hLOs are especially suitable for the investigation of PN associated effects because they display hepatic cellular diversity, including the presence of hepatocytes, hepatic stellate cells, Kupffer cells, and endothelial cells, recapitulating the

What is Known

- Infants receiving parenteral nutrition (PN) are at risk of developing PN associated liver disease (PNALD) which include steatosis, cholestasis, and gallbladder sludge/stones.
- Composition of lipid emulsions affect risk of PNALD.

What is New

- Induced pluripotent stem cell (iPSC) derived liver organoids can provide a testing platform for PN.
- High concentrations of both intralipid and clinoleic decreased Cytochrome P450 3A4 (CYP3A4) activity.
- High concentrations of intralipid and clinoleic reduced the expression of *CYP1A2* and *CYP3A4* RNA.
- High concentrations of clinoleic caused significant upregulation of interleukin 6 expression.

cellular complexity of the organ. They express glutamine synthetase, which is absent from foetal hepatocytes, indicating that our organoids are in a developmental stage past the fetal liver. Importantly, they have both basal and inducible CYP1A2 and CYP3A4 making them relevant for drug testing.[21-23] The aim of this study was to assess the utility of hiPSC derived hLOs to model PNALD. To achieve this we tested the PNs intralipid (Fresenius Kabi), 100% lipid, derived solely from soybean oil, and clinoleic (Baxter), a lipid emulsion composed of olive oil (80%)/soybean oil (20%).[24] We assessed a number of parameters which included lipid droplet accumulation (steatosis), effect of PN on CYP activity and transcription, viability of the organoids under different lipid concentrations, high glucose and assessment of proinflammatory markers.

2 | METHODS

2.1 | Pluripotent stem cell culture

The human iPSC line AG27 (reprogrammed using retrovirus from AG05836B fibroblasts, obtained from Coriell Cell Repositories) was used for the experiments.[25,26] Human iPSCs were maintained under feeder free conditions on geltrex (Life Technologies) or recombinant vitronectin (Life Technologies) coated tissue culture plates using Essential 8 medium made in house as described previously.[27]

2.2 | Liver organoid differentiation

Liver organoids were differentiated based on the protocol from Harrison et al.[18]

2.3 | Lipid emulsion treatment of liver organoids

Organoids were cultured in L15 medium[18] with either 0.45 g/L (low) or 4.5 g/L (high) glucose or no addition. L15 medium was also supplemented with either clinoleic or intralipid with both high and low glucose. Organoids were maintained under these conditions for 7 days in nonadherent six-well plates on a shaker at 70 rpm in an incubator at 37°C and 5% CO_2, with medium changes every 48 h. The calculation for the amount of lipid supplement is based on a 2000 g neonate with 160 mL of blood. For clinoleic a rate of 0.05 mL per minute for the first 10–30 min with 200 mg/mL concentration in the infusion bag, equals 300 mg of lipids administered in first 30 min, meaning 1.875 mg/mL lipid concentration in the newborn.[28] When extrapolated to cell culture this gives 0.94% lipids in the medium. The concentration of intralipid is 200 mg/mL and 200 mg per minute administered in the first 30 min, the final blood concentration is 12.5 mg/ml in the newborn[29] resulting in 6.25% of lipid in the cell culture medium. Based on this we used 0.94%, an intermediate concentration of 3.5% and 6.25% to show the effects of lipid concentration on the liver organoids.

2.4 | CYP3A4 activity assay

After 7 days of culture in the lipid emulsions, the organoids were collected and washed-twice with L15 differentiation medium before resuspension in a half volume of the same, samples were then divided into four technical replicates. The Promega P450-Glo™ CYP3A4 Assay with Luciferin-IPA (V9002) was performed according to the manufacturer's instructions. Wells without organoids were measured to determine background levels of detection and were subtracted from the wells with organoids.

2.5 | CYP1A2 activity assay

After 7 days of culture in the lipid emulsions, the organoids were collected and washed with L15 differentiation medium before resuspension in a half volume of PBS with magnesium and calcium, containing 3 mM salicylamide, samples were then divided into four technical replicates. The Promega P450-Glo™ CYP1A2 Assay (V8421) was performed according to the manufacturer's instructions. Wells without organoids were measured to determine background levels of detection and were subtracted from the wells with organoids.

2.6 | Immunofluorescence

Samples were fixed at room temperature for 45 min with 4% PFA. 10% Goat serum (G9023, Sigma) was used for blocking in PBS with 0.1% Triton (PBS Triton) for 1 h, and incubation with primary antibody in 5% goat serum in PBS Triton over night at 4°C. The samples were washed three times for 10 min in PBS Triton. The secondary antibody diluted in 1% goat serum (G9023, Sigma) in PBS Triton was added for 1 h at 37°C and washed for 1 h afterwards in PBS Triton. For a list of all antibodies, see Table S1.

2.7 | Bodipy staining and live imaging

After 6 days of culture in the different lipid emulsions, a 0.5 mL aliquot of suspension was taken, washed

three times in L15 differentiation medium and then incubated in a final concentration of 3.8 mM BODIPY 493/503 (Thermofisher, D3922) for 30 min in the cell culture incubator. Organoids were then washed three more times in L15 differentiation medium and one drop of NucBlue DNA counterstain (Thermofisher, R37605) was added. Cells were then imaged on an Andor Dragonfly spinning disk confocal microscope.

2.8 | Reverse transcription quantitative real time polymerase chain reaction (RT-qPCR) gene expression analysis

Organoids were collected in TRI Reagent® (T9424, Sigma-Aldrich) and RNA was isolated according to the manufacturer's instructions and RNA was quantified using the NanoDrop ND-1000 Spectrophotometer System (NanoDrop). One microgram of RNA was converted to cDNA using the High-capacity cDNA Reverse Transcription kit (Life Technologies). Commercially available validated TaqMan™ gene expression assays were used with the BioRad SsoAdvanced Universal Probes Supermix (#1725284). The RT-qPCR was performed using TaqMan Reagents (Life Technologies) with the only modification being that the reaction volume was reduced from 20 to 15 µL. In all cases a minimum of four biological replicates were analyzed using 5 ng of cDNA per reaction. In all cases three technical replicates were performed for all samples/genes. In all cases gene expression was normalized to (beta-actin) ACTB. Data are presented as the average of four independent experiments ± the standard deviation. All TaqMan™ gene expression assays are presented in Table S2.

2.9 | Statistical analysis

Analysis was carried out using Graphpad Prism (version 10.1). Statistical significance was determined using ordinary one-way analysis of variance and Tukey's and Dunnett's multiple comparison tests to compare means. Stars delineate significant results as shown: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

2.9.1 | Ethical approval information

The human iPSCs line AG27, is registered on the hPSCreg-UIOi001-A. The parental fibroblast line AG05836B was obtained from the Coriell Cell Repositories and reprogramed into hiPSCs and characterized as previously described in.[30] All ethical approvals are in place.

3 | RESULTS

3.1 | Assessing if PN impinges liver organoid function

To investigate whether liver organoids could be utilized as a testing platform for PN, we first generated iPSCs derived hLOs. We validated the hLOs by staining with HNF4a (hepatic nuclear factor 4) which is expressed in the hepatocytes (Figure 1A). Next, the hLOs were treated with two commercial lipid solutions (intralipid and clinoleic), at either 0.94% or 6.25% final concentration in the media. First, we explored if lipid droplet accumulation occurred in hLOs after 6 days treatment. We observed a dose dependent accumulation of lipid under both treatments as compared to control (Figure 1B). Next, the hLOs were either cultured in maintenance medium (L15 for control organoids) or L15 supplemented with either clinoleic or intralipid, at either 0.94% or 6.25%. After 7 days treatment, RNA was isolated, and RT-qPCR performed. Of note, the hLOs varied in size (Figure 1A), but in all experiments the control organoids (L15) expressed *HNF4a*, *albumin*, *SERPINA* (alpha-1 antitrypsin), and *AT3* (*SERPINC*/antithrombin) at comparable levels (Figure 1C). On treatment with both clinoleic and intralipid, the gene expression levels at the 0.94% treatment group saw a marginal change in levels of *HNF4a* and *A1AT* but these were not significant while a small but significant decrease in expression of *ALB* and *AT3* where observed (Figure 1C). However, at the higher concentration of lipid treatment (6.25%) we observed significantly decreased gene expression levels in all groups, *HNF4a, ALB*, and *A1AT and AT3* (Figure 1C). Next, we wanted to ensure that the observed decrease in gene expression was not a function of cell death/loss of viability. So, we looked at total ATP to assess the effect of the PN treatments on cell viability. Interestingly, we observed no significant changes between the control and all PN treatment groups (Figure 1D). PN is also supplemented with glucose along with other trace element, with supplementation of up to 18 g/kg being tolerated.[31] We investigated the effect of high glucose alone and in combination with the PN groups on cell viability. Again, we observed no significant changes in cell viability, indicating that the treatment had no impact on cell viability (Figure S1).

3.2 | Do lipid emulsions influence de novo lipogenesis (DNL)?

DNL plays an essential role in low-birth-weight infants because they have a high energy demand for growth which is met either with triglycerides from diet or from DNL.[32] Moreover, lipid emulsion composition might

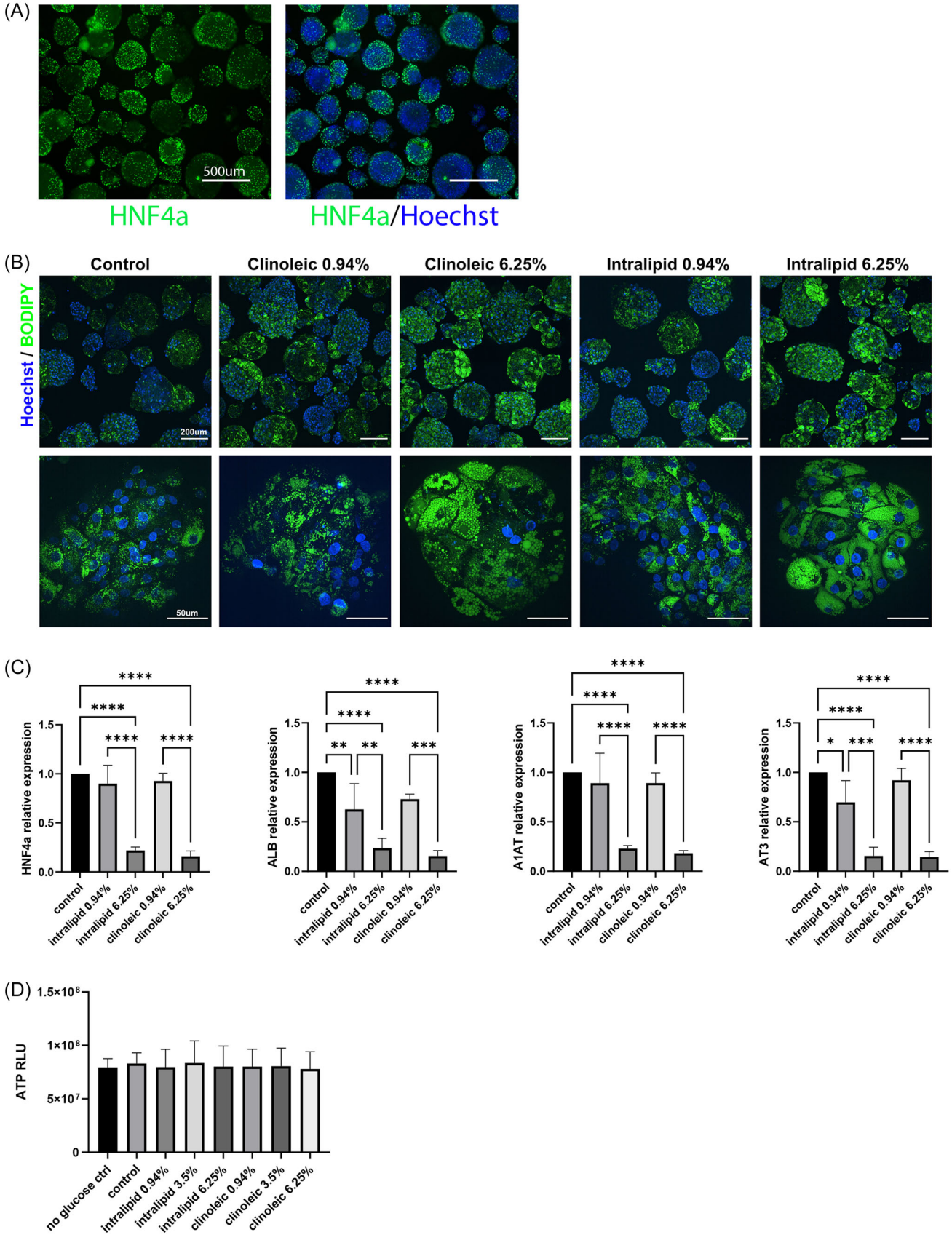


FIGURE 1 (See caption on next page).

influence lipogenesis[33] and we wanted to assess whether our organoid system was suitable for studying the effect of lipid emulsions on DNL. We assessed markers of DNL with RT-qPCR; In active sites of DNL, the transcription factor *MLXIPL*/*CHREBP (*MLX Interacting Protein Like/carbohydrate-responsive element-binding protein*)* is expressed[34] and we observed upregulation in the 0.94% PN treatment conditions (Figure 2A). However, on challenge with the high PN treatments (6.25%), we observed a significant down regulation of *MLXIPL*/*CHREBP*. Among the target genes of *MLXIPL*/*CHREBP* are fatty acid synthase (*FASN*), stearoyl CoA desaturase-1 gene (*SCD-1*), and ATP-citrate lyase (*ACLY*).[35] Interestingly, these DNL markers were not significantly affected by any of the treatments, apart from *ACLY* where we observed an increase in expression (Figure 2A). We also assessed mRNA levels of the scavenger receptor *CD36*,[36] it showed an upwards trend but was not significant (Figure 2B).

3.3 | Effect of PN on the cytochrome p450s

PN has been studied for its effect on cytochrome p450s (CYP), for example, cholesterol 7a-hydroxylase (CYP7A1) was found to be suppressed in infants

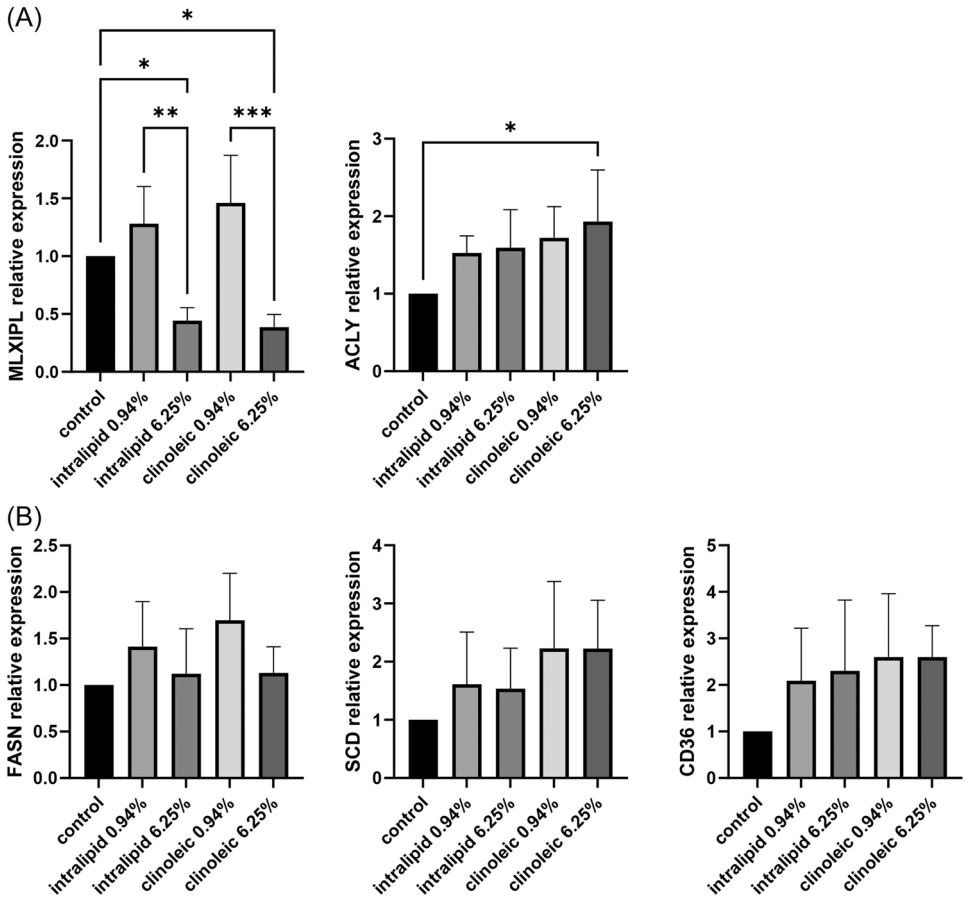


FIGURE 2 Effects of parenteral nutrition treatment on de novo lipogenesis in liver organoids. (A) RT-qPCR of de novo lipogenesis markers, *MLXIPL*, *ACLY*, and (B) *FASN*, *SCD* and *CD36* after 7 days of PN treatment. Normalized to *B-actin*, $n = 4$ independent experiments. Error bars show standard deviation. p Value, *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$), ****($p < 0.0001$).

FIGURE 1 Effects of parenteral nutrition on hepatocyte marker expression and cell viability. (A) Immunostaining of liver organoids before PN treatment. HNF4a in green, Hoechst in blue. 500 µm scale bar. (B) Intracellular fat deposition in liver organoids after PN treatment. Live imaging of BODIPY 493/503 staining (green) and Hoechst (blue), showing neutral lipids and nuclei respectively, in organoids treated with 0.94% or 6.25% intralipid or clinoleic acid for 6 days. Bottom row shows higher magnification. Scale bar represents 200 µm in the top row and 50 µm in the bottom row. (C) RT-qPCR of hepatocyte gene expression markers after parenteral nutrition (PN) treatment for 7 days with either clinoleic or intralipid in comparison to untreated/control (L15). *HNF4a*, *albumin* (*ALB*), *alpha -1-antitrypsin* (*A1AT*), and *antithrombin* (*AT3*) normalized to *B-actin*, $n = 4$ independent experiments. (D) ATP measured in organoids after PN treatment for 7 days, $n = 4$ independent experiments. Error bars show standard deviation. p value, *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$), ****($p < 0.0001$).

requiring prolonged PN.[37] In a mouse study intralipid was shown to reduce the expression of genes involved in drug metabolism, including *Cyp3a11*, (homolog to human cytochrome P450 Family 3 Subfamily A Member 4 (CYP3A4)). Consequently, we explored the effects of PN treatment on CYP450 gene expression and activity in liver organoids. Organoids were treated with either intralipid or clinoleic (0.94% or 6.25%) for 7 days. We first looked at the gene expression level of *CYP3A4* and *CYP1A2*, where we observed a significant reduction in the gene expression level of both CYPs at the highest concentration (6.25%), but at the lower concentration (0.94%) we a saw significant reduction in the *CYP3A4* and *CYP1A2* RNA levels with clinoleic treatment, but intralipid only gave a significant reduction in CYP3A4. However, the trend for intralipid (0.94%) treated hLOs for *CYP1A2* RNA levels was down but not significant (Figure 3A). We next assessed the CYP activity, first CYP3A4 activity was assayed at Day 7 post-treatment with either intralipid or clinoleic (0.94%, 3.5%, or 6.25%) (Figure 3B). The CYP3A4 activity was significantly decreased at the highest concentration (6.25%) with an overall downward trend in a dose dependent manner, albeit not statistically significant at the lower concentrations. We observed a similar trend for CYP1A2 activity with the same treatments but the observed changes between control and PN treatments groups were not significant (Figure 3B). We also assessed the effect of high glucose (PN is also supplemented with high glucose) applied to the same lipid formulations described above on CYP1A2 and CYP3A4 activity. In short, we observed the same pattern as above with significant inhibition of CYP3A4 activity at the highest PN concentration (6.25%) and an overall downward trend in a dose dependent manner (Figure S1). While for CYP1A2 we observed the same trend as above of reduced CYP1A2 activity, but this was not statistically significant (Figure S1).

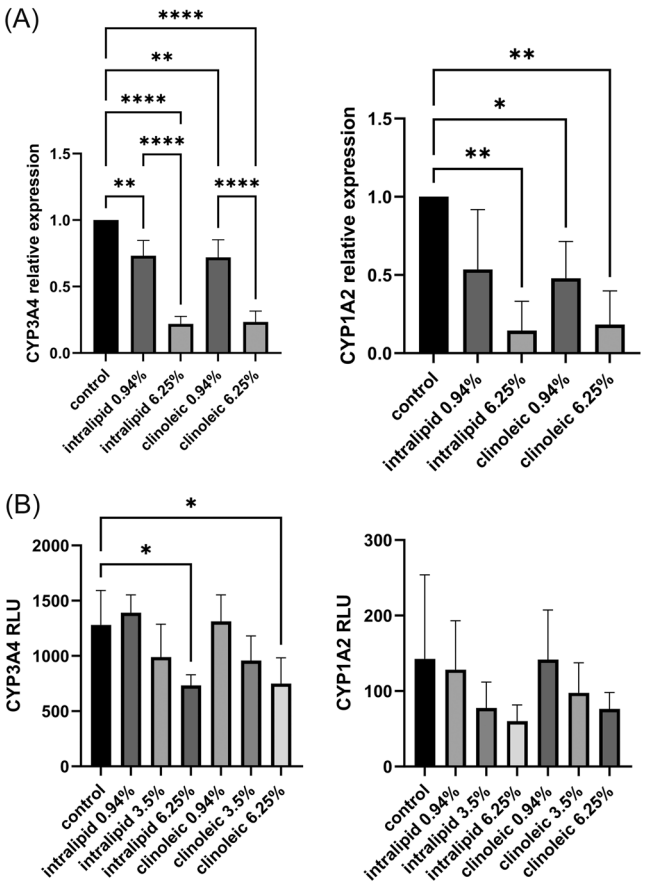


FIGURE 3 Effects of parenteral nutrition treatment of liver organoids on CYP450 gene expression and activity. RT-qPCR of (A) *CYP3A4* and *CYP1A2* gene expression in liver organoids treated with 0.94% or 6.25% intralipid or clinoleic for 7 days in comparison to untreated control organoids. Normalized to *B-actin*, $n = 4$ independent experiments. Error bars show standard deviation. p Value, *($p < 0.05$), **($p < 0.01$), **($p < 0.001$), ****($p < 0.0001$). (B) Luminescence based CYP450 assays showing CYP3A4 and CYP1A2 activity levels in the liver organoids treated with 0.94%, 3.5%, or 6.25% intralipid or clinoleic for 7 days in comparison to untreated control organoids. $n = 4$ independent experiments. Error bars show standard deviation. p Value, *($p < .05$), **($p < 0.01$), ***($p < 0.001$), ****($p < 0.0001$).

3.4 | PN effect on inflammation and fibrosis

It is well acknowledged that lipids can invoke an inflammatory response in the liver and other tissues.[38] To address this, we investigated if PN formulas applied to hLOs had any effect on inflammatory or fibrotic markers. First, we looked at the toll-like receptor 4 (*TLR4*) and interleukin 6 (*IL6*) levels of expression, where we observed an upward trend in expression for all treatment, but was not statistically significant, however the 6.25% clinoleic treatment we observed the significant upregulation of both *TLR4* and *IL6* expression (Figure 4A,B and Figure S2). We also assessed several fibrotic markers including smooth muscle actin (*aSMA*), collagen 1A1 (*COL1A1*), and Transforming growth factor beta (*TGF-b*), however in all cases we did not observe any significant changes in expression for any of the test regimes (Figure 4C–E).

4 | DISCUSSION

PNALD is a severe complication after PN in infants and in the efforts to optimize PN relevant testing, new models are required. Using hiPSCs derived hLOs we demonstrated that PN treatment with clinoleic or intralipid did not affect cell viability even with high glucose, indicating that our calculated physiologically relevant PN concentrations are tolerated by the hLOs (Figure 1D). We also observed accumulation of lipid

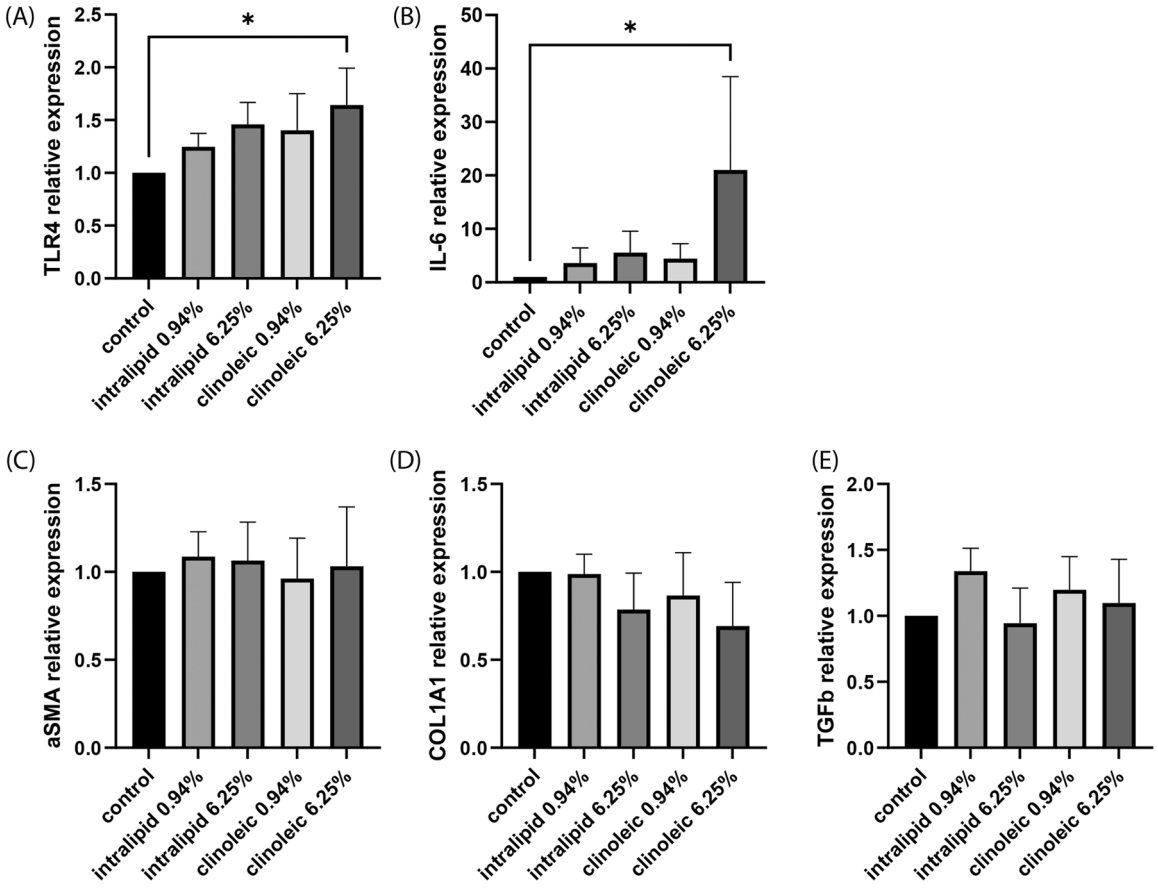


FIGURE 4 Effects of parenteral nutrition treatment of liver organoids on markers of inflammation and fibrosis. RT-qPCR of (A) *IL6*, (B) *TLR4*, (C) *aSMA*, (D) *COL1A1*, and (E) *TGFb* gene expression in liver organoids treated with 0.94% or 6.25% intralipid or clinoleic for 7 days in comparison to untreated control organoids. Normalized to *B-actin*, $n = 4$ independent experiments. Error bars show standard deviation. p Value, *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$), ****($p < 0.0001$).

droplets in hLOs in a dose dependent manner, presenting with a nonalcoholic fatty liver disease (NAFLD) like appearance (Figure 1B). Additionally, we observed a dose dependent decrease in the expression of the hepatic markers *HNF4a*, *ALB*, *A1AT* and *AT3*. This has been observed in a number of models, including rabbit under a high fat diet, where albumin mRNA levels are reduced significantly.[39] In addition, *HNF4a*, a master regulator of liver metabolism, is down regulated in fatty liver diseases such as NAFLD and diabetes.[40–42] This may account for the decrease in *A1AT* and *AT3* expression, which has been shown to regulate the expression of these factors.[43,44]

The liver plays a role in the regulation of lipid metabolism/metabolic function and disruption of these can lead to obesity, metabolic syndrome and NAFLD,[45-51] making the analysis of DNL and regulation of lipid metabolism important, as fat infiltration in the liver is implicated in the onset of PNALD. On assessment of DNL, we observed that 0.94% PN treatments marginally increased *MLXIPL*/*CHREBP* levels, but this was not significant. Contrary, reduced expression was observed for high dose 6.25% PN treatment, for both intralipid and clinoleic. It has been shown that diminishing MLXIPL, reduced lipid accumulation.[52] One could speculate that this maybe a feedback loop to antagonize the observed lipid accumulation. We found a dose dependent increase in expression of *ACLY* in keeping with the induction of *ACLY* by free fatty acid.[53] For *FASN, SCD*, and *CD36*, we did not observe any significant increase in expression. This is in alignment with the diminishing *MLXIPL* levels, as MLXIPL has been shown to regulate genes involved in DNL such as *FASN* and *SCD1*.[54]

There are links connecting liver disease with DNL and changes in CYP450 activity. Reduced CYP3A4 protein expression has been observed in NAFLD and diabetes mellitus.[21,55] We investigated how two key CYPs (1A2 and 3A4) were affected by PN treatments at the transcriptional level, observing PN treatment dose dependently reduced mRNA levels. CYP3A4 activity was significantly affected at the high lipid concentration (both PN treatments at 6.25%). While CYP1A2 activity showed a downward trend in activity, but not statistically significant. Interestingly, a study investigating how high fat diets in mice effected the drug metabolizing

enzymes (*Cyps*) observed reduced Cyp1a2 but unfortunately the activity levels were not assessed.[56] The consequences of lowered CYP3A4 activity can be severe in newborns, as it has not yet reached adult levels, taking 3 years to do so.[57–59] Therefore, drugs administered and metabolized by CYP3A4 might not have the intended effect i.e., prodrugs will have delayed activity due to lower CYP3A4 activity. Another potential problem is the genetic background of a patient and the expression of other CYPs, for example, CYP3A5. It can represent up to 50% of total hepatic CYP3A protein,[60,61] but it is only detected in the livers of 10–30% of adult Caucasians due to genetic polymorphism.[62–66] A reduction of CYP3A4 activity could be deleterious in a combination with the absence of CYP3A5.

Additionally down regulation of CYP3A4 can have serious consequences, for liver health in association with cholestasis.[67,68] CYP3A4 catalyses the hydroxylation of bile acids, thereby increasing the hydrophilicity and lowering toxicity.[68] As a response to cholestasis CYP3A4 is up regulated[69] to fulfill its role in bile acid detoxification, but bile acids can directly inhibit CYP3A4 due to their detergent effect, creating a vicious circle. Cholestasis has been observed in infants (40–60%) who receive long-term PN,[70] which can progress to cirrhosis and liver failure.[3]

We observed that 6.25% clinoleic treatment caused significant upregulation of *TLR4 and IL6* in the hLOs, however the other PN treatment groups caused upregulation, but was not significant (Figure 4A,B). This is not unexpected as TLR4 signaling through NFkB and MAPK leads to the production of proinflammatory cytokines such as IL-6.[71] Also, this could contribute to the observed down regulation of CYP3A4 as CYPs are down regulated in response to inflammation.[72] Interestingly, other cytokines affect the expression of other CYPs, in the case of CYP1A2 TNF-alpha was shown to be very potent.[73] This would be an interesting avenue to explore, with regard to cytokine profiling and the effect on expression of CYPs.

IL6 is also presumed to be a proinflammatory cytokine contributing to obesity-driven insulin resistance[74,75] and a regulator of immune function.[76] However, another role for IL6 is emerging as a potent regulator of fat metabolism in adipose and muscle tissues.[77,78] But paradoxically increased plasma levels of IL6 correlate with metabolic syndrome.[74,79,80] A study by Vida and colleagues,[81] show the levels and duration of IL6 are important. For example, a moderate increase in IL6 maintains fatty acid β-oxidation, balancing the increased accumulation of liver fatty acids. But high levels of IL6, associated with obese patients, promote lipogenesis and further steatosis/NAFLD progression. The differences in the levels of IL6 expression under intralipid and clinoleic PN treatments may have important implications to patient outcome.

Infants who receive long-term PN treatment can progress to cirrhosis and liver failure.[3] However, in our hLO model, we did not observe elevation of fibrotic markers. This is contrary to a study from Fitzgibbons and colleagues,[82] using liver biopsies from children undergoing PN, where they observed PN-associated liver fibrosis. However, these patients had undergone PN for 4.7 months (median duration). Our results can be explained by the duration of treatment, as another study showed PN treatments of <2 weeks presented with no fibrosis, requiring 6 weeks treatment to develop moderate-to-severe fibrosis.[83] This warrants further investigation in the context of acute (days) and chronic (weeks) PN treatment in hLOs.

5 | CONCLUSION

A limitation of this study is that we only assessed the effect of different intravenous lipid formulas and high glucose, which had no contributory effect. But under clinical use these lipid emulsions are co-administered with a plethora of other nutritional and non-nutritional components, including carbohydrates, amino acids, polyvalent electrolytes as well as prescription drugs. This is worthy of further future exploration to understand the impact of these.

Our hLO model offers the possibility to investigate how PN impacts overall liver functions and activities such as the drug metabolizing enzymes. This potentially will aid how prescription drugs are administered in the context of CYP activity. Our model will potentially allow a more personalized approach, for example by generating hiPSC derived hLOs with different CYP variants and assessing their activities under different PN treatments. Another interesting area to further develop is cytokine profiling upon PN treatment. This would impact at several levels, including CYP activity assessment, as well as the potential beneficial effects of IL6 levels in the context of promotion of fatty acid β-oxidation, thus addressing increased lipid accumulation. Our model could be fine-tuned to identify the sweet spot in PN delivery in the context of lipid levels that promote moderate levels that are beneficial to the patient. Finally, as we can culture our hLOs long term, for many months,[18,84] making it possible to study long term PN treatments and the development of fibrosis. In short, the described hLO model will aid the development of safer PN treatments.

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CONFLICT OF INTEREST STATEMENT

The author declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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