# A Study on Liver Metabolism

## Investigating altered metabolic fluxes in patients with non-alcoholic fatty liver disease (NAFLD)

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## 1. Introduction

Non-alcholic fatty liver disease (NAFLD) is one of the leading causes of chronic liver disease worldwide. It is defined as **macrovesicular steatosis** in > 5 % of hepatocytes, in the absence of a secondary cause. If untreated, NAFLD can progress into **fibrosis** and **inflammation** of the liver (Figure 1), and ultimately develop into **liver cirrhosis.** At this end stage, the scarring and loss of function of the tissue is so severe that the organ must be transplanted in order to ensure the continued health of the patient.

Several risk factors have been associated with the development of NAFLD, such as **obesity, type 2 diabetes mellitus (T2D)** as well as **increasing age**.

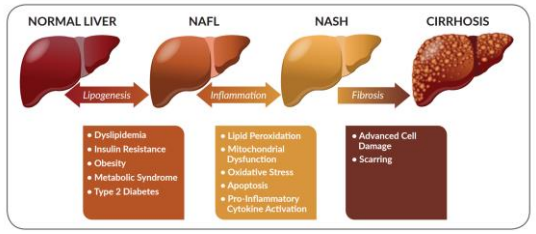


**Figure 1.** The progression of NAFLD.

Previous studies have shown that the metabolic activity of the liver is altered in individuals with NAFLD. However, as of yet, no studies have targeted alterations to **metabolic fluxes** in **live liver tissue**.

## 2. Objectives

There are two primary aims of this study:

- To develop a method that enables studies of metabolic fluxes in liver tissue (*ex vivo*) in patients with different degrees of NAFLD.
- To investigate what metabolic pathways are **affected** and **altered** in patients with NAFLD.

**Figure 2.** Liver tissue samples that have been sectioned into 250 μm thin sections are cultivated in a carbon13 enriched culture media.

**Figure 4.** A digital twin is a digitalised version of a person or an organ based on experimental data and computer models.

## 3. Method

Metabolic flux analysis is performed on liver tissue (*ex vivo*) that is cultivated in a culture medium containing carbon 13 (Figure 2). The carbon 13 isotope is stable and non -radioactive, which makes it ideal to be used as a tracer in cellular reactions. When the cells in the tissue consume the medium, the tracer is incorporated into the various metabolic reactions known to take place in the liver.

In order to cultivate the tissue under optimal conditions, it is sectioned into 250 μm thick slices (Figure 3), which allow for nutritional and oxygen uptake to be maintained throughout the different cellular layers.

Metabolic flux patterns are established through analysis with mass spectrometry. By the use of computational models, this data is converted into a digital version of the liver, referred to as a **digital twin** (Figure 4). The digital twin can then be used to make predictions regarding the different stages of the disease.

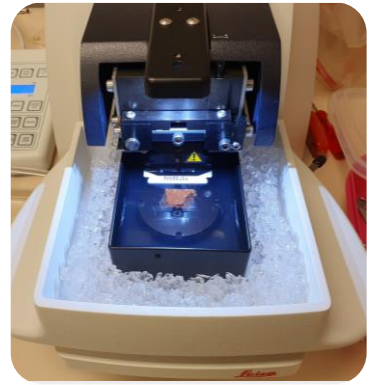

**Figure 3.** Surgically removed liver tissue is sectioned with a vibratome, in order to produce 250 μm thin slices.

## 4. Preliminary results and next step

Results from the pilot study display a satisfactory level of C13 labeled metabolites, which indicate that metabolite tracing is working. Furthermore, the mRNA expression of several key metabolic enzymes appears to be stable, suggesting that these processes can be maintained in the tissue despite the interference of surgical intervention.

However, the results also show an increased inflammatory response in the tissue, which emphasize a need to further optimize the method. This will consequently be the next step of the study.

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