# MASTER BIOLOGIE-SANTE – SUJETS PROPOSES PAR UMR 1011
# PARCOURS PRECISION HEALTH

## The role of ER-mitochondria contact sites (MAM) in GLP-1 secretion by L cells in the human intestinal organoid model/Diabetes and Cardiovascular Diseases and Precision Health courses

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Type 2 diabetes, linked to dysregulation of glucose metabolism, is a global health emergency. In long term, it can lead to cardiometabolic complications.
The intestine plays a major endocrine role by secreting hormones including the incretin GLP-1 (Glucagon-Like Peptide 1), which ensures glycaemic balance by potentiating the secretion of insulin by pancreatic β-cell in response to glucose (Lu *et al.,* 2021). The contact sites between the endoplasmic reticulum and mitochondria (MAM: Mitochondria-Associated Membranes) and their dynamics are essential for ensuring insulin sensitivity in liver and muscle and insulin secretion by the pancreas (Rieusset, 2018). Preliminary *in vitro* results in a murine L cell line show that MAMs are also involved in GLP-1 secretion.
The aim of the M2 internship is to study the role of MAMs in GLP-1 secretion by intestinal L cells using an original and complex *ex vivo* model of human intestinal organoids. The organoids will be exposed to various GLP-1 secretagogues (glucose, bile acids, fatty acids, amino acids, etc.). GLP-1 will be measured by ELISA in the supernatant and MAMs will be quantified by PLA (Proximity Ligation Assay) specifically in L cells using GLP-1 immunolocalization. These techniques are mastered in the laboratory. Following the development of adenovirus transfection of organoids, MAMs will be inhibited by a protein, the FATE1 spacer, in order to confirm the role of MAMs in GLP-1 secretion by human intestinal epithelium.
These results should help to position MAMs as potential therapeutic targets in type 2 diabetes to restore insulin sensitivity and increase insulin secretion.

## RevErbα in the gut as target in the control of metabolic disorders-related complications

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Nuclear receptors are transcription factors that modulate the expression of target genes in response to specific ligands. Among these, Rev-Erbα is highly expressed in the body and

participates in energy homeostasis, coordinating lipid, carbohydrate and bile acid metabolism with the biological clock.

The intestine plays a unique role in metabolic defense, regulating the absorption of dietary lipids and also contributing to glucose homeostasis. As an endocrine organ, it secretes large quantities of hormones and bioactive peptides that regulate various metabolic processes such as energy homeostasis, intestinal motility and local immunity, as well as its barrier function toward the microbiota. Significant abnormalities in intestinal function favor type 2 diabetes and obesity, leading to atherosclerosis and steatohepatitis.

The project we are proposing for an M2 is part of our research into the molecular mechanisms by which the nuclear receptor RevErbα, expressed in intestinal cells, controls specific intestinal functions such as dietary lipid absorption, barrier function or enteroendocrine response. It is based on important preliminary results in the human enterocyte model Caco-2/TC7 and in murine intestinal organoids, showing disruption of dietary lipid metabolism in the absence of RevErbα.

The approaches employed involve cell and molecular biology techniques (gene and protein expression analysis, indirect immunofluorescence and video microscopy, protein half-life, gene invalidation and overexpression, etc.) and the use of omics approaches. This project is based on cell culture work (filter culture of the human enterocyte line Caco-2/TC7 and murine and human intestinal organoids) and the use of animal models.

## Role of « ubiquitin-like protein » FAT10 in the dévelopment of hepatic insulin resistance during MASH developement

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Metabolic associated steatotic liver disease (MASLD) is now considered the hepatic component of metabolic syndrome and is associated with the development of insulin resistance (IR). This IR is defined as the reduction in the cellular and tissue response to insulin and develops following an accumulation of hepatic triglycerides (steatosis) and chronic inflammatory stress, characteristics of metabolic steatohepatitis (MASH), at high risk of rapid progression to cirrhosis. Although there are clear links, the mechanisms contributing to the development of MASH and hepatic IR remain complex and still poorly understood. Interestingly, transcriptomic analysis of liver biopsies from obese patients developing different grades of MASLD showed that FAT10/UBD expression was positively correlated with MASH severity. Modulation of FAT10 expression in human and murine hepatocytes decreases lipid droplet accumulation during MASLD, and FAT10 deficiency in aged mice has been shown to promote insulin sensitivity, suggesting that FAT10 may contribute to the development of hepatic IR. However, no study to date has demonstrated a direct role for FAT10 in the regulation of the insulin signaling pathway and the development of hepatic IR during MASH. In order to better understand the role of FAT10 in the development of IR during MASH, we propose as part of Master 2, 1) to study the role of FAT10 and its mechanism of action in the response hepatocytes to insulin and IR in a context of MASH *in vitro*, 2) to determine the role of FAT10 in hepatocytes in IR induced in a context of MASH *in vivo* in mice.

## Study of FAT10/PPARα interaction during NASH development

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The prevalence of non-alcoholic fatty liver disease (NAFLD) is on the rise. NAFLDs are initiated by steatosis progressing to non-alcoholic steatohepatitis (NASH) characterized by inflammation, ballooning of hepatocytes, and sometimes fibrosis which can progress to more serious stages ranging from cirrhosis to hepatocellular carcinoma. No pharmacological treatment is currently available. The laboratory has shown that the activation of PPARα, a nuclear receptor strongly expressed in the liver, known for its anti-inflammatory, anti-fibrotic effects and for promoting lipid metabolism, is a promising therapeutic strategy. However, the gene expression of PPARα as well as its activity are reduced in the livers of patients with NASH, partly explaining the ineffectiveness of PPARα agonists in clinical studies treating NASH. It is therefore crucial to better understand the mechanisms underlying this modulation of PPARα during the progression of NASH. Transcriptomic analysis of liver biopsies from obese patients showed that the expression of the FAT10 (UBD) gene, an ubiquitin-like protein, increases during the progression of NASH, and is inversely correlated with the expression of PPARα. FAT10 is known to be responsible for FATylation controlling the stability/degradation and activity of various proteins. Thus, FAT10 could interact with PPARα to modulate its activity during NASH. Our preliminary results show that FAT10 interacts with PPARα in hepatocytes during NASH progression *in vivo* in murine and human NASH liver biopsies and *in vitro* in HepG2 cells and contributes to inhibit PPARα activity by its agonist, pemafibrate *in vitro* and *in vivo*. FAT10 could therefore promote the progression of NASH by inducing the degradation and/or deactivation of PPARα, making any therapeutic strategy targeting PPARα ineffective. The Master 2 project therefore aims to study the FAT10/PPARα interaction during the development of NASH *in vitro* and *in vivo* in order to contribute to the identification of a new therapeutic treatment.

## Evaluation of pharmacological therapies for MASH in a new preclinical mouse model of MASLD

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MASLD (metabolic dysfunction-associated steatotic liver disease) is the most common liver disease in the world, with a prevalence estimated at 25% of the general population, but reaching 80-90% in obese adults and 50-70% in patients with type 2 diabetes. This pathology has now become a veritable global “epidemic” whose incidence continues to increase, in parallel with the growing epidemic of obesity and diabetes. MASLD is characterized in its first stage by an excessive accumulation of fat in the liver, considered as benign steatosis, in the absence of excessive alcohol consumption. During the progression of MASLD, simple steatosis can progress to MASH (Metabolic dysfunction-associated steatohepatitis), diagnosed as a combination of steatosis, inflammation and ballooning of hepatocytes. In the worst cases, liver damage can progress to fibrosis, cirrhosis and hepatocellular carcinoma, which can lead to the death of the patient. Currently, there is no approved therapeutic treatment for patients with MASH, the aggressive form of NAFLD.
In the laboratory, we developed a new mouse model which presents all stages of human MASLD pathology (liver steatosis, inflammation, ballooning and fibrosis) under high fat diet for 12 weeks. The

project aims to better understand MASH physiopathology and to test novel therapeutic targets for MASH in this model. Histological, biochemical and molecular analyzes will be carried out on the various technical platforms of the laboratory.

## Characterization of haematoma in an innovative ex vivo model, towards optimization of the management of patients with intracerebral hemorrhage

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Intracerebral hemorrhages (ICH) account for 10-20% of strokes and affect 3.5 million people worldwide every year. Only 50% of patients survive, and half of survivors suffer significant handicap. This poor prognosis is due to the lack of effective treatment for ICH. One way to improve prognosis is to increase haematoma evacuation using a fibrinolytic agent. At present, this approach is not very effective and is contraindicated in patients at high risk of hemorrhage. To optimize this approach and offer it to a greater number of patients, we need to know more about the characteristics of these haematoma. The aim of this project is to characterize haematoma using an innovative ex vivo model developed in the laboratory. The haematoma will be prepared using blood from healthy subjects and patients at high risk of ICH (patients on anticoagulants or with hemorrhagic disease). The effect of antidotes and clotting factor concentrates used in ICH will also be studied. Haematoma will be characterized by several approaches: study of the kinetics of formation, of spontaneous retraction over time and of composition by immunostaining (red blood cells, platelets, leucocytes, fibrin, etc.) combined with a 3D fluorescence imaging approach. The permeability of haematoma and the characteristics of the fibrin network will be assessed by scanning electron microscopy coupled with image analysis. The results obtained will be compared between the different groups of patients and controls. This project will provide important information that will ultimately enable to propose fibrinolytic strategies adapted to each patient. This project is part of the TIPITCH project, which aims to radically transform the prognosis of patients with ICH (https://medecine.univ-lille.fr/fileufr3s/user_upload/ufr3s-actualites/2023/recherche/2023-11-28-rhu-laureat-lillois-projet-tipitch-v4.pdf).

## Endothelium-immune cell cross-talk in metabolic-associated steatohepatitis.

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**Background**. Metabolism-associated steatohepatitis (MASH) is a pathology that can progress to cirrhosis and then to hepatacocarcinoma. In the absence of pharmacological treatment, it is the leading cause of liver transplantation in the USA. The presence of inflammatory infiltrates is one of the hallmarks of MASH, and plays an essential role in the progression of the disease.

The hepatic recruitment of immune cells by diapedesis directly depends on the interactions between immune cells and vascular endothelium which acquires a pro-inflammatory phenotype in this context. However, the precise mechanisms of this cross-talk are still not fully understood.

**Objective**. This project will examine the potential contribution of immune cell-endothelium interactions to the development of MASH, with the ultimate goal of modulating these interactions for therapeutic purposes.

**Methods**. Endothelium-immune cell interactions will be studied *in vitro*. Hepatic endothelial cells and blood immune cells will be isolated from mice developing non-alcoholic steatohepatitis (NASH) following a diet enriched in fats, cholesterol and carbohydrates, and from control mice. Phenotypic alterations in endothelium and immune cells will be analyzed by scRNAseq and validated by *in vitro* cell activation experiments, including analysis of permeability and transendothelial migration.

**Keywords.** MASH, Endothelium, Immune cells, Metabolism, Inflammation.

## Transcriptional control of the hepatocyte response to liver injury by Ubiquitin D

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https://u1011.pasteur-lille.fr/lunite/theme-4-analyse-transcriptionnelle-integree-des-maladies-hepatiques/

The liver is characterized by its regenerative potential allowing to cope with various insults and replenish the mass of functional hepatocytes. However, acute or chronic diseases can nevertheless promote liver dysfunction underlain by altered hepatocyte regeneration potential and function. Alterations to the hepatocyte transcriptome are involved in this process. In this context, we are seeking to define the role of Ubiquitin D (UBD also known as FAT10). FAT10 is a member of the eukaryotic ubiquitin-like protein family, weakly expressed in normal liver but increased by inflammatory signals upon injury. FAT10 contains two UBL domains enabling covalent interaction (FATylation) via ligases (USE1 and UBA6), or non-covalent interaction, leading its substrates to proteasomal or lysosomal degradation. Our laboratory has already developed a panel of tools to assess how FAT10 controls the hepatocyte transcriptional program (e.g. stable cell-line with FAT10 overrexpression).

We propose a Master 2 internship where the goal will be to perform in vitro assays using our already established cell-lines to define how FAT10 overrexpression or silencing impacts on expression/activity of hepatocyte transcription factors. Cellular and molecular biology approaches to define gene expression, protein levels and subcellular localization will be implemented.

## Role of the mitochondrial protein import machinery in angiogenesis in health and disease

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Mitochondria exert central functions in bioenergetics, metabolism, and apoptosis. The correct function of these organelles requires the import of > 1000 nucleus-encoded proteins as the mitochondrial genome provides only 13 proteins. A key component of the mitochondrial protein import machinery is the evolutionarily conserved CHCHD4 oxidoreductase that catalyzes the oxidative folding of targeted proteins after they cross the outer mitochondrial membrane. This mechanism is finely tuned and it is affected in disease.

Using a multidisciplinary approach, combining molecular and cellular biology techniques, this project aims at i) studying the role and functional relevance of CHCHD4 in endothelial cells, and ii) characterizing the signaling pathways that impact on the CHCHD4-dependent import pathway in angiogenesis in disease. The working hypothesis is that aberrant activity of this import pathway drives pathological angiogenesis.

The results generated with this project promise to provide unprecedented insights that will be useful for the development of novel therapeutic strategies for a variety of human diseases characterized by dysfunctional vasculature, such as cardiovascular disorders and cancer.