# The entanglement of plasma Cathepsin D and metabolic disturbances

# Appendices

## Impact paragraph

### Social-economical and clinical relevance

With the global increase of obesity, MetS has become a global and escalating public health threat.[1] Currently, it is estimated that 12–37% of the Asian population, 12–26% of the European population and more than 30% of Northern American population suffers from MetS.[2,3] Moreover, individuals suffering from MetS have high risks to develop NAFLD, T2DM and other metabolic diseases,[4,5] emphasizing the public health threat that MetS brings to the world.

Being the hepatic event of MetS, NAFLD has become a common cause of chronic liver disease in the world.[6] The prevalence of NAFLD is currently estimated to be 25% in the world, among which NASH makes up 5% of the world population. Similarly, diabetes is one of the largest global public health concerns, whose prevalence has been rising in recent decades. According to the recent report of the International Diabetes Federation (IDF), 451 million adults live with diabetes in the world in 2017, which is expected to increase to 693 million by 2045 if no effective prevention is conducted.[7,8] Among the prevalence of diabetes, T2DM accounts for about 90% of all cases. In line with the high prevalence of NAFLD and T2DM, the economic burden of NAFLD and T2DM are also huge. For instance, the United States spent approximately 103 billion dollars per year on NAFLD-related costs[9] and the healthcare costs of diabetes accounts for 10% of global health care expenditure (USD 760 billion).[10] Besides the economic burden, NAFLD and T2DM also bring huge social and clinical burdens to the world. For instance, more social or medical workers are needed to take care of NAFLD and T2DM patients. As there is no available non-invasive biomarker to diagnosis NASH and no effective pharmaceutic therapy for the treatment of NAFLD due to the largely unknown underlying mechanism of NAFLD progression (i.e., hepatic inflammation), clinicians also face a major challenge in diagnosing and treating patients with NAFLD. Likewise, the treatment of T2DM in the clinic also has some limitations due to the high price and side effects of antidiabetic reagents. Therefore, there is an enormous demand for developing novel diagnostic markers and effective therapeutics for NAFLD and T2DM as well as for MetS in general.

In the current thesis, we propose that insulin resistance might contribute to NAFLD progression via elevation of plasma CTSD activity, suggesting that plasma CTSD activity might be a promising target for the prevention and treatment of NAFLD. This finding is valuable from a clinical point of view as it might lead to the development of new effective pharmaceutic therapies for NAFLD, thus reducing social and economic burden. Additionally, as previously discussed, determining organ-specific insulin resistance rather than whole-body insulin resistance is beneficial to achieve higher intervention efficacy via organ-specific interventions. The current thesis indeed demonstrated that plasma CTSD activity might be useful as a marker

to determine hepatic insulin sensitivity in pre(diabetic) individuals, thereby providing support for clinicians to prevent the disease progression and apply the organ-specific therapy for T2DM patients. Furthermore, this thesis also indicated that plasma CTSD activity that is increased in T2DM patients compared to healthy controls, also associates with metabolic parameters of T2DM, suggesting that plasma CTSD activity is likely involved in the pathogenesis of T2DM. Therefore, from a therapeutic point of view, these findings imply that plasma CTSD activity is a promising and potential target for the treatment of T2DM. Moreover, the findings of this thesis might also be interesting for other related metabolic diseases, such as, atherosclerosis and lysosomal storage disorders as these diseases have similarities in the perspective of pathology (i.e., lipid accumulation and lysosomal dysfunction).[11-13] For instance, in the context of atherosclerosis, studies have shown that CTSD is upregulated in atherosclerotic lesions.[14] Besides CTSD, other cathepsins including cathepsin B and X have also been found to play important roles in atherosclerosis, where these cathepsins can participate in the modification and accumulation of LDL cholesterol, cellular targeting of inflammatory cells, and extracellular matrix (ECM) remodelling, thereby contributing to the pathogenesis and progression of atherosclerosis.[15] This confirms the previous suggestion that CTSD and other cathepsins could be potential targets of intervention for the prevention of atherosclerosis. Likewise, CTSD has also been found to be involved in lysosomal storage disorders such as neurodegenerative disorders (i.e., Alzheimer's disease).[16,17] Indeed, current studies have demonstrated that CTSD is closely associated with the mechanisms of neurodegeneration by playing a role in the processing of Alzheimer's disease pathogenic proteins and autophagy.[18-20] Given this fact, researchers suggest CTSD as a therapeutic target for the treatment of Alzheimer's disease.[21] In addition to CTSD, other lysosomal enzymes, such as cathepsin B and cathepsin S are also reported to be involved in Alzheimer's disease [22-24] likely via initiating apoptotic cell death and activating inflammatory processes[25,26]. As such, our findings in this thesis could be extrapolated to other metabolic diseases where the clinical relevance of targeting other lysosomal enzymes in the context of those metabolic-related diseases should be further considered for investigation. Altogether, the successful translation of our findings from this thesis into the clinic in the future would probably improve the life quality of NAFLD, T2DM and MetS patients in general and alleviate the social, economic and clinical burdens that these diseases bring to the world.

**Novelty of the concept**

In this thesis, we investigated the link between plasma CTSD to different aspects of MetS (i.e., NAFLD and T2DM) in clinical cohorts. Here, we demonstrated for the first time that except for the liver, myosteatosis also links to plasma CTSD levels in NAFLD patients, further confirming the important role of the muscle in the context of MetS. Additionally, while studies have shown that insulin resistance contributes to the pathogenesis of NAFLD, the underlying mechanism is still not yet fully understood. Together with our previous findings, this thesis

proposes that insulin resistance might contribute to the progression of NAFLD via elevation of plasma CTSD activity, thereby elucidating part of a potential mechanism for the progression of NAFLD. Moreover, as previously discussed, assessing organ-specific insulin resistance is urgent for achieving organ-specific intervention for (pre)diabetic individuals. While currently there is no available non-invasive tool to determine organ-specific insulin resistance, this thesis suggests that plasma CTSD activity might be a potential non-invasive marker to determine hepatic insulin sensitivity, which is a non-invasive, easier-performed and faster approach. Thus, our finding provides a novel concept for a non-invasive tool to identify organ-specific insulin resistance. Finally, this thesis also demonstrated that plasma CTSD activity is likely involved in the pathogenesis of T2DM, thereby adding new mechanistical insight into T2DM progression and suggesting that targeting plasma CTSD activity might be of therapeutic value for T2DM patients.

**Future plan**

While the findings in this thesis provide novel diagnostic and therapeutic options for T2DM and NAFLD as well as MetS in general, more efforts have to be put to achieve the clinical translation of our findings to patients and public health. In order to implement the use of plasma CTSD activity as a marker to assess hepatic insulin sensitivity in the clinic, prognostic analyses need to be performed and cut-off values should be determined in other cohorts. Additionally, the value of plasma CTSD as biomarkers should also be investigated in combination with other (organ-specific) markers. Additionally, while there are commercial CTSD inhibitors available, so far, they are not capable of targeting circulating CTSD activity specifically. Hence, further research to develop specific inhibitors for targeting plasma CTSD activity which will have less side effects is particularly attractive for the treatment of NAFLD and T2DM. Apart from these two aspects, another one of our focuses is to perform validation studies in additional models and cohorts also in the context of other metabolic diseases. This is already in progress as this project is supported by a competitive grant from the TKI (a collaborative project between national and international academic researchers together with companies and patient organizations). For instance, *in-viv*o experiments in insulin resistance mouse models (i.e., *ob/ob* mice) will be performed with specific CTSD inhibitors to validate the finding that plasma CTSD plays an important role in insulin resistance. In addition to animal models, our group also has access to plasma samples of a hepatocellular carcinoma (HCC) cohort and alcoholic liver disease (ALD) cohort which we will use to validate our findings with respect to plasma CTSD in lipid metabolism, inflammation and insulin resistance. Besides the role of CTSD in metabolic diseases, investigating the role of other lysosomal enzymes in the context of metabolic diseases is another valuable direction for future research. As mentioned earlier, other lysosomal enzymes also play important roles in metabolic diseases. For instance, cathepsin S has been suggested as a potential biomarker and therapeutic target in inflammatory bowel diseases (IBD) as it has been shown that cathepsin S is a key driver of

intestinal inflammation[27]. Therefore, investigating the role of cathepsin S in other metabolic diseases in the context of inflammation would be very promising. Finally, given the fact that CTSD has dual mechanisms of action either through ligand (CTSD levels) interactions or protease activity (CTSD activity),[28] figuring out the difference between CTSD levels and activity, especially from the perspective of underlying mechanisms (i.e., molecular signalling pathway) is also one of our points of interest for further research. Based on our finding that plasma CTSD activity is always associated with insulin resistance in different cohorts, it would be interesting to first investigate the effect of CTSD enzymatic activity on insulin signalling pathway via utilizing specific CTSD activity inhibitors. Additionally, LDL receptor-related protein-1 (LRP1) receptor interactions with CTSD is a good start to explore the effect of CTSD as a ligand in terms of lipid metabolism and inflammation signalling pathways[29,30]. Overall, our findings from this thesis provide multiple ideas for future research which can be built upon to better understand the link between lysosomal enzymes and metabolic diseases.

# Appendices

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