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

Christa de Ruiter[1]
Joline Attema[1]
Nanda Keijzer[1]
Wim van Duyvenvoorde[1]
Aswin Menke[1]
Martine Morisson[1]
Jan Verheijen[2]
Reinout Stoop[1]
Natascha van Lent[2]
Roeland Hanemaaijer[1,2]

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.

Easy quantification of collagen using a chromogenic assay is hampered by the low levels of collagen in liver, especially in chronic preclinical models where no severe fibrosis is observed.

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.

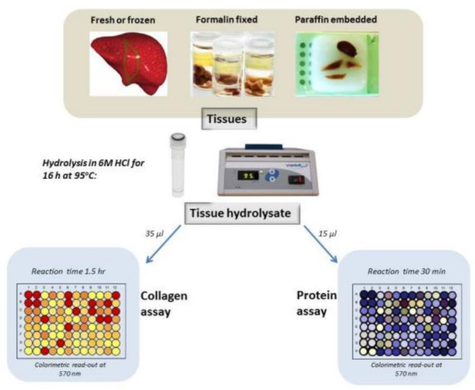
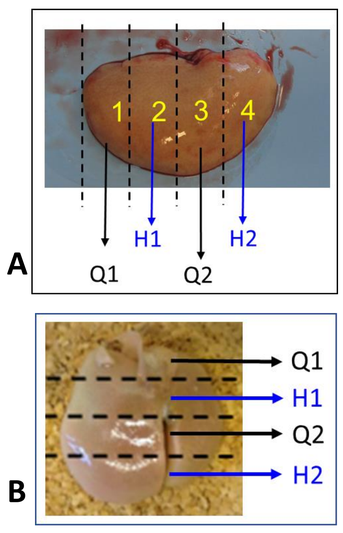


Fig. 1. Method collagen and protein quantification on FFPE tissue samples

Methods

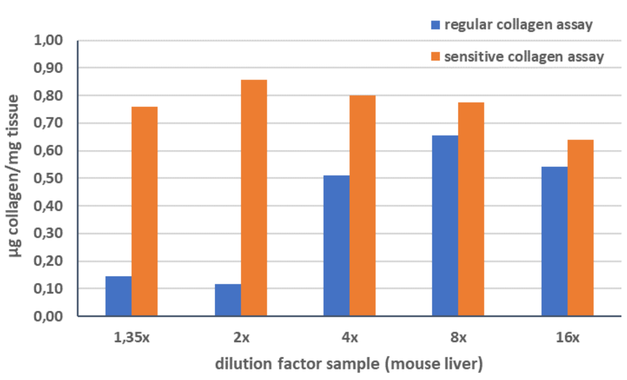


Ldlr-/-.Leiden mice were fed on chow or high-fat diet (no added cholesterol) for 40 weeks. The sinister and the medial lobes were isolated and sectioned as shown in Fig. 2. Q1 and Q2 were snap-frozen and H1 and H2 were formalin fixed and paraffin-embedded. Q1, Q2, H1 and H2 were used for collagen quantification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

Fig. 2. Sectioning of the sinister (A) and medial liver lobe (B).

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

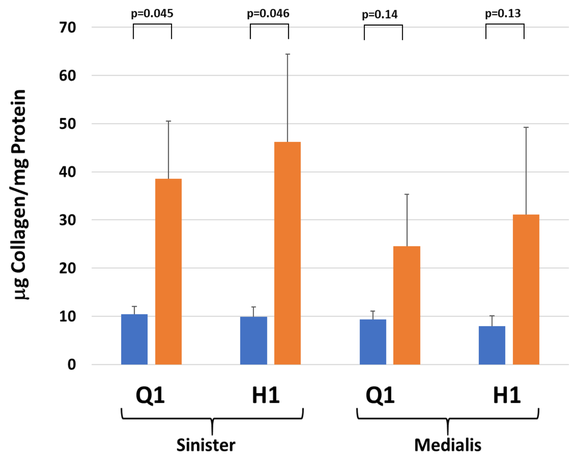


We developed a new chromogenic collagen assay in which the matrix effect is prevented and thus no further sample dilution is needed, making it possible to measure low collagen levels in liver tissue.

Fig. 3. Regular and sensitive collagen assay: effect of sample dilution

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.



A trend was observed that collagen content was higher in the sinister lobe than in the medial lobe (Fig. 4). In addition, the variation in the sinister lobe was lower, may be due to possible gall bladder effects present in the medial lobe. The data also show that FFPE tissue (Q1) can be used for biochemical collagen analysis.

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

Histological vs biochemical collagen analysis

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

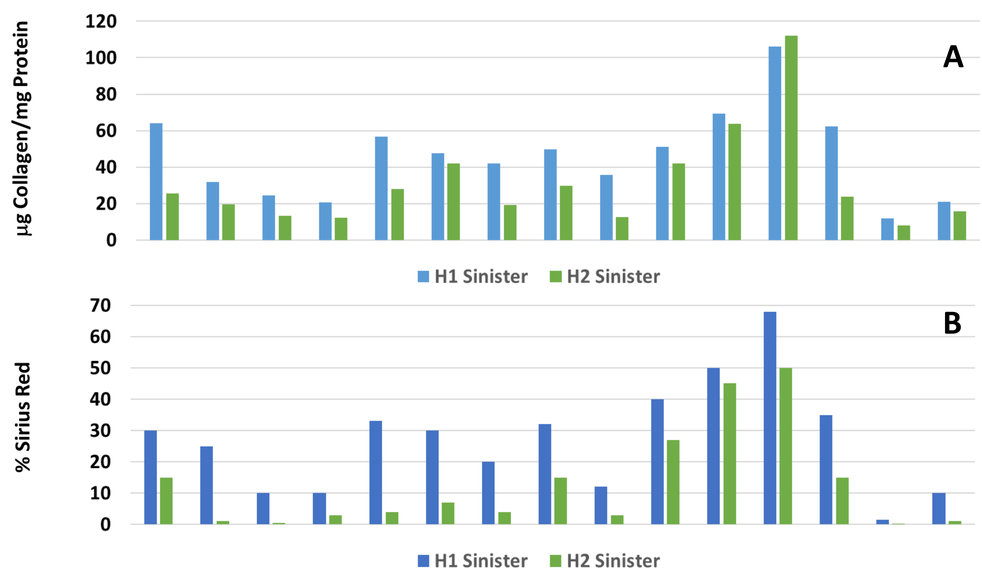
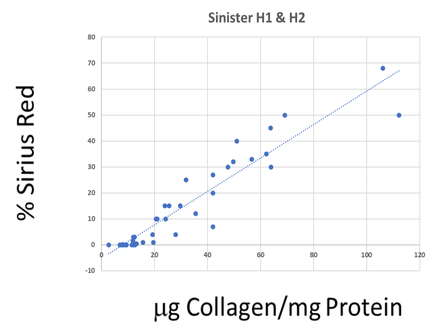


Fig. 5. Fibrosis quantification by biochemical (A) and histological (B) analysis.

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 .

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

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.

[1]TNO Metabolic Health Research, Leiden, The Netherlands; [2]QuickZyme Biosciences B.V., Leiden, The Netherlands
contact: Roeland.Hanemaaijer@tno.nl