Lysosomes ‘in control’

Valorisation

Socio-economical and clinical relevance

Metabolic syndrome is one of the largest health challenges of the 21st century with currently more thanr **600 million** adult individuals suffering from this condition.[1] While the term was initially used to exclusively identify obese patients at risk for cardiovascular diseases and type 2 diabetes, an increasing amount of evidence demonstrates the influence of metabolic syndrome on other physiological and pathological processes.[2] Moreover, in the last 5 years, a staggering increase of **pediatric and adolescent metabolic syndrome**[3-5] has been diagnosed, emphasizing the gigantic health challenge metabolic syndrome presents for society.

In this thesis, the main research goal was to provide translational insight into the mechanisms leading to NASH. NASH is a disorder characterized by hepatic inflammation and is mostly present in obese subjects [6]. Though exact numbers remain obscure, latest findings estimate 25% of the global population suffering from a form of fatty liver disease, of which about one third suffers from NASH.[6] The recent evidence that NASH is also becoming more prevalent in children has supported the expert prediction that **NASH will become the leading liver disorder** by the year 2019.[7] This is worrying since NASH patients progress to more advanced liver disorders such as fibrosis, cirrhosis and liver failure. Currently, the only effective treatment option for these advanced liver disorders is liver transplantation.[7] In addition, diagnosis of NASH can only be made by histological assessment of a liver biopsy, in itself a risky procedure which can lead to severe complications.[8] Taking all this into account, Younossi *et al*. recently estimated the economic and clinical burden of fatty liver disease in the United States and in 4 European countries (Germany, France, Italy and United Kingdom).[9] In the **United States**, over 64 million people were projected to suffer from NAFLD, leading to an **annual cost of about $103 billion**. In the **4 European countries**, 52 million NAFLD patients are expected to run up **an annual bill of €35 billion.**[9] Therefore, there is an enormous demand for better diagnostic and therapeutic tools for NASH and metabolic syndrome in general. This is also reflected by the increasing amount of projects dedicated to the identification of better therapeutic compounds and biomarkers.[10]

We propose plasma **cathepsin D** as a novel **non-invasive** tool to diagnose NASH in an **early stage** of the disease. This finding is exceptionally **promising from an economic point of view** as it has the potential to strongly **reduce healthcare costs** for patients and society in general. Furthermore, early diagnosis of NASH can be a huge **support for clinicians** in preventing the development of NASH but also its progression into more advanced liver diseases. From a therapeutical point of view, reducing oxLDL levels, blocking plasma cathepsin D activity and/or changing the composition of gut microbiota are suggested as novel ways to tackle NASH and metabolic syndrome and can be alternatives to treat patients in the clinic.

The findings of this thesis should also be considered of interest with respect to **other related diseases**. Firstly, excessive lysosomal lipid storage in macrophages has also been shown in the context of atherosclerosis,[11] suggesting that our findings can be extrapolated to **cardiovascular diseases**. Secondly, lipid disturbances induced by lysosomal dysfunction in microglia, the macrophages of the brain, were previously linked to **Alzheimer's, Parkinson's and Huntington's disease**, strongly suggesting that the observations of this thesis are also relevant for these disorders.[12,13] Finally, **lysosomal storage disorder's** central feature is the accumulation of digested or undigested material in lysosomes.[14] Our findings might therefore also be of interest for these disorders.

Knowledge dissemination among different target groups

The observations described in this thesis have enormous clinical potential and are, therefore, of great interest for clinicians, industrial partners and particularly, patients. In order to reach these target groups, we have published our observations in **peer-reviewed scientific and clinical journals** such as *The American Journal of Gastroenterology* and *Scientific Reports*. To discuss our findings with the scientific community, we also presented at multiple national and international conferences by means of **oral or poster presentations**. Furthermore, by **organizing the 1st European Fatty Liver Conference**, we facilitated fruitful meetings of scientists with clinicians and industrial representatives. To make our findings available to the general public, we have recently published an article in the Science & Technology magazine (issue of April 2017) of **Pan European Networks**. This is a media group devoted to provide the most relevant information about European advances made in the fields of politics, technology and research to the general public.

Originality and/or innovative elements

Many diagnostic and therapeutic tools for NASH that are currently used in the clinic are the result of an explorative-driven research approach. Unfortunately, this approach has resulted in non-specific diagnostic (i.e. ALT) and therapeutic (i.e. exercise) options. In contrast, we employed a **hypothesis-driven approach** that identified lysosomal lipid accumulation in macrophages at the basis of NASH development. This finding was achieved by performing experiments at ***in vitro*** and ***in vivo*** both in mouse models and in **human subjects**. This approach is **innovative** and promising as it offers a **translational** platform for the development of novel diagnostic and therapeutic tools for NASH and metabolic syndrome in general. By identifying plasma levels of the lysosomal enzyme

cathepsin D as a marker for NASH, we also prove the successfulness of our translational and hypothesis-driven approach, emphasizing the enormous clinical and industrial potential of our findings.

A main obstacle that prevents progress in the field of NASH is the wide-spread use of animal models that do not adequately mimic (patho-)physiology and metabolism of humans.[15] In contrast, we here used the *Ldlr*$^{-/-}$ mouse model, which is a **physiological model** that mimics hepatic inflammation (and other features of metabolic syndrome) in humans. The findings that are obtained from these *in vivo* experiments can therefore be **applied to the human situation**.

Also, while most diagnostic and therapeutic research of NASH focuses on alleviating and reducing advanced NASH,[16] we here provide information of the **early molecular mechanisms** at the fundament of the disease. As such, our observations have the **potential to prevent**, rather than cure advanced NASH.

Products and activities

Up until now, a liver biopsy is considered the golden standard for assessing NASH. This is known as an invasive, painful and risky procedure, underlining the need for non-invasive methods to improve early diagnosis of NASH. This thesis identifies **plasma cathepsin D** as a novel, non-invasive marker to diagnose and monitor NASH. In contrast to the liver biopsy, this approach is **safer, more rapid and associated with less discomfort** for the patient. This finding has also resulted in a **filed patent application**. Our finding that oxLDL particles substantially contribute to lysosomal lipid accumulation in hepatic macrophages (Chapter 4), strongly suggests that **plasma oxLDL** levels should be investigated for their potential to predict NASH. In line, low levels of anti-oxLDL IgM antibodies were correlated with the level of hepatic inflammation in humans.[17]

Besides novel diagnostic tools, this thesis also provides several therapeutical alternatives for NASH and metabolic syndrome. In chapter 7, we showed that modulation of cathepsin D reduced hepatic inflammation, thereby identifying **cathepsin D as a potential therapeutic target** for NASH. In chapter 4, we demonstrated that an **immunization** protocol with **heat-inactivated pneumococci** increased anti-oxLDL IgM antibody levels, resulting in reduced hepatic inflammation. This type of immunization might be further refined for application in patients at risk of developing NASH and metabolic syndrome as oxLDL has been shown a risk factor in several metabolic syndrome-associated pathologies.[18] Finally, we found that lysosomal lipid storage in

macrophages resulted in a shift in **gut microbiota composition** (chapter 8), suggesting that gut microbiota potentially contributes to lysosomal lipid storage-associated problems. Future research should point out the effect of gut microbiota on physiology and pathology, and assess whether modulating gut microbiota can be an attractive alternative for therapeutic intervention.

Future planning

While this thesis offers several novel diagnostic and therapeutic options for NASH and metabolic syndrome, more action has to be undertaken to translate our findings to patients and the general public. One of our main goals for the future is to create a novel non-invasive approach to diagnose all stages of fatty liver disease in the clinic. We recently **obtained a competitive grant from the TKI**, which will support us in translating our findings to the clinic and general public. This project involves collaborations between national (Groningen, Amsterdam, Maastricht) and international (Antwerp, BE) academic partners together with companies (Echosens, FR and Tobira, US) and patient organizations. Therefore, the first step has already been made in the direction of implementation of a novel, non-invasive approach to diagnose NASH. As mentioned, this project is also **closely connected to the Dutch patient organization of the liver,** a collaboration that we intend to intensify in the future. Furthermore, as follow-up on the 1st European Fatty Liver Conference, we are currently planning the **2nd European Fatty Liver Conference** to be held in Maastricht in March 2018. Besides close collaboration between clinicians, researchers and industrial partners, a contribution of patient organizations will also be included within the program of the conference.

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