Easy collagen quantification in a diet (HFD)-induced mouse model of NASH-fibrosis

Background
NAFLD encompasses a wide spectrum of liver diseases ranging from steatosis to non-alcoholic steatohepatitis (NASH) associated with fibrosis, which can eventually evolve to cirrhosis and hepatocellular carcinoma. Reliable quantification of fibrosis is of key importance in fibrosis research, with collagen being one of the major matrix components.

The aim of this project was to identify conditions for reliable collagen / fibrosis quantification in chronic NASH-related fibrosis models.

Approach
Collagen was analyzed by histology (Sirius Red staining in formalin fixed liver tissue) and by biochemical analysis (QuickZyme Sensitive Tissue Collagen assay in both frozen tissue and formalin-fixed paraffin embedded (FFPE) liver tissue).

The biochemical collagen assay protocol was adapted such that it could also be applied for FFPE liver tissue. A slice of 100 μm was sufficient for reliable collagen quantification. To correct for the amount of tissue a Total Protein assay (QuickZyme) was used which could be applied in the same acid hydrolysate.

Methods
Ldr/-/-Leiden mice were fed on chow or high-fat diet (no added cholesterol) for 40 weeks. The sinister and the medial lobes were isolated and sectioned as shown in Fig. 2. Q1 and Q2 were snap-frozen and H1 and H2 were formalin fixed and paraffin-embedded. Q1, Q2, H1 and H2 were used for collagen quantification using the biochemical assay. H1 and H2 were used for histological collagen quantification using Sirius Red staining.

Preventing assay matrix effect
Matrix effect includes interference between an assay and components in the sample. This often results in erroneous results. It occurs with all kinds of biochemical assays such as GC, HPLC, ELISA or fluorescent / colorimetric biochemical assays. It can be prevented by further dilution of the sample, which may be difficult in tissues with high matrix effect and low collagen levels such as liver.

We developed a new chromogenic collagen assay in which the matrix effect is prevented and thus no further sample dilution is needed, making it possible to measure low collagen levels in liver tissue.

Collagen levels in sinister and medial lobe
Fibrosis in the diet-induced NASH model was analyzed upon 40w feeding with chow or HFD. Biochemical collagen analysis was applied in frozen (Q1) and FFPE (H1) tissue both in the sinister and the medial liver lobes.

It was observed (Figs 5 and 6) that the biochemical and histological analysis gave similar results. It was observed (Figs 5 and 6) that the biochemical and histological analysis gave similar results. At low levels of fibrosis some variation between both methods was observed. Also, sections from H1 show a trend of higher collagen levels than sections from H2.

Histological vs biochemical collagen analysis
Fibrosis was analyzed in FFPE tissue by both biochemical quantification and histology (Sirius-Red).

Conclusions
• Low collagen levels in liver tissue can easily be quantified using either a chromogenic collagen assay or histology
Both assays can be performed in formalin fixed paraffin embedded (FFPE) tissue and give similar results, if analysed in the same piece of tissue.
• Early fibrosis in liver is ‘patchy’, probably starting from the outside of the lobe
• The sinister lobe shows a trend of higher collagen levels than the medial lobe with lower variability in analysis

For collagen quantification in diet-induced fibrosis models: 4 μm FFPE sections for histology and 100 μm FFPE sections for biochemical analysis.

Fig. 4. Collagen levels in sinister and medial lobes (biochemical method). Blue bars: chow-fed animals, Orange bars: HFD-fed animals.

Fig. 5. Fibrosis quantification by biochemical (A) and histological (B) analysis.

Fig. 6. Comparison of fibrosis quantification by biochemical and histological analysis.