Project 8: Dietary interventions in prevention of infectious diseases

The current project will be conducted through collaboration between Technische Universität Dresden (TUD), the University of Zurich (UZH), and ETH Zurich. It is part of an International Research Training Group (IRTG3019) titled "Metabolic and Endocrine Drivers of Infection Susceptibility" comprising a total of 9 projects. Within this collaboration, students will have the opportunity to obtain a joint certificate from TUD and the universities in Zurich. The current project will take place in Dresden, with the option of an exchange to Zurich, under the supervision of Prof. Dr. Hani Harb and Prof. Dr. Nikolaos Perakakis.

(Hani Harb, Nikolaos Perakakis & Philipp Gerber, Annelies Zinkernagel)

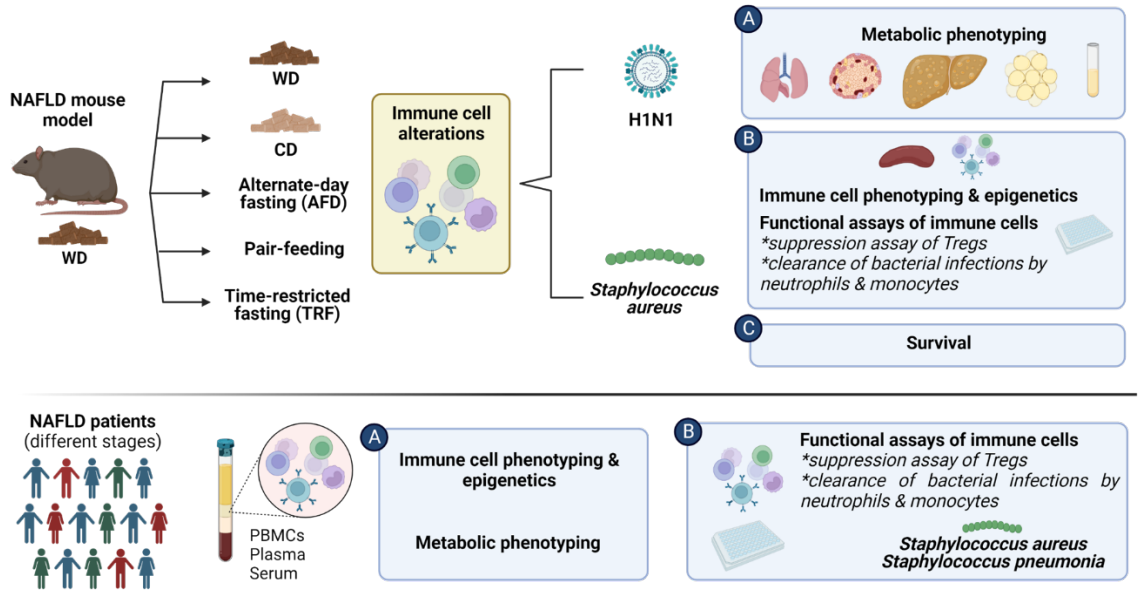

Figure 8P: *NAFLD may induce systemic changes in immune system function which they may increase the risk of severe infection. Intermittent fasting may restore these perturbations thus improving the response to infections Created with BioRender.com.*

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease and is considered the hepatic manifestation of metabolic syndrome (1). NAFLD is characterized by the accumulation of liver fat (steatosis), that can progress to liver inflammation (non-alcoholic steatohepatitis, NASH) and in later stages to liver fibrosis and cirrhosis (2; 3). Recent studies have shown that NAFLD is associated with increased risk of severe infection (4-6). This risk is evident in all stages of NAFLD and increases as disease progresses. Importantly, this elevated risk remains after adjusting for all parameters of metabolic syndrome and after performing respective sensitivity analyses (4). The underlying mechanisms of this association remain largely unknown and it is speculated that a dysregulation of the innate and adaptive – cellular immune system may occur, which might be similar to the perturbations in immune system response observed in type 2 diabetes (7-9). Intermittent fasting (i.e. alternate-day fasting [ADF] or time restricted feeding [TRF]) has been shown to improve hepatic state and promote weight loss in adults with NAFLD (10). Additionally, intermittent fasting has been shown to exert beneficial immunomodulatory effects in healthy populations and in people with obesity (11). Whether improvement of hepatic state with intermittent fasting can restore immune system response and can reduce infection susceptibility and severity in patients with NAFLD remains a critical and yet unresolved question. Similarly, it remains unclear whether ARD or TRF might be more beneficial for immune system response to infection and whether any protective effects against severe infection are partially or exclusively dependent on concomitant weight – loss due to the diet.

Aims: First aim of this project is to assess whether development and progression of NAFLD due to overnutrition is associated with alterations in both innate and adaptive immune system

(neutrophils, monocytes, innate lymphoid cells and T-cells) response that might increase the risk of severe infection. Second aim of this project is to evaluate whether intermittent fasting (ARD or TRF) is able to restore the NAFLD-related perturbations due to overnutrition in immune system, thus attenuating the risk of severe infection. Third aim is to assess whether the restoration of immune system response differs according to the type of intermittent fasting (ARD or TRF), as well as whether it is partially or completely weight-loss dependent.

Approach: Aim 1 => For this aim, we will employ our already established mouse model of NAFLD. C57BL6 mice will be fed a high fat (40%), high fructose (20%) and cholesterol (2%) diet (WD) or Chow diet (CD) for up to 36 weeks. The suggested WD demonstrates several advantages: a) Mice develop NAFLD which progresses from steatosis to NASH and liver fibrosis, thus resembling the human course of the disease (12; 13), b) The transcriptomic/metabolomic analysis supports the high translatability of the model to humans (14; 15), c) Mice additionally present a metabolic phenotype with obesity and insulin resistance that it is also often present in patients with NAFLD (12; 13; 16). Perakakis group has experience with the WD model and has used it to evaluate the impact of different treatments in NAFLD **(16-18)**. Following the 36 weeks diet a viral (H1N1) (Harb group) or a bacterial infection (Staphylococcus aureus) (Zinkernagel group) will be induced. Survival, immune cell activity, epigenetic regulation of immune cells (Harb and Zinkernagel group), as well as metabolic and hormonal effects (both systemic and tissue-specific, such as insulin sensitivity, glucose tolerance, lipid accumulation, lipidomic/metabolomics profile – Perakakis group) will be evaluated and compared between groups before and during the infection as well as after the resolving of the infection (6 weeks follow-up).
In parallel, in order to elucidate the translational potential of these findings, we will utilize blood samples from subjects at different NAFLD stages (healthy, steatosis, steatosis with fibrosis). The samples will derive from studies that the Perakakis group will be performing (protocol approved by the ethic committee of Dresden) and not only the hepatic but also the metabolic state of the subjects will be thoroughly evaluated. Furthermore, we are going to perform a complete immune and metabolic phenotyping which includes assessment of cytokine levels, quantitative and qualitative characteristics of immune cells, including epigenetic modifications (Harb group). Similarly, we are going to evaluate clearance of intracellular bacteria by neutrophils and monocytes of the above study participants upon challenge with Staphylococcus aureus, in line with our previous work (Zinkernagel group) **(19; 20)**. Furthermore, we will assess the suppressive capacity of T regulatory cells in controlling the immune system as shown by us previously (Harb group) **(21; 22)**. We are going further to investigate the factors that may affect intracellular killing capacity of immune cells upon bacterial stimulation, describe the functional characteristics of immune cells and signalling alterations involved in dysfunctional phenotypes.
Aim 2 & Aim 3 => We are going to use the same model described in Aim 1, but after 28 weeks of WD, mice will be randomized to be fed either ad libitum or to follow an ADF or a TRF diet protocol for 8 weeks. A pair-fed group for ADF will be also included in order to dissect between weight-loss dependent and weight-loss independent effects. After 8 weeks of intermittent fasting (36 weeks in total) a viral (H1N1) or a bacterial infection (Staphylococcus aureus) will be induced. Metabolic, immunologic and infection-related outcomes will be assessed as described in Aim 1.
In order to evaluate the translational potential of these findings, we will also utilize blood samples from individuals undergoing ADF, TRF vs control (Gerber group). The Gerber group has expertise in nutritional-clinical studies **(23; 24)** and is currently performing a study evaluating the impact of ADF and TRF in overweight individuals (LIMITFOOD, NCT04732130). A similar study will be performed in patients with obesity who will be also screened non-invasively for the presence of NAFLD (Study protocol currently under evaluation by the ethic committee in UZH). Similar to Aim 1, possible restoration of immune cell phenotyping and function as well as of the ability of monocytes and neutrophils to clear intracellular bacteria will be investigated ex vivo following an ADF or a TRF diet.

Topics for the PhD studentships will be:

Ad In vivo assessment of metabolic and immune system alterations in response to dietary interventions (ADF, TRF, reversal chow, pair-fed to ADF) in a mouse model of NAFLD (with concomitant obesity, insulin resistance due to overnutrition) before and after induction of a:

i) Staphylococcus aureus infection (Zurich), **ii)** H1N1 infection (Dresden)

Ad iii) Quantitative and qualitative assessment of plasma immune profile and immune cell function, including upon bacterial challenge, in metabolically characterized patients with NAFLD vs healthy ones, as well as before and after intermittent fasting (Dresden & Zurich)

Work to be performed in Dresden: Students will work on a mouse model of NAFLD which will undergo different dietary interventions. They will learn different techniques of molecular biology, perform relevant assays, and consecutive analysis of metabolic and hepatic outcomes (Perakakis group). Furthermore, the students will conduct the mouse H1N1 model, combined with the previously mentioned mouse models (Harb group). Additionally, students will assist with the clinical study of Aim 1 and will perform relevant metabolic and immunologic phenotyping (Perakakis group). Students will further assess innate lymphoid cells and T-cell function, including epigenetic modifications of the respective cells both in the animal model and in the human samples along with suppression assays for Treg cells (Harb group).

Work to be performed in Zurich: Students will work on the mouse model of Staphylococcus aureus infection performing all the relevant immunologic analysis (Zinkernagel). Furthermore students will assist with the human study of Aim 2 and perform the metabolic and hormonal characterization (Gerber group). Furthermore, they are going to perform the ex-vivo assessment of neutrophils and monocytes, including relevant bacterial challenges, from animal and human samples (Zinkernagel).

Added value through the collaboration between Dresden & Zurich: Perakakis group has expertise in metabolism/NAFLD **(16-18; 25-30)** and Harb group **(21; 22; 31-34)** in infectious immunology and they will be responsible for the NAFLD mouse models of viral infection. The Zinkernagel group has great expertise in bacterial infections **(19; 20; 35-38)** and will be responsible for the Staphylococcus aureus infection model and for the ex vivo assessments after bacterial challenge. Gerber group **(23; 24; 39; 40)** has expertise in clinical nutrition/metabolism and will be responsible for the diet intervention in humans.

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