Mind the gatekeeper

Impact paragraph

Scientific and socio-economic advances have brought on a number of innovations that have irremediably transformed human lives. While many of these advances have improved the average individual's quality of life and lifespan, they carry their own set of challenges, such as high levels of stress and loneliness, as well as the increasing prevalence of metabolic diseases. A staggering portion of the population suffers from, or will at some point develop, overweight/obesity and Metabolic Syndrome. In 2016, the World Health Organization estimates that 1.9 billion adults were overweight, 650 million of which were obese (1). In addition, nearly 378 million adolescents and children under five years of age are either overweight or obese worldwide (1, 2). Furthermore, it is estimated that nearly a quarter of the population presents Metabolic Syndrome, a group of metabolic disease risk factors that includes hyperlipidemia, hyperglycemia and high blood pressure (3).

While the combination of overnutrition and low energy expenditure underlies the development of overweight/obesity and Metabolic Syndrome, there are a number of factors that can influence this ratio, including cultural and socio-economic elements; genetic variants and mutations impacting energy metabolism; and medication, as well as disability (4). Nonetheless, socio-economic factors and education are the most important contributors to the development of overweight and Metabolic Syndrome, and these disorders are becoming more and more prevalent in developing countries and low-income populations (5). Regardless of the underlying causes, the development of effective preventive strategies and therapies is more important than ever. If untreated, overweight/obese and Metabolic Syndrome patients are at high risk of developing non-communicable diseases (NCDs) such as type 2 diabetes mellitus, non-alcoholic steatohepatitis, cardiovascular disease, musculoskeletal disorders, neurodegenerative and neuropsychiatric disorders (Alzheimer's disease, depression) and several types of cancer. Altogether, the abovementioned NCDs account for more than 70% of preventable, premature deaths worldwide, and this number is expected to increase in years to come (6). Metabolic disorders and NCDs cause a tremendous burden on affected individuals and their families on both an emotional and economical level, as patients struggle to remain employed and keep up with health costs. As such, in addition to posing a threat to public health, metabolic disorders and associated NCD burden are important components of the pervasive vicious cycle of poverty, and thus interfere with socio-economic development where it is often needed the most. Of note, health costs and loss of economic activity associated with NCDs were estimated to result in the loss of nearly 38 trillion euros between 2010 and 2030 worldwide (i.e., nearly half of the global gross domestic product in 2019) (7), further emphasizing the impact of metabolic disorders and NCDs on socio-economic development. Another important factor associated with increased

development of metabolic disorders and associated NCDs is aging. In 2018, the estimated number of individuals over the age of 64 surpassed the number of infants for the first time in human history, and it is well known that the risk of metabolic dysfunction and development of NCDs is positively associated with aging. Ironically, although life expectancy has increased by several years in many countries worldwide, such trends are slowing down, possibly due to increased mortality associated with NCDs, among other factors (8). Nonetheless, it is undeniable that current demographics and increasing numbers of older individuals represent an important factor to the increasing prevalence of metabolic disorders and NCDs, particularly in developed countries. Of note, metabolic disorders and NCDs make patients more vulnerable to infectious diseases and associated comorbidities and mortality (9, 10), a fact worth mentioning in light of recent epidemic and pandemic events, such as those mediated by SARS and, recently, COVID-19. All considered, research into pathways underlying disease development, in parallel with policy changes, is essential for patients, clinicians and societies alike, in order to develop efficient preventive and therapeutical strategies to improve life quality of metabolic disease patients.

Despite the different pathophysiology of NCDs such as NASH, Alzheimer's disease and cancer, they often feature overlapping underlying pathological mechanisms, including inflammation, oxidative stress, dysfunctional energy metabolism and lysosomal dysfunction. Of note, lysosomal dysfunction is both an important trigger and contributor to disease burden, as is clear in the case of NPC1 disease, a severe genetic disease. Although rare, NPC1 disease takes a great toll in patients, triggering the development of liver, spleen and lung dysfunction. In addition, NPC1 disease patients suffer from debilitating and progressive neurodegeneration which impairs their motor and cognitive abilities. While some NPC1 disease patients suffer from relatively mild and late-onset symptoms, many experience disabling peripheral and neurological disease manifestations from a very young age. Due to a combination of disease severity, delayed diagnosis and limited therapeutical tools, NPC1 disease patients suffer from strongly reduced life quality and lifespan. As such, further research into NPC1 disease is required, in order to develop more efficient diagnostic and therapeutic strategies. In addition, given the aforementioned overlap between pathophysiological mechanisms of NPC1 disease and metabolic NCDs, findings pertaining to NPC1 disease may be applicable to a wide range of NCDs, and vice-versa. In addition, several studies have found genetic *NPC1* SNPs and heterozygous *NPC1* loss-of-function mutations in overweight and obese individuals, as well as patients with type 2 diabetes and cardiovascular disease. These studies further link (mild) NPC1 impairment and lysosomal lipid accumulation in highly prevalent metabolic disorders and NCDs, further emphasizing the wide application value of NPC1 disease findings.

In this thesis, we reported for the first time that NPC1 impairment in blood-derived immune cells shifts the gut microbiome composition in a HFC diet setting (**chapter 4**). Future studies should further explore whether NPC1 dysfunction, as well as lifestyle-associated lysosomal lipid accumulation, is also associated with gut microbiome composition in humans. In addition, further studies should assess whether the interaction between NPC1 impairment and HFC diet results in a well-defined gut microbiome landscape. If so, the detection of such a characteristic gut microbiome via stool samples could translate into a non-invasive diagnosis tool for lysosomal cholesterol accumulation in individuals and allow for clinicians to put in practice more targeted and efficient therapeutic strategies. In addition, while the role of the gut microbiome in health and disease has yet to be fully elucidated, altered gut microbiome composition has been associated with NCDs such as liver disease and cancer. As such, it is possible that host NPC1 impairment/HFC diet and the resulting gut microbiome structure contributes to disease burden severity. Therefore, tools to reshape the gut microbiome may be promising, affordable and accessible tools to improve NPC1-related host features, such as dyslipidemia and inflammation, and thus prevent the development of severe NCDs in individuals with lysosomal cholesterol accumulation, as observed in obesity and MetS.

Furthermore, in **chapter 2**, we showed that increasing plant stanol consumption improves liver lipid metabolism and as well as liver and peripheral inflammation in a mouse model for NPC1 disease. Dietary plant stanol supplementation, via supplements or plant stanol-enriched margarines, is a tool commonly used to reduce hypercholesterolemia in patients. As such, it is a widely available and affordable tool which may be beneficial to a portion of NPC1 disease patients, in combination with other therapeutic compounds. Of note, dysphagia, i.e., difficulties swallowing, is a symptom that many NPC1 disease patients suffer from; as such, dietary supplementations and oral pharmacological interventions are unlikely to be applicable to patients with severe NPC1 disease. Nonetheless, our findings from **chapter 2** indicate that dietary plant stanol supplementation is an effective tool to reduce liver inflammation in a disease model characterized by severe lysosomal lipid accumulation. This observation suggests that dietary plant stanol supplementation may be a valuable tool to prevent or reduce systemic inflammation in a wider range of NCDs characterized by lysosomal lipid accumulation, such as obesity, MetS and NASH, in addition to reducing hypercholesterolemia.

Moreover, in **chapter 5**, we demonstrated that increasing antibody levels against oxLDL reduces systemic and neurological symptoms of NPC1 disease. While oxLDL is not a primary source of NPC1 disease, our findings emphasize that oxLDL contributes to NPC1 disease severity by exacerbating metabolic dysfunction, oxidative stress and inflammation. As such,

reducing oxLDL levels may remove further challenges (i.e. inflammation, oxidative stress) that altogether contribute to further lysosomal dysfunction and severe tissue and organ dysfunction. Indeed, previous studies found that compounds targeting parallel disease mechanisms in many cases work in synergy, enhancing the overall benefits regarding disease progression and burden. As such, the combination of tools targeting parallel disease mechanisms is likely to benefit individuals with complex diseases for which there are no definite curative treatments, such as obesity and MetS and related NCDs.

Similarly to NPC1 disease, high levels of oxLDL have been associated with the progression of highly prevalent NCDs, such as NASH, cardiovascular disease, Alzheimer's disease and some types of cancer. As such, tools to reduce oxLDL show great promise to improve quality of life for a great number of patients. Importantly, while increasing antibody levels against oxLDL may be an effective tool to reduce inflammation, oxidative stress and overall disease burden, our observations from **chapter 6**, in line with reports from other investigators, indicate that pneumococcal vaccination does not increase antibodies against oxLDL in human patients. As such, our results emphasize the need to develop alternative strategies to target oxLDL in human patients, such as vaccines tailored specifically to trigger antibodies against oxLDL or even nano compounds targeting this molecule.

Overall, the findings reported in this thesis highlight the consequences of lysosomal lipid accumulation and the potential of different therapeutic strategies in ameliorating NPC1 disease. In addition to being applicable to NPC1 disease, such findings may also come to benefit patients with metabolic disorders and associated NCDs.

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