Exploring anti-fibrotic drugs

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Chapter B1

Pathophysiological model of non-alcoholic fatty liver disease using precision-cut liver slices

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Manuscript in preparation

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Abstract

Background and purpose

Current *in vitro* non-alcoholic fatty liver disease (NAFLD) models fail to capture the essential interactions between liver cell-types and do not reflect the pathophysiological status of NAFLD patients. Precision-cut liver slices (PCLS) are an effective *ex vivo* model to study multicellular diseases, since the interplay between liver cell-types is maintained. Therefore, this study aimed to develop PCLS to study NAFLD.

Experimental approach

Steatosis was induced by culturing rat PCLS in modified culture media which contained pathophysiological serum concentrations of glucose, fructose, insulin and palmitic acid as seen in NAFLD patients, for up to 48h. The steatosis was evaluated using Oil Red O staining. Furthermore, expression of carbohydrate/lipid metabolism-related genes was assessed by quantitative real-time PCR.

Key results

Liver steatosis, characterized by intracellular microvesicular lipid droplets, was successfully induced in PCLS cultured in medium containing insulin. The microvesicular steatosis was in line with the down-regulation of genes encoding carnitine palmitoyltransferase 1 (*Cpt1*) which plays a role in fatty acid transport and mitochondrial β-oxidation. On the other hand, the up-regulation of markers of *de novo* lipogenesis: acetyl-CoA carboxylase 1 (*Acc1*), carbohydrate responsive element binding protein (*Chrebp*) and sterol regulatory element binding protein 1c (*Srebp-1c*), was not always paralleled by steatosis observed in the PCLS.

Conclusion and implications

Steatosis can be induced in PCLS using modified culture media, which reflects the pathophysiological development of NAFLD. The disruption of fatty acid transport and mitochondrial β-oxidation are probably the main pathways responsible for the accumulation of microvesicular steatosis in our PCLS model. This novel pathophysiological *ex vivo* model can be used as an effective tool to study NAFLD-associated conditions and test the efficacy of anti-NALFD drugs.

Key words: NAFLD; non-alcoholic fatty liver disease; *ex vivo*; pathophysiological model.

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; VLDL, very low-density lipoprotein; PCLS, precision-cut liver slices; BSA, bovine serum albumin; Ywhaz, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta; Acc, acetyl-CoA carboxylase; Srebp, sterol regulatory element binding protein; Chrebp, carbohydrate responsive element binding protein; Cpt, carnitine palmitoyltransferase.

Introduction

Non-alcoholic fatty liver disease (NAFLD), or hepatic steatosis, is characterized by excessive deposition of lipids in the liver in the absence of excessive alcohol intake [1, 2]. Although NAFLD, the most common liver disorder worldwide, may be benign, chronic and unresolved liver steatosis can progress to non-alcoholic steatohepatitis (NASH), cirrhosis and even hepatocellular carcinoma [1-3]. Furthermore, the clinical burden of NAFLD is not only confined to liver-related problems, uncontrolled NAFLD can aggravate extra-hepatic diseases such as diabetes mellitus, cardiovascular disease and chronic kidney disease [4]. To date, no NAFLD- or NASH-specific pharmacological interventions are clinically approved, and the only well-established option for the management of NAFLD are life-style modifications [5, 6].

The biggest risk factors for NAFLD are metabolic disorders, in particular hyperglycemia and insulin resistance [7-11]. Chronic hyperglycemia and hyperinsulinemia disrupt the systemic physiological regulation of carbohydrate/lipid metabolism leading to the development of NAFLD [10-12]. Recently, several studies have shown that fructose also plays a role in the pathogenesis of hepatic steatosis [13-15]. Excessive intake of fructose alters carbohydrate/lipid metabolism in the liver, adipose tissue, gastrointestinal tract and nervous system [16]. In the liver, fructose activates *de novo* lipogenesis and increases very low-density lipoprotein (VLDL) synthesis, which ultimately contributes to the accumulation of lipid droplets [15]. Besides dysglycemia, dyslipidemia is an important risk factor in the development of NAFLD [9]. Fatty acids directly promote lipotoxicity and play a role in the progression to NASH [17, 18]. The lipotoxicity of fatty acids was frequently illustrated *in vitro* by using palmitic acid, the most abundant saturated fatty acid in the human body [19-21]. Palmitic acid is converted to palmitoyl-CoA in the cytosol before entering the mitochondrial matrix, via the L-carnitine shuttle system, where it undergoes β-oxidation [18]. In addition, disruption of mitochondrial β-oxidation is key in the progression of NAFLD [11, 22].

Currently, several *in vitro* models are used to study the pathogenesis of NAFLD [23, 24]; however, most of them fail to capture the essential interactions between hepatocytes and other cell-types such as hepatic stellate cells and Kupffer cells. In addition, *in vitro* and animal models often do not reflect the pathophysiological status of NAFLD patients [11, 22, 25]. Precision-cut liver slices (PCLS) have been proven to be an ideal *ex vivo* model to study multicellular diseases, since the interplay between the various liver cell-types is maintained [26, 27]. Therefore, we aimed to develop an *ex vivo* NAFLD model using PCLS, by mimicking pathophysiological risks factors in NAFLD patients.

Methods

Animals

Male Wistar rats, 12-16 weeks old, were purchased from Charles River (Sulzfeld, Germany). The experiments were approved by the Animal Ethical Committee of the University of Groningen.

Precision-cut liver slices (PCLS)

PCLS, with an estimated thickness of 250-300 μm, were prepared and cultured under continuous supply of 80% O_2/5% CO_2 up to 48h, as previously described [27]. Culture media was refreshed every 24h.

Modification of culture medium

Williams medium E with Glutamax (Invitrogen, Bleiswijk, the Netherlands) supplemented with gentamycin (50 mg/mL; Invitrogen) was used for PCLS culture. Five different culture media were prepared to mimic the pathophysiological concentrations of glucose (Merck, Darmstadt, Germany), fructose (Merck), human insulin (Sigma, St. Louis, US) and palmitic acid (Sigma) in the serum of NALFD patients (Table 1).

Table 1: Modification of culture medium

Medium	Characteristic	Component and final concentration			
		Glucose	Fructose	Insulin	Palmitic acid
M1	Unmodified	11.1 mM	-	-	-
M2	Additional glucose	25 mM	-	-	-
M3	Additional glucose and fructose	25 mM	5 mM	-	-
M4	Additional glucose, fructose and insulin	25 mM	5 mM	1 nM	-
M5	Additional glucose, fructose, insulin and palmitic acid	25 mM	5 mM	1 nM	240 μM

Palmitic acid solutions were prepared via complexation of palmitic acid with bovine serum albumin (BSA; Sigma). Briefly, palmitic acid was dissolved in 0.1 M sodium hydroxide (Merck) at 70 °C and subsequently mixed with preheated BSA solution at 55 °C. In addition, BSA solution without palmitic acid was added into M1-M4 to mimic the concentration of BSA (0.04%) in M5.

Oil Red O lipid staining

Steatosis was evaluated by Oil Red O staining. Briefly, 4 μm cryosections of PCLS were fixed with 4% formaldehyde/PBS for 10 minutes before staining with Oil Red O solution (0.6% Oil Red O in 36% 2-propanol) for 10 minutes at room temperature. The sections were counterstained with hematoxylin and examined using light microscopy. Three PCLS from each treatment-group were used per experiment. The fat content (fat-ratio) was determined by measuring the amount of lipid droplets (red area) per nucleus (blue area) using ImageJ software (US National Institutes of Health).

Quantitative real-time PCR

Expression of key genes involved in carbohydrate/lipid metabolism was assessed by quantitative real-time PCR. From each experiment, three PCLS were pooled, snap frozen in liquid nitrogen and stored at -80 °C until analysis. Total RNA was isolated using the RNeasy Lipid Tissue Mini Kit (Qiagen, Venlo, the Netherlands). Reverse transcription was performed using the Reverse Transcription System (Promega, Leiden, the Netherlands). Gene expression was determined using specific primers (Table 2) and the SensiMix™ SYBR Hi-ROX kit (Bioline, Luckenwalde, Germany). Quantitative real-time PCR was performed using a 7900HT Real Time PCR apparatus (Applied Biosystems, Bleiswijk, the Netherlands) with 1 cycle at 95 °C/10 minutes followed by 45 cycles of 95 °C/15 seconds, 60 °C/30 seconds and 72 °C/30 seconds. Ct values were corrected for the *Ywhaz* (ΔCt) and compared to medium M1 ($\Delta\Delta$Ct). Results are displayed as fold induction ($2^{-\Delta\Delta Ct}$).

Table 2: Primer sequences used in quantitative real-time PCR.

Acc1 (Acetyl-CoA carboxylase 1)		
	Forward:	GCCATCCGGTTTGTTGTCA
	Reverse:	GGATACCTGCAGTTTGAGCCA
Acc2 (Acetyl-CoA carboxylase 2)		
	Forward:	AATTTGTCACCCGCTTTGGA
	Reverse:	CATACACTTGACCGCAGCGATA
Srebp-1a (Sterol regulatory element binding protein 1a)		
	Forward:	GAGCTACCCTTCGGTGAGG
	Reverse:	CAAATAGGCCAGGGAAGTCA
Srebp-1c (Sterol regulatory element binding protein 1c)		
	Forward:	GGAGCCATGGATTGCACATT
	Reverse:	AGGCCAGGGAAGTCACTGTCT
Chrebp (Carbohydrate responsive element binding protein)		
	Forward:	GAAGACCCAAAGACCAAGATGC
	Reverse:	TCTGACAACAAAGCAGGAGGTG
Cpt1 (Carnitine palmitoyltransferase 1)		
	Forward:	TCTTGCAGTCGACTCACCTT
	Reverse:	TCCACAGGACACATAGTCAGG
Ywhaz (Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta)		
	Forward:	TTGAGCAGAAGACGGAAGGT
	Reverse:	GAAGCATTGGGGATCAAGAA

Statistics

The results are expressed as means ± standard error of the mean (SEM) and compared to the control group using ANOVA followed by Dunnett's post hoc analysis. A *p*-value less than 0.05 was considered significant. Statistical differences were determined on relative value of ATP and ΔCt for mRNA expression.

Results

Liver steatosis

Oil Red O staining revealed an abundance of small-size lipid droplets in PCLS cultured in medium containing additional glucose, fructose and insulin (M4), as well as in PCLS cultured in medium containing additional glucose, fructose, insulin and palmitic acid (M5) at both 24h and 48h (Figure 1). Conversely, lipid-rich droplets were not observed in PCLS cultured in unmodified culture medium (M1), medium containing additional glucose (M2) and medium containing additional glucose and fructose (M3).

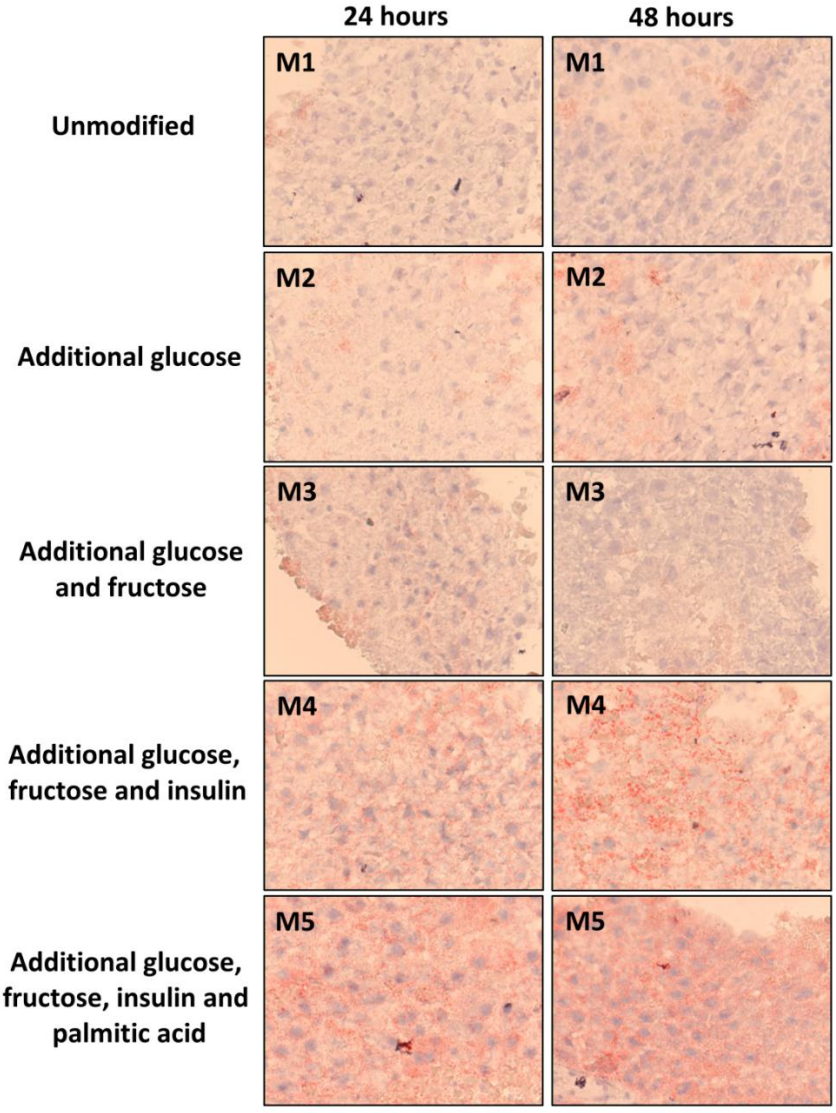

Figure 1: Representative Oil Red O-stained sections of PCLS cultured in modified culture media (400X).

To confirm the microscopic evaluation, liver steatosis was quantified by using a fat-ratio calculation (Figure 2). As shown, after 24h and 48h, the fat-ratio of PCLS cultured in M4 increased with 12.7% and 8.5%, respectively, as compared to M1. Likewise, the fat-ratio of PCLS cultured in M5 was 16.3% and 9.1% when compared to M1 after culturing for 24h and 48h, respectively. It should be noted that the fat-ratio of PCLS cultured in M4 was not statistically different from PCLS cultured in M5.

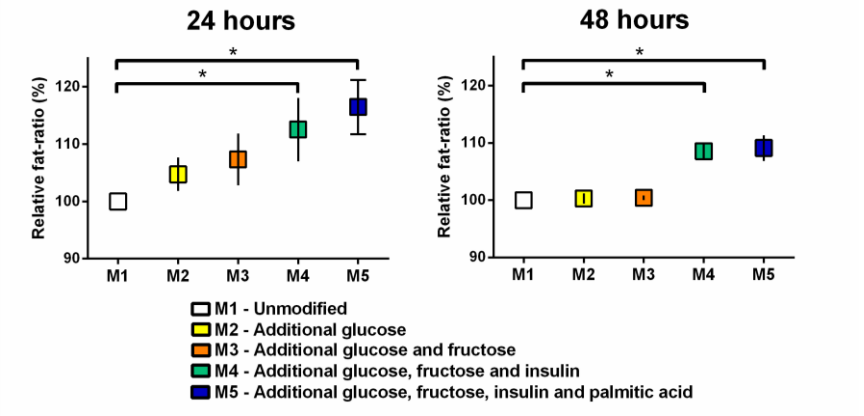

Figure 2: Fat-ratio of PCLS cultured in modified culture media. The fat-ratio was determined by measuring the amount of lipid droplets (red area) per nucleus (blue area) using ImageJ software. Data are expressed as means ± SEM (n=3). **p*<0.05 compared to unmodified culture medium (M1).

Viability

Next, we elucidated whether the induction of steatosis impacted the viability of PCLS (Figure 3). The results showed that the modified media did not influence the viability of PCLS at both 24h and 48h. However, at 48h, the viability of PCLS cultured in M4 was non-significantly reduced compared to control.

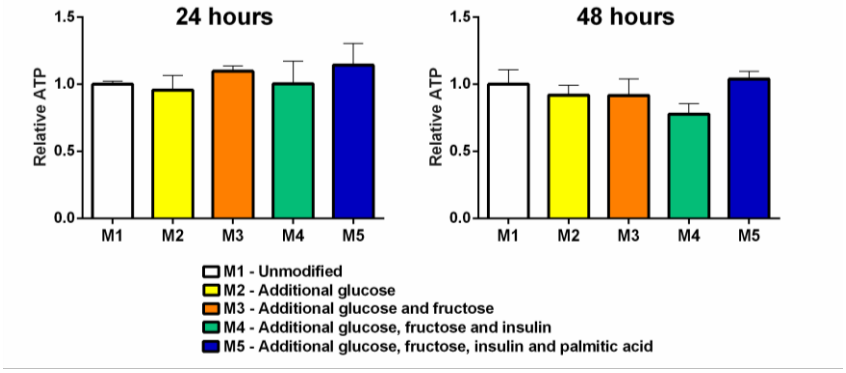

Figure 3: ATP level/protein of PCLS cultured in modified culture medium at 24h and 48h. Data are expressed as means + SEM (n=3).

Expression of carbohydrate/lipid metabolism-related genes

Next, we studied change in the expression levels of carbohydrate/lipid metabolism-related genes. As shown in Figure 4, the expression of acetyl-CoA carboxylase 1 (*Acc1*) was significantly increased 3.1-, 3.7- and 3.4-fold in PCLS cultured for 24h in M3, M4 and M5, respectively. Similarly, the expression of carbohydrate responsive element binding protein (*Chrebp*) gene was up-regulated 3.4-, 3.2- and 3.0-fold in PCLS cultured in M3, M4 and M5, respectively. Although the gene expression of sterol regulatory element binding protein 1c (*Srebp-1c*) was increased 2.4-, 3.9- and 3.4-fold in PCLS cultured in M3, M4 and M5, respectively, the differences were not statistically significant. On the other hand, the mRNA level of carnitine palmitoyltransferase 1 (*Cpt1*) decreased 0.35- and 0.29-fold in PCLS cultured in M4 and M5, respectively. Even though the influence of culture medium modification on the change of carbohydrate/lipid-related gene expression was not statistically different at 48h, the expression profile was similar to the profile at 24h of incubation.

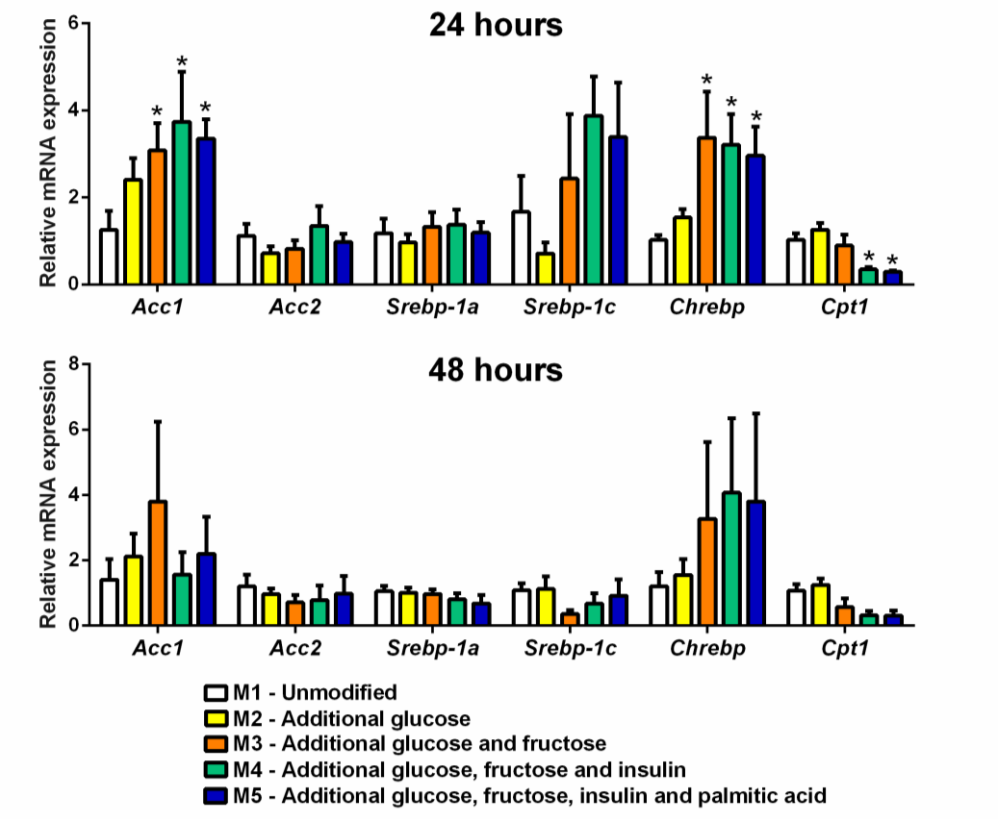

Figure 4: Expression of carbohydrate/lipid metabolism-related genes of PCLS cultured in the modified culture medium at 24h and 48h. Data are expressed as means + SEM (n=3-5). **p*<0.05 compared to M1.

Discussion

Pathophysiological model of NAFLD

To induce liver steatosis, we used supraphysiological concentrations of glucose, fructose, insulin and palmitic acid in the modified culture media to mimic pathophysiological serum concentrations measured in NAFLD patients [11, 22, 25]. Therefore, in addition to the preserved multicellular environment and interplay between various liver cell-types as in the intact liver of PCLS [29], the process in the formation and accumulation of lipid droplets in PCLS could recapitulate the pathophysiological development of the disease. However, it should be noted that it remains to be investigated whether serum levels reflect the portal concentration range of each component.

Insulin acts as the master regulator

Our results demonstrated that insulin is essential for the formation and accumulation of lipid droplets in PCLS [30, 31]. Moreover, a pilot study showed that the steatosis was not observed in PCLS cultured in the medium containing glucose, fructose and palmitic acid, in the absence of insulin (Figure 5). This finding emphasizes again that insulin is the master modulator in the formation and accumulation of lipid droplets in our pathophysiological PCLS model, and probably not palmitic acid. In addition, it is worthwhile to note that palmitic acid did not alter PCLS viability, while clear lipotoxicity was observed at the same concentration in other *in vitro* studies [19-21]. This discrepancy might be due to the protective effect of insulin which promotes the conversion of free palmitic acid into triglycerides that may accumulate in lipid droplets, thereby decreasing the free fatty acid concentration that might induce lipotoxicity [32].

Disruption of mitochondrial β–oxidation is the main pathway

In our study, the lipid droplets in PCLS can be characterized as microvesicular steatosis which is in line with other *in vitro* NAFLD studies [33-35]. This differs from the macrovesicular steatosis found in various *in vivo* models of NAFLD [24, 36, 37]. The main reason why the size of lipid droplets is different *in vitro* could be the time-frame for induction of steatosis, which in our PCLS, as similar to other *in vitro* models, are restricted to 48h, and takes many months in *in vivo* studies. Furthermore, this dissimilarity might also be depended on the main processes for the formation of lipid droplets: mitochondrial β-oxidation and *de novo* lipogenesis [18, 38]. It was shown in rats-treated with valproate, an anti-epileptic drug, that the development of microvesicular steatosis was due to the mitochondrial β–oxidation impairment [39]. Additionally in clinical practice, microvesicular steatosis is a manifestation in patients who received long-term treatment of various drugs affecting mitochondrial β-oxidation such as tetracycline, amiodarone, amineptine and pirprofen [40]. On the other hand, macrovesicular steatosis in *in vivo* models is the result of multiple systemic factors such as eating habits and endocrine effects which chronically and synergistically enhance *de novo* lipogenesis [23, 24].

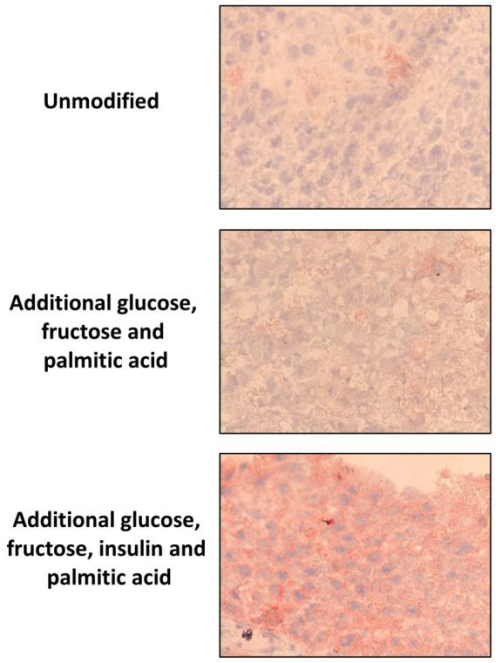

Figure 5: Representative Oil Red O stained sections of PCLS cultured in the modified culture medium at 48h (400X).

The association of both mitochondrial β-oxidation and *de novo* lipogenesis in the formation and accumulation of lipids in PCLS was supported by the expression profile of gene encoding carbohydrate/lipid-related metabolism. Most importantly, the expression of *Cpt1*, an enzyme responsible for fatty acid transportation from the cytosol to the mitochondrial matrix where β-oxidation takes places [41], was down-regulated in steatotic PCLS. Furthermore, although the up-regulation of *Acc1*, *Chrebp* and *Srebp-1c* which are key enzymes responsible for the *de novo* lipogenesis [42-44] was observed in PCLS cultured in medium containing additional glucose and fructose, steatosis was not observed in these liver slices. Taken together, we postulated that the promotion of *de novo* lipogenesis alone may be insufficient to induce microvesicular steatosis, and therefore the disruption of fatty acid transport and mitochondrial β-oxidation are probably the main pathways responsible for the accumulation of microvesicular steatosis in our PCLS model [45]. Nevertheless, future studies are required to confirm these findings.

Conclusion

Our preliminary study demonstrates that, by mimicking the main risk factors of NAFLD, steatosis can be induced *ex vivo* in PCLS. The developed model can be an effective tool to study NAFLD and associated conditions, and could possibly be used to test the efficacy of anti-NALFD drugs. Additionally, since PCLS can be prepared from human livers, implementation of a human steatotic PCLS model would be able to overcome some of the limitations of rodent NAFLD/NASH models.

Acknowledgements

This work was kindly supported by ZonMw, grant number 114021010. We would like to thank V. Starokozhko for her help with the Oil Red O staining technique. In addition, multiple experimental procedures and equipment were greatly supported by Department of Pharmacokinetics, Toxicology and Targeting, University of Groningen. We are very grateful for all the help and suggestions during this study.

References

1. Neuschwander-Tetri, B.A., Non-alcoholic fatty liver disease. BMC Med, 2017. 15(1): p. 45.
2. Rinella, M.E., Nonalcoholic fatty liver disease: a systematic review. JAMA, 2015. 313(22): p. 2263-73.
3. Sherif, Z.A., et al., Global epidemiology of nonalcoholic fatty liver disease and perspectives on US minority populations. Dig Dis Sci, 2016. 61(5): p. 1214-25.
4. Byrne, C.D. and G. Targher, NAFLD: a multisystem disease. J Hepatol, 2015. 62(1 Suppl): p. S47-64.
5. Corey, K.E. and M.E. Rinella, Medical and surgical treatment options for nonalcoholic steatohepatitis. Dig Dis Sci, 2016. 61(5): p. 1387-97.
6. Ganesh, S. and V.K. Rustgi, Current pharmacologic therapy for nonalcoholic fatty liver disease. Clin Liver Dis, 2016. 20(2): p. 351-64.
7. Buzzetti, E., M. Pinzani, and E.A. Tsochatzis, The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism, 2016. 65(8): p. 1038-48.
8. Bhatt, H.B. and R.J. Smith, Fatty liver disease in diabetes mellitus. Hepatobiliary Surgery and Nutrition, 2015. 4(2): p. 101-108.
9. Katsiki, N., D.P. Mikhailidis, and C.S. Mantzoros, Non-alcoholic fatty liver disease and dyslipidemia: An update. Metabolism, 2016. 65(8): p. 1109-23.
10. Ress, C. and S. Kaser, Mechanisms of intrahepatic triglyceride accumulation. World Journal of Gastroenterology, 2016. 22(4): p. 1664-1673.
11. Berlanga, A., et al., Molecular pathways in non-alcoholic fatty liver disease. Clinical and Experimental Gastroenterology, 2014. 7: p. 221-239.
12. Alam, S., et al., Insulin resistance in development and progression of nonalcoholic fatty liver disease. World Journal of Gastrointestinal Pathophysiology, 2016. 7(2): p. 211-217.
13. Jegatheesan, P. and J.-P. De Bandt, Fructose and NAFLD: The multifaceted aspects of fructose metabolism. Nutrients, 2017. 9(3): p. 230.
14. Basaranoglu, M., G. Basaranoglu, and E. Bugianesi, Carbohydrate intake and nonalcoholic fatty liver disease: fructose as a weapon of mass destruction. Hepatobiliary Surgery and Nutrition, 2015. 4(2): p. 109-116.
15. Softic, S., D.E. Cohen, and C.R. Kahn, Role of dietary fructose and hepatic de novo lipogenesis in fatty liver disease. Dig Dis Sci, 2016. 61(5): p. 1282-93.
16. Campos, V.C. and L. Tappy, Physiological handling of dietary fructose-containing sugars: implications for health. Int J Obes (Lond), 2016. 40 Suppl 1: p. S6-11.
17. Leamy, A.K., R.A. Egnatchik, and J.D. Young, Molecular mechanisms and the role of saturated fatty acids in the progression of non-alcoholic fatty liver disease. Prog Lipid Res, 2013. 52(1): p. 165-74.
18. Rui, L., Energy metabolism in the liver. Comprehensive Physiology, 2014. 4(1): p. 177-197.
19. Yao, H.-R., et al., Lipotoxicity in HepG2 cells triggered by free fatty acids. American Journal of Translational Research, 2011. 3(3): p. 284-291.
20. Pardo, V., et al., Role of hepatocyte S6K1 in palmitic acid-induced endoplasmic reticulum stress, lipotoxicity, insulin resistance and in oleic acid-induced protection. Food and Chemical Toxicology, 2015. 80: p. 298-309.
21. Jiang, X.-s., et al., Autophagy protects against palmitic acid-induced apoptosis in podocytes in vitro. Scientific Reports, 2017. 7: p. 42764.
22. Liu, W., et al., Pathogenesis of nonalcoholic steatohepatitis. Cell Mol Life Sci, 2016. 73(10): p. 1969-87.
23. Chavez-Tapia, N.C., N. Rosso, and C. Tiribelli, In vitro models for the study of non-alcoholic fatty liver disease. Curr Med Chem, 2011. 18(7): p. 1079-84.
24. Kanuri, G. and I. Bergheim, In vitro and in vivo models of non-alcoholic fatty liver disease (NAFLD). International Journal of Molecular Sciences, 2013. 14(6): p. 11963-11980.
25. Magee, N., A. Zou, and Y. Zhang, Pathogenesis of nonalcoholic steatohepatitis: Interactions between liver parenchymal and nonparenchymal cells. BioMed Research International, 2016. 2016: p. 5170402.
26. Westra, I.M., et al., Human precision-cut liver slices as a model to test antifibrotic drugs in the early onset of liver fibrosis. Toxicol In Vitro, 2016. 35: p. 77-85.

27. Olinga, P. and D. Schuppan, Precision-cut liver slices: A tool to model the liver ex vivo. Journal of Hepatology, 2013. 58(6): p. 1252-1253.
28. de Graaf, I.A., et al., Preparation and incubation of precision-cut liver and intestinal slices for application in drug metabolism and toxicity studies. Nat Protoc, 2010. 5(9): p. 1540-51.
29. Ravi, M., et al., 3D cell culture systems: advantages and applications. J Cell Physiol, 2015. 230(1): p. 16-26.
30. Titchenell, P.M., et al., Direct hepatocyte insulin signaling is required for lipogenesis but is dispensable for the suppression of glucose production. Cell Metab, 2016. 23(6): p. 1154-66.
31. Wilcox, G., Insulin and insulin resistance. Clinical Biochemist Reviews, 2005. 26(2): p. 19-39.
32. Listenberger, L.L., et al., Triglyceride accumulation protects against fatty acid-induced lipotoxicity. Proceedings of the National Academy of Sciences of the United States of America, 2003. 100(6): p. 3077-3082.
33. Antherieu, S., et al., Induction of vesicular steatosis by amiodarone and tetracycline is associated with up-regulation of lipogenic genes in HepaRG cells. Hepatology, 2011. 53(6): p. 1895-905.
34. Zhang, D.-D., et al., Nuciferine downregulates Per-Arnt-Sim kinase expression during its alleviation of lipogenesis and inflammation on oleic acid-induced hepatic steatosis in HepG2 cells. Frontiers in Pharmacology, 2015. 6(238).
35. Cui, W., S.L. Chen, and K.-Q. Hu, Quantification and mechanisms of oleic acid-induced steatosis in HepG2 cells. American Journal of Translational Research, 2010. 2(1): p. 95-104.
36. Roh, J.S., et al., Effect of Gangjihwan on hepatic steatosis and inflammation in high fat diet-fed mice. J Ethnopharmacol, 2017. 206: p. 315-326.
37. Jang, J.E., et al., Protective role of endogenous plasmalogens against hepatic steatosis and steatohepatitis in mice. Hepatology, 2017.
38. Sanders, F.W.B. and J.L. Griffin, De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. Biological Reviews of the Cambridge Philosophical Society, 2016. 91(2): p. 452-468.
39. Natarajan, S.K., et al., Oxidative stress in experimental liver microvesicular steatosis: role of mitochondria and peroxisomes. J Gastroenterol Hepatol, 2006. 21(8): p. 1240-9.
40. Schumacher, J. and G. Guo, Mechanistic review of drug-induced steatohepatitis. Toxicology and applied pharmacology, 2015. 289(1): p. 40-47.
41. Longo, N., C.A. di San Filippo, and M. Pasquali, Disorders of carnitine transport and the carnitine cycle. American journal of medical genetics. Part C, Seminars in medical genetics, 2006. 142C(2): p. 77-85.
42. Wang, Y., et al., Transcriptional regulation of hepatic lipogenesis. Nature reviews. Molecular cell biology, 2015. 16(11): p. 678-689.
43. Xu, X., et al., Transcriptional control of hepatic lipid metabolism by SREBP and ChREBP. Seminars in liver disease, 2013. 33(4): p. 301-311.
44. Brownsey, R.W., et al., Regulation of acetyl-CoA carboxylase. Biochem Soc Trans, 2006. 34(Pt 2): p. 223-7.
45. Fromenty, B. and D. Pessayre, Impaired mitochondrial function in microvesicular steatosis. Effects of drugs, ethanol, hormones and cytokines. J Hepatol, 1997. 26 Suppl 2: p. 43-53.