Nonalcoholic fatty liver disease in relation to cardiovascular disease

Valorisation addendum

1. Introduction

Cardiovascular disease (CVD) is a major threat to global health.[1] In 2017, approximately 17.8 million people died as a consequence of CVD.[2] Several risk factors for CVD have been identified, including – but not limited to – age, male sex, smoking, high blood pressure, high cholesterol level, obesity and diabetes mellitus.[3,4]

Recently, another risk factor has been added to the list; nonalcoholic fatty liver disease (NAFLD) is a histological spectrum comprising hepatic steatosis (presence of fat in the liver), steatohepatitis (presence of fat *and* inflammation in the liver) and fibrosis[5], in the absence of excessive alcohol intake. It occurs in 25% of the adult population[6] and the prevalence of NAFLD is even higher in specific populations, such as type 2 diabetes mellitus.[7] The first stage of NAFLD (i.e., hepatic steatosis) results from an imbalance between the formation and degradation of fat in the liver, either due to (1) increased flux of free fatty acids towards the liver (the backbones of fat), (2) increased *de novo* lipogenesis (the formation of fat from glucose), (3) impaired beta-oxidation (the breakdown of fatty acids as energy source) or (4) impaired production and secretion of VLDL particles (the transporters of fat through the body).

Already much research has been performed on the role of NAFLD in the development of CVD. However, it remains unclear whether NAFLD is an active contributor to cardiovascular risk or just an innocent bystander. This distinction is essential as it requires a different prevention and treatment approach. In the current thesis, we have tried to gain insight into the causal relationship between NAFLD and CVD, and possible underlying mechanisms.

This chapter describes the value of the performed research for society in general and for clinicians and researchers in specific.

2. (Cardiovascular) risk management in NAFLD patients

In spite of the increasing prevalence and associated morbidity and mortality, NAFLD is an asymptomatic disease with low awareness among patients and clinicians. In order to adequately treat patients, a good understanding of disease etiology is essential. This thesis has gained insight into the causal relationship between NAFLD and CVD. We have shown that NAFLD per se does not cause CVD and that the cardiovascular risk in NAFLD patients

depends on the pathway that leads to intrahepatic lipid accumulation (IHL) accumulation. Specifically, pathways that result in increased secretion of triglyceride-rich VLDL particles (and hence a more atherogenic lipid profile) - such as *de novo* lipogenesis - are deleterious for cardiovascular health. This finding has relevant consequences for the daily clinical practice. Since not all NAFLD patients carry the same cardiovascular risk, disease management should be tailored to each individual patient. Measurement of plasma lipids (and assessment of the overall cardiovascular risk profile) could be leading in the decision to start cardiovascular treatment in individuals with NAFLD, as we have shown that the increased risk of developing CVD is mainly attributed to plasma lipid levels. The current guidelines recommending screening for dyslipidemia are in line with the results of this thesis.[5] Aside from standard treatment with HMG-CoA-Reductase Inhibitors (statins), pharmacological options that have proven to affect both NAFLD and CVD, such as pioglitazone and liraglutide[8-11], could be considered (although the former recommendation is hampered by other side-effects, such as heart failure and fractures[12], and the latter recommendation first needs to be supported by more large-scale randomized controlled trials).

In addition to prevention of CVD in NAFLD patients, there is an ongoing search for noninvasive biomarkers for feasible prognostication and disease monitoring of NAFLD itself, as it could progress into cirrhosis and hepatocellular carcinoma.[13] Moreover, NAFLD is expected to become the primary indication for liver transplantation by 2025.[14] However, the current gold standard for NAFLD, in particular NASH (i.e., liver histology), is invasive and poorly tolerated. This thesis has provided insight into a potential new biomarker for NAFLD severity; plasma soluble E-selectin. We showed that: 1) NAFLD severity, in particular the inflammatory stage, was associated with hepatic E-selectin expression; 2) E-selectin mRNA expression in liver, but not in other organ tissue, was associated with plasma sE-selectin levels; and 3) NAFLD susceptibility genes and liver parenchyma damage (reflected by plasma alanine aminotransferase levels levels) were associated with plasma sE-selectin levels, independent of potential confounding factors. These results, however, need to be replicated in cohort studies with biopsy proven NAFLD before sE-selectin could be considered as part of the biomarker panel for NAFLD.

3. Fructose-1-phosphate and glucokinase regulatory protein as potential treatment targets

The phenotypic manifestation and severity of NAFLD is the result of multiple gene-environment interactions.[15] Fructose has been suggested to play a key role in the development of NAFLD as it is primarily metabolized in the liver.[16] Yet, trials on the effect of fructose on IHL content in humans show conflicting results.[17] This thesis has gained insight into the role of fructose in the development of NAFLD. We have found indications that specifically fructose-1-phosphate (an intermediate of fructose metabolism) is an important factor in IHL accumulation (via *de novo* lipogenesis). This insight provides a new starting point for future research, more specific on molecules that block the formation of fructose-1-phosphate, for example via inhibition of ketohexokinase (the enzyme responsible for the conversion of fructose to fructose-1-phosphate). Indeed, by reducing the intrahepatic fructose-1-phosphate concentration, both the direct lipogenic effects (i.e., increased *de novo* lipogenesis) and indirect lipogenic effects (i.e., increased disruption of the glucokinase-glucokinase regulatory protein complex) of fructose could be prevented. The subsequent elevated levels of plasma fructose (which also occurs in patients with a ketohexokinase deficiency known as essential fructosuria (OMIM# 229800)) is clinically asymptomatic and harmless, which further emphasize ketohexokinase as potential new therapeutic target. This new type of drug would not only reduce IHL accumulation in NAFLD patients, but could also be of benefit for HFI patients that are currently still bound to a lifelong diet that is devoid of fructose. Besides emphasizing the need for additional treatment, this thesis also provided new information on the long-term consequences of HFI for practice guidelines, which up to now were primary based on case reports (of children) at time of diagnosis.

An alternative target for NAFLD treatment could be the glucokinase-glucokinase regulatory protein complex. Recently, small molecules that disrupt the binding between glucokinase and glucokinase regulatory protein have been proposed as a potential new class of antidiabetic drugs. These small molecule disruptors have indeed been shown to affectively lower plasma glucose levels in mice.[18] This thesis, however, strongly suggests that increased glucokinase-glucokinase regulatory protein dissociation will have disadvantageous side-effects, such as IHL accumulation and subsequent dyslipidemia and CVD development. Glucokinase-glucokinase regulatory protein *enhancement*, on the other hand, could be an alternative pharmacological target for NAFLD treatment. The occurrence of hyperglycemia as potential negative side effect

would, of course, require additional blood glucose lowering medication (for example with the use of sodium-glucose co-transporter-2 inhibitors).

For both potential new treatment targets, mouse models are available.[19,20] The results of this thesis have directly led to the set-up of new studies, aimed at reducing the IHL content by interfering with either hepatic fructose metabolism or the glucokinase-glucokinase regulatory protein complex, of which the results will hopefully follow shortly.

4. The toxic truth about fructose

Last, this thesis has gained insight into the metabolic consequences of fructose in comparison to glucose. In the late 1960s, fructose was introduced to the food industry as a sweeter alternative for glucose. Since then, it has been widely distributed and used in sugar-sweetened beverages and processed foods. Since the 1980s, however, there have been doubts about the negative health consequences of this monosaccharide as the rise in fructose intake paralleled the increase in obesity and its related metabolic complications, such as NAFLD.[21-23] For a long time, fructose has been the scope of research and although there is abundant evidence from animal studies on the prominent role and putative mechanism by which fructose causes IHL accumulation[24-28], a previous meta-analysis of controlled trials in humans did not show any effects of fructose on IHL content.[17] The included studies, however, often used a fructose overdose in combination with a hypercaloric diet.[29-32] We are the first that studied the effects of fructose *restriction* in overweight adults in a randomized controlled setting and found that removal of fructose from the diet does have additional health benefits (i.e., reducing IHL content). Thus, in a certain way one could argue if in the current Western society the phrase ‘an apple a day keeps the doctor away’ is outdated.

5. Conclusion

In conclusion, the results reported in this thesis may benefit society in general and clinicians and researchers in specific by providing new information on 1) the relationship between NAFLD, plasma lipids and CVD, which can be used in NAFLD practice guidelines; 2) a potential new biomarker for NAFLD, which in time can potentially be used for disease prognostication and monitoring; 3) potential new treatment targets for NAFLD and HFI, which in time can delay

disease progression and improve quality of life; 4) the health benefits of fructose restriction, which can contribute to the current vision on excessive sugar intake and the need to reduce sugar processing by the industry.

Literature

1. McAloon CJ, Boylan LM, Hamborg T, et al. The changing face of cardiovascular disease 2000-2012: An analysis of the world health organisation global health estimates data. Int J Cardiol 2016;224:256-64.
2. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2018;392:1736-88.
3. Hennekens CH. Increasing burden of cardiovascular disease: current knowledge and future directions for research on risk factors. Circulation 1998;97:1095-102.
4. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937-52.
5. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388-402.
6. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73-84.
7. Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. J Hepatol 2019;71: 793-801.
8. Cusi K, Orsak B, Bril F, et al. Long-Term Pioglitazone Treatment for Patients With Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus: A Randomized Trial. Ann Intern Med 2016;165:305-15.
9. Dormandy JA, Charbonnel B, Eckland DJ, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. Lancet 2005;366: 1279-89.
10. Armstrong MJ, Gaunt P, Aithal GP, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. Lancet 2016;387:679-90.
11. Marso SP, Daniels GH, Brown-Frandsen K, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 2016;375:311-22.
12. Liao HW, Saver JL, Wu YL, Chen TH, Lee M, Ovbiagele B. Pioglitazone and cardiovascular outcomes in patients with insulin resistance, pre-diabetes and type 2 diabetes: a systematic review and meta-analysis. BMJ Open 2017;7:e013927.
13. Beste LA, Leipertz SL, Green PK, Dominitz JA, Ross D, Ioannou GN. Trends in burden of cirrhosis and hepatocellular carcinoma by underlying liver disease in US veterans, 2001-2013. Gastroenterology 2015;149:1471-82.e5; quiz e17-8.
14. Wong RJ, Aguilar M, Cheung R, et al. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. Gastroenterology 2015;148:547-55.
15. Eslam M, George J. Genetic and epigenetic mechanisms of NASH. Hepatol Int 2016;10: 394-406.
16. Jensen T, Abdelmalek MF, Sullivan S, et al. Fructose and sugar: A major mediator of non-alcoholic fatty liver disease. J Hepatol 2018;68:1063-75.
17. Chiu S, Sievenpiper JL, de Souza RJ, et al. Effect of fructose on markers of non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of controlled feeding trials. Eur J Clin Nutr 2014;68:416-23.

18. Lloyd DJ, St Jean DJ, Jr., Kurzeja RJ, et al. Antidiabetic effects of glucokinase regulatory protein small-molecule disruptors. Nature 2013;504:437-40.
19. Grimsby J, Coffey JW, Dvorozniak MT, et al. Characterization of glucokinase regulatory protein-deficient mice. J Biol Chem 2000;275:7826-31.
20. Oppelt SA, Sennott EM, Tolan DR. Aldolase-B knockout in mice phenocopies hereditary fructose intolerance in humans. Mol Genet Metab 2015;114:445-50.
21. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab (Lond) 2005;2:5.
22. Nakagawa T, Tuttle KR, Short RA, Johnson RJ. Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. Nat Clin Pract Nephrol 2005;1:80-6.
23. Dhingra R, Sullivan L, Jacques PF, et al. Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. Circulation 2007;116:480-8.
24. Ackerman Z, Oron-Herman M, Grozovski M, et al. Fructose-induced fatty liver disease: hepatic effects of blood pressure and plasma triglyceride reduction. Hypertension 2005;45:1012-8.
25. Armutcu F, Coskun O, Gürel A, et al. Thymosin alpha 1 attenuates lipid peroxidation and improves fructose-induced steatohepatitis in rats. Clin Biochem 2005;38:540-7.
26. Koo HY, Miyashita M, Cho BH, Nakamura MT. Replacing dietary glucose with fructose increases ChREBP activity and SREBP-1 protein in rat liver nucleus. Biochem Biophys Res Commun 2009;390:285-9.
27. Roglans N, Vilà L, Farré M, et al. Impairment of hepatic Stat-3 activation and reduction of PPARalpha activity in fructose-fed rats. Hepatology 2007;45:778-88.
28. Samuel VT, Liu ZX, Wang A, et al. Inhibition of protein kinase Cepsilon prevents hepatic insulin resistance in nonalcoholic fatty liver disease. J Clin Invest 2007;117:739-45.
29. Sobrecases H, Le KA, Bortolotti M, et al. Effects of short-term overfeeding with fructose, fat and fructose plus fat on plasma and hepatic lipids in healthy men. Diabetes Metab 2010;36:244-6.
30. Le KA, Ith M, Kreis R, et al. Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. Am J Clin Nutr 2009;89:1760-5.
31. Kechagias S, Ernersson A, Dahlqvist O, Lundberg P, Lindstrom T, Nystrom FH. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. Gut 2008;57:649-54.
32. Sevastianova K, Santos A, Kotronen A, et al. Effect of short-term carbohydrate overfeeding and long-term weight loss on liver fat in overweight humans. Am J Clin Nutr 2012;96:727-34.