Project 5: Gut infection modulating glucose metabolism

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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. Nikolaos Perakakis.

Background: We have recently demonstrated that the gut plays a crucial role in regulating insulin sensitivity by affecting hepatic gluconeogenesis and muscle insulin resistance (Mingrone group) **(1, 2)**. Additionally, gut-secreted hormones such as incretins are involved in the regulation of insulin secretion, appetite, energy homeostasis and glucose metabolism (Perakakis group) **(3-6).** Furthermore, we have shown that gut inflammation, characterized by the presence of pro-inflammatory intestinal macrophages, plays an important role in metabolic diseases such as obesity by affecting glucose metabolism (Cavelti-Weder group) **(7, 8)** (Figure 1A). We could show that this shift towards inflammatory intestinal macrophages is linked to glucose homeostasis as colon-specific macrophage depletion by intrarectal administrations of clodronate liposomes led to improved glucose tolerance (Fig. P5).

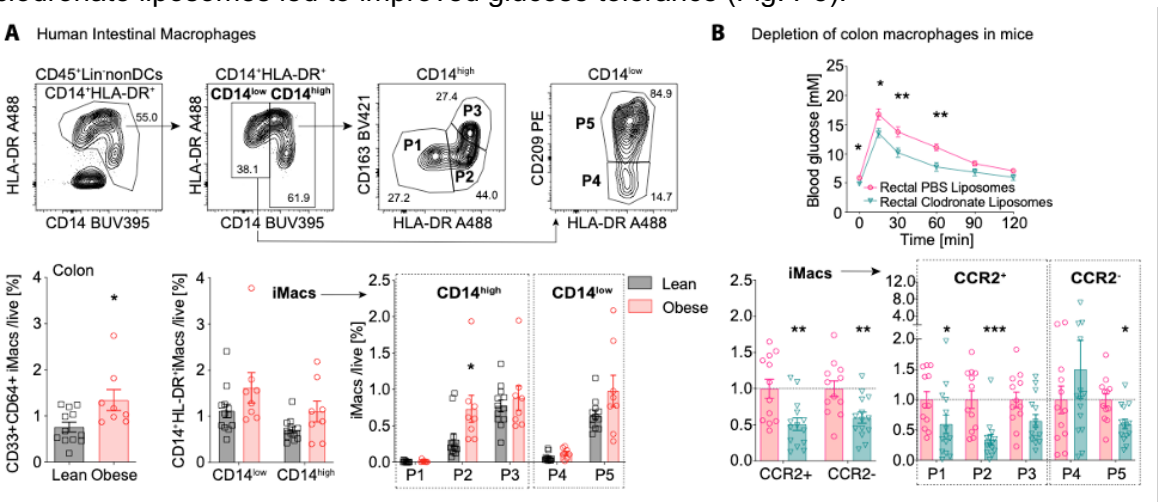

*Fig. P5**: Increased inflammatory (P2) intestinal macrophages in obesity.** (**A:** Humans, **B:** improved glycemia by mac depletion in mice).*

Aims: We aim to test whether gut inflammatory responses elicited either by an infectious or non-infectious agent are able to regulate glucose and lipid homeostasis, thus contributing to the development or aggravation of metabolic diseases and specifically to progression of hyperglycaemia.

Approach: First, we will use mouse models of gastrointestinal infections that do not elicit a systemic inflammatory response. For example, trichomonas infections of the gut are considered as “non-pathogenic” as they are limited to the gastrointestinal tract. We (Zürich group) will characterize the metabolic phenotype of mice infected with trichomonas (mouse model recently established in our lab) compared to uncolonized controls. We will perform immune cell phenotyping of the gut, other organs and the blood to better understand the role of gut immunity in regulating glucose homeostasis. The gut microbiome will be also studied by 16s rRNA gene amplicon sequencing to assess interactions between trichomonas and gut bacteria. We will perform the above experiments both in metabolically healthy mice fed chow-diet, as well as in high fat diet - fed mice (12 weeks of high fat diet) that become metabolically unhealthy by developing obesity, insulin resistance and mild hyperglycemia. Additionally, we (Dresden group – Perakakis group with Mingrone group) will perform two human studies. In the first cross-sectional study, we will recruit subjects with obesity in different glycemic control,

ranging from normal glucose tolerance to prediabetes and treatment-naïve diabetes and collect baseline characteristics including gender, age, comorbidities, and medications. Patients will undergo a colonoscopy (as part of preventive measures) with biopsies from the colon as well as a metabolic phenotyping including a 5-time point oral glucose tolerance test, a mixed-meal test, a body composition analysis and liver fat assessment with Fibroscan-CAP. Intestinal macrophages and peripheral blood mononuclear cells will be isolated and sorted for identifying specific macrophage subpopulation and compared between study groups. RNAseq in gut biopsies and proteomic/metabolomic/lipidomic profiling in biopsies and blood will be used to evaluate the inflammatory and lipid-lipoprotein status. Gut microbiome analysis by using 16S rRNA gene amplicon sequencing will be performed. Gut hormones (Glucagon, GLP-1, GLP-2, GIP, glicentin, oxyntomodulin) will be assessed during the OGTT and mixed meal. Insulin sensitivity assessed with Matsuda index and β-cell function with insulinogenic and disposition index will be correlated with both peripheral and gut-related inflammatory status. This study will elucidate whether gut inflammation is a major characteristic of metabolic dysregulation. In the second prospective study, we will recruit patients with an inflammatory process of the gastrointestinal tract of infectious (e.g. infection with clostridium difficile) or non-infectious origin (e.g. diverticulitis, microscopic colitis). Patients will serve as their own controls and undergo the above metabolic testing during and after the remission of the disease, i.e. 3-6 months after the initial visit. Resolution of the gastrointestinal disease will be determined as by clinical standard of care (i.e. clinical status, fecal calprotectin, ultrasound). This second study will answer the question how gut inflammatory processes due to gastrointestinal tract diseases may affect glucose homeostasis in humans.

Topics for the PhD studentships will be:

Ad i) Mouse study of gut-specific infection by non-pathogenic trichomonas to characterize gut-specific peripheral inflammatory response and related metabolic phenotype in metabolically unhealthy mice (Zurich)

Ad ii) Human study of patients with obesity at different metabolic state (i.e. normoglycemia, prediabetes, diabetes) to assess whether the level of glucose homeostasis/ metabolic state is associated with the level of gut inflammation. (Dresden)

Ad iii) Human study of infectious (i.e. infection with clostridium difficile) or non-infectious diseases (i.e. colitis ulcerosa, diverticulitis, microscopic colitis) to characterize glucose metabolism before and after remission of the disease (Dresden & Zurich)

Work to be performed at TUD: The student will be trained in all aspects of a clinical study, multiomics analysis, FACS sorting, working with human tissues, i.e. IHC, RNAseq, isolation of macrophages and detection of macrophage subpopulations.

Work to be performed in Zurich: The student will be trained in all aspects of a mouse study, including techniques of working with mouse tissues, i.e. IHC, RNAseq, isolation of macrophages and detection of macrophage subpopulation.

Added value through the collaboration between Dresden & Zurich: Dresden groups (Perakakis, Mingrone) have great experience with clinical studies involving patients with metabolic diseases and in the evaluation of glucometabolic status in different energy states **(3, 4, 6, 9-12)** . The two groups will join forces and will be responsible for the clinical studies of the project. The Zurich group has great experience on immunologic mouse studies, focusing on gut inflammation and will be responsible for performing the mouse studies **(7, 8, 13-16)**. Additionally the Zürich group will advise Dresden groups and contribute to the immunologic phenotyping of the clinical studies. Combining these areas of expertise will provide important knowledge on the possible involvement of the inflammatory state of the gut due to infectious diseases in the regulation of glucose homeostasis.

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