# Lysosomal Disturbances in Metabolic Disorders

# Impact paragraph

## Social-economical and clinical relevance

Metabolic syndrome (MetS) has emerged as a major health challenge, with its prevalence steadily rising across adults and younger populations. Modern risk factors for MetS, including aging, physical inactivity, and poor diet, contribute to its estimated prevalence of 20-30% in the adult population globally [1]. Additionally, the occurrence of MetS, a collection of risk factors that heightens the likelihood of developing type 2 diabetes, cardiovascular diseases, and fatty liver disease, is becoming increasingly common among adolescents [2, 3]. Currently, over 340 million children and adolescents aged 5 to 19 are classified as overweight or obese, predominantly due to alterations in dietary patterns and lifestyle behaviors [4]. This trend reflects a major public health concern, as the early onset of MetS in youth is likely to result in lifelong health complications. This condition imposes significant health, social, and economic burdens. This increasing burden on healthcare systems is becoming obvious, emphasizing the urgent need for effective strategies to prevent and manage this condition to reduce its impact on future generations.

Being the hepatic event of MetS, chronic liver disease has become a common global health issue. Chronic liver disease, including cirrhosis, viral hepatitis, and liver cancer, profoundly affects global mortality, morbidity, and economic costs, accounting for over two million deaths annually and representing 4% of all deaths worldwide [5]. According to the World Health Organization and the Global Burden of Disease, the burden of chronic liver disease is increasing, mainly due to the rise in MASLD/MASH and ALD [6]. Currently MASLD affects approximately 25%-30% in the world, among which MASH ranges from 3%-5% of the world population [7]. The rate of cirrhosis is growing more rapidly than any other liver disease worldwide, while HCC rates are rising, especially in high-income countries [5, 8]. The United States, in particular, has the highest prevalence of ALD-related HCC cases [9]. Understanding the burden of HCC by country and its underlying causes is crucial to addressing this escalating health issue effectively. MASH and ALD have brought huge social, clinical and economic burden to the world [10, 11]. For instance, the increasing prevalence of MASH and ALD necessitates a greater number of healthcare and social workers to provide comprehensive care and support for patients, ensuring effective management and improved outcomes for these complex conditions [12]. Currently there are no available non-invasive biomarkers to diagnosis early ALD and limited effective pharmaceutic therapy for the treatment of MASH. While the recent approval of Rezdiffra by the FDA represents a promising advancement in treating MASH, not all patients experience improvement in liver histology during clinical trials [13]. Therefore, continued research into additional therapeutic targets remains crucial for enhancing patient outcomes and addressing this escalating health challenge.

In the current thesis, we propose that lysosomal enzymes (i.e. CTSD and AP) correlate with the development of metabolic disorders including MetS, MASH, and ALD. For example, we demonstrated that AP could be a potential parameter for detecting MetS in obese adolescents and adults, which is valuable from a clinical point of view as it has the potential to improve early diagnosis for MetS. Additionally, plasma CTSD levels could potentially be used as an early biomarker to diagnose MASH and ALD, thereby providing support for clinicians to prevent disease progression and apply specific

therapy for patients. Moreover, due to the elevated levels of CTSD in MASH patients, this thesis also investigates the effects of inhibition of CTSD (extracellular CTSD inhibition and intracellular CTSD inhibition) on the treatment of MASH, suggesting that extracellular CTSD inhibition has the beneficial effects for the treatment of MASH. Furthermore, CTSD is also used as a therapeutic target in MASH-HCC [14] and other cancers [15, 16]. Moreover, the findings of this thesis might also be interesting for other related metabolic disorders, such as lysosomal storage disorders, cardiovascular disease as these diseases have similarities in the perspective of pathology (i.e. lipid accumulation and lysosomal dysfunction). For instance, in the context of lysosomal storage disorders such as NPC1 disease, the accumulation of cholesterol and sphingolipids in lysosomes may disrupt the acidic environment necessary for lysosomal enzymes [17], such as acid sphingomyelinase, which is crucial for breaking down sphingomyelin. Consequently, the impaired enzyme activity exacerbates the buildup of sphingomyelin, leading to cellular dysfunction and neurodegeneration [18]. Additionally, current studies have demonstrated that lysosomal cathepsins are also closely associated with the mechanisms of NPC1 by playing a role in the processing the cholesterol trafficking [19]. Likewise, lysosomal enzymes have also been found to be involved in atherosclerosis, studies have shown that lysosomal enzymes such as cathepsins regulate cellular homeostasis in atherosclerosis-based cardiovascular disease [20-22]. Cathepsins influence atherosclerosis by modulating chemokine production, which recruits various inflammatory and immune cells to the vascular wall [22]. They are involved in critical processes such as collagen and elastin degradation, endothelial cell migration, and the formation of vasa vasorum [23]. Additionally, cathepsins impact lipid metabolism, including the degradation of oxLDL and HDL, and contribute to foam cell formation, smooth muscle cell proliferation, and apoptosis in both vascular and inflammatory cells [22]. As such, our findings imply that targeting lysosomal enzymes may have broader applications in metabolic disorders beyond those studied, suggesting that further investigation into their clinical relevance across various conditions is warranted. Altogether, successfully translating these insights into clinical practice could improve the quality of life for patients with MetS, MASH, and ALD, while also mitigating the extensive social, economic, and clinical burdens associated with these conditions.

## Novelty of the concept

In this thesis, we made a novel observation regarding the dynamic behavior of CTSD across the various stages of ALD. Our research is the first to reveal that plasma CTSD levels increase significantly in the early stages of ALD, but subsequently decrease accordingly as the disease progresses into more advanced stages in patients. These observations suggest that CTSD is dynamic over different stages of ALD and that the severity of ALD has a significant impact on plasma CTSD levels. These discoveries are particularly important, offering the insight of a strong association between plasma CTSD levels and specific hepatic pathophysiological processes. The discovery of this CTSD dynamic in human subjects highlights its clinical relevance and potential implications for managing ALD. Furthermore, this finding sets a new foundation for understanding the stages of ALD and its progression, contributing to the advancement of assessment tools and patient care.

In addition, we investigate extracellular CTSD inhibition as a novel therapeutic strategy for MASH based on *in vitro* and *in vivo* experiments conducted in both cell cultures and mouse models. We used the *Ldlr*$^{-/-}$ mouse model, which closely mimics key pathological features of MASH including hepatic steatosis

and inflammation. This model serves as a reliable translational system for studying human disease. We also employed a novel lipidome technique, matrix-assisted laser desorption-ionization mass spectrometry imaging (MALDI-MSI), to investigate alterations in hepatic lipid composition and distribution in *Ldlr*$^{-/-}$ mice fed a high-fat, high-cholesterol diet. Inhibition of extracellular CTSD resulted in significant improvements in MASH compared to intracellular CTSD inhibition, with these effects linked to the regulation of specific lipid composition. This innovative approach provides a platform for the development of therapeutic tools for MASH, offering a comprehensive understanding of the lipidomic consequences associated with CTSD inhibition and its potential as a targeted therapeutic intervention for this disease.

Additionally, besides CTSD, we investigated the lysosomal enzyme AP, which showed a strong positive association with triglycerides and could serve as a potential parameter for detecting MetS in adolescents. While previous study established this relationship in adults with MetS, our study is the first to identify this association in obese adolescents and in a combined population of adolescents and adults. These findings can help in diagnosing and managing MetS in an early stage, potentially reducing the risk of diseases such as type 2 diabetes, MASH, and cardiovascular disease in both adolescents and adults.

Lastly, we here demonstrated that the *Npc1*$^{nih}$ loss-of-function mutation increased plasma granulocyte colony-stimulating factor (G-CSF) levels and consequently, induced hematopoietic stem cell (HSC) mobilization. Also, patients with NPC1 disease show elevated G-CSF in plasma. This finding may open up new therapeutic options to mitigate splenomegaly, addressing peripheral symptoms of NPC1 diseases more effectively.

## Future planning

This thesis introduces several novel diagnostic and therapeutic strategies for MetS, ALD, and MASH, and thus, translating these findings into practical applications for patients and the general public remains a priority. Future work will focus on several key areas to enhance the clinical relevance of our discoveries. One of our goals for the future is exploring CTSD as a novel, non-invasive diagnostic tool for monitoring all stages of ALD, which could precisely stage the progression of this disease. The next important step would be to validate the ability of another lysosomal enzyme AP for detecting MetS in a large cohort. Although extracellular CTSD inhibitors have shown promise in MASH treatment, optimizing the dosage through evaluation in diverse patient cohorts will be crucial for creating effective treatment regimens with minimal side effects. Further research will also be directed at investigating the exact mechanisms by which CTSD affects specific lipid composition during MASH treatment. Given the shared pathogenic mechanisms between MASH and atherosclerosis, it is essential to validate and extend the findings of this thesis in atherosclerosis models. Besides the role of CTSD in metabolic disorders, exploring the role of other cathepsins and lysosomal enzymes in metabolic disorders could provide additional insights into the broader implications in these conditions. For instance, considering the crucial role of cathepsins in HCC, it is also important to explore the effects of both intracellular and extracellular cathepsins, specifically CTSD, CTSB and CTSS, in the progression of HCC. In addition, all results of the current project should be validated in a big cohort and in more human models. Lastly, based on the mechanistic study of NPC1 presented in this thesis, future research should validate the findings related to HSC mobilization using fresh blood samples from NPC1 patients. Overall, the findings from this thesis offer a foundation for

future research that will advance our understanding of the relationship between lysosomal enzymes and metabolic disorders, paving the way for improved diagnostic and therapeutic approaches.

## References

[1] Li W, Qiu X, Ma H, Geng Q. Incidence and long-term specific mortality trends of metabolic syndrome in the United States. Front Endocrinol (Lausanne) 2022;13:1029736.
[2] Chiarelli F, Mohn A. Early diagnosis of metabolic syndrome in children. The Lancet Child & Adolescent Health 2017;1:86-88.
[3] Mohamed SM, Shalaby MA, El-Shiekh RA, El-Banna HA, Emam SR, Bakr AF. Metabolic syndrome: risk factors, diagnosis, pathogenesis, and management with natural approaches. Food Chemistry Advances 2023;3:100335.
[4] Dragoumani K, Troumbis A, Bacopoulou F, Chrousos G. Childhood and Adolescent Obesity with Somatic Indicators of Stress, Inflammation, and Dysmetabolism before and after Intervention: A Meta-Analysis. J Pers Med 2023;13.
[5] Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. Journal of Hepatology 2023;79:516-537.
[6] Younossi ZM, Wong G, Anstee QM, Henry L. The Global Burden of Liver Disease. Clin Gastroenterol Hepatol 2023;21:1978-1991.
[7] Eskridge W, Cryer DR, Schattenberg JM, Gastaldelli A, Malhi H, Allen AM, et al. Metabolic Dysfunction-Associated Steatotic Liver Disease and Metabolic Dysfunction-Associated Steatohepatitis: The Patient and Physician Perspective. J Clin Med 2023;12.
[8] Wu X-N, Xue F, Zhang N, Zhang W, Hou J-J, Lv Y, et al. Global burden of liver cirrhosis and other chronic liver diseases caused by specific etiologies from 1990 to 2019. BMC Public Health 2024;24:363.
[9] Younossi ZM, Wong G, Anstee QM, Henry L. The Global Burden of Liver Disease. Clinical Gastroenterology and Hepatology 2023;21:1978-1991.
[10] Witkowski M, Moreno SI, Fernandes J, Johansen P, Augusto M, Nair S. The Economic Burden of Non-Alcoholic Steatohepatitis: A Systematic Review. Pharmacoeconomics 2022;40:751-776.
[11] Julien J, Ayer T, Tapper EB, Chhatwal J. The Rising Costs of Alcohol-Associated Liver Disease in the United States. Am J Gastroenterol 2024;119:270-277.
[12] Zarska A, Avgar AC, Sterling MR. Relationship Between Working Conditions, Worker Outcomes, and Patient Care: A Theoretical Model for Frontline Health Care Workers. Am J Med Qual 2021;36:429-440.
[13] Harrison SA. Use of Resmetirom in Patients With Metabolic Dysfunction-Associated Steatohepatitis. Gastroenterology & Hepatology 2024;20.
[14] van Mourik H, Li M, Baumgartner S, Theys J, Shiri-Sverdlov R. All Roads Lead to Cathepsins: The Role of Cathepsins in Non-Alcoholic Steatohepatitis-Induced Hepatocellular Carcinoma. Biomedicines 2022;10:2351.
[15] David T, Mallavialle A, Faget J, Alcaraz LB, Lapierre M, Du Roure PD, et al. Anti-cathepsin D immunotherapy triggers both innate and adaptive anti-tumour immunity in breast cancer. British Journal of Pharmacology 2023.
[16] Lin X, Dong L, Miao Q, Huang Z, Wang F. Cycloheptylprodigiosin from marine bacterium Spartinivicinus ruber MCCC 1K03745T induces a novel form of cell death characterized by Golgi disruption and enhanced secretion of cathepsin D in non-small cell lung cancer cell lines. European Journal of Pharmacology 2024;974:176608.
[17] Brown RD, Mahawar U, Wattenberg BW, Spiegel S. ORMDL mislocalization by impaired

autophagy in Niemann-Pick type C disease leads to increased de novo sphingolipid biosynthesis. Journal of Lipid Research 2024;65.
[18] Tirelli C, Rondinone O, Italia M, Mira S, Belmonte LA, De Grassi M, et al. The Genetic Basis, Lung Involvement, and Therapeutic Options in Niemann–Pick Disease: A Comprehensive Review. Biomolecules 2024;14:211.
[19] Ahmad I, Fatemi SN, Ghaheri M, Rezvani A, Khezri DA, Natami M, et al. An overview of the role of Niemann-pick C1 (NPC1) in viral infections and inhibition of viral infections through NPC1 inhibitor. Cell Communication and Signaling 2023;21:352.
[20] Li H, Zhao Q, Liu D, Zhou B, Liao F, Chen L. Cathepsin B aggravates atherosclerosis in ApoE-deficient mice by modulating vascular smooth muscle cell pyroptosis through NF-κB/NLRP3 signaling pathway. Plos one 2024;19:e0294514.
[21] Fang F, Feng T, Li J, Zhang H, Wang Q, Chen Y, et al. Cathepsin K contributed to disturbed flow-induced atherosclerosis is dependent on integrin-actin cytoskeleton–NF–κB pathway. Genes & Diseases 2023;10:583-595.
[22] Cheng XW, Narisawa M, Wang H, Piao L. Overview of multifunctional cysteinyl cathepsins in atherosclerosis-based cardiovascular disease: from insights into molecular functions to clinical implications. Cell & Bioscience 2023;13:91.
[23] Kettunen S, Ruotsalainen A-K, Örd T, Suoranta T, Heikkilä J, Kaikkonen MU, et al. Deletion of the murine ortholog of human 9p21. 3 locus promotes atherosclerosis by increasing macrophage proinflammatory activity. Frontiers in Cardiovascular Medicine 2023;10:1113890.