COMPARISON OF VARIOUS QUANTITATIVE AND QUALITATIVE COLLAGEN ANALYSIS METHODS IN EARLY LIVER FIBROSIS

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BACKGROUND

Non-alcoholic steatohepatitis (NASH) is a chronic progressive liver disease that can progress to liver fibrosis. Fibrosis is considered the most important predictor of NASH-related mortality.
The main read-out parameter of fibrosis is collagen analysis. Collagen can be measured both quantitatively and qualitatively. Various methods for collagen analysis exist, but much remains unknown regarding the significance of these methods.

AIM

To compare various collagen analysis techniques in a diet-induced NASH fibrosis model as well as a CCL4-induced model.

METHODS

- Chronic liver fibrosis was studied in the diet-induced Ldlr-/-.Leiden mouse. Mice were fed a NASH-inducing high-fat diet (45 kcal% fat, 35 kcal% carbohydrate, no cholesterol) for 16 weeks to induce very early-stage hepatic fibrosis. Acute liver fibrosis was studied in a CCl_4 model (4-6 weeks).
- Hepatic collagen content was measured biochemically using the Quickzyme Total Collagen assay.
- Multiphoton and second harmonic generation (SHG) imaging of hepatic collagen was performed using a Genesis 200 imaging system and subsequent computer-assisted data analysis (Clinnovate Health, UK).
- Collagen was visualized histologically by Picro Sirius red staining and immunohistochemical staining of collagen type I and type III.

RESULTS

Low solubility of hepatic collagen

Table 1: *Extraction efficiency of collagen from liver tissue using various extraction solutions*

	Control liver		CCl_4 liver	
Extraction method	ng/mg wet tissue	fraction of total	ng/mg wet tissue	fraction of total
Total tissue (no extraction)	1400	100%	3400	100%
Sup 0.5M Acetic acid	140	10%	544	16%
Sup 0.5M Acetic acid + 0.5 mg/ml trypsin	126	9%	612	18%
Sup 1% SDS	84	6%	153	5%
Sup 0.5M lactic acid	56	4%	510	15%

Collagen (hydroxyproline) was analyzed biochemically in tissue as well as in the solubilized fractions.

Comparison of various collagen extraction methods showed that only a small fraction of tissue collagen can be solubilized. These results indicate that complete tissue hydrolysis followed by a hydroxyproline-based assay is required to ensure accurate quantification of tissue collagen.

Diet-induced fibrosis is detectable biochemically at an early timepoint

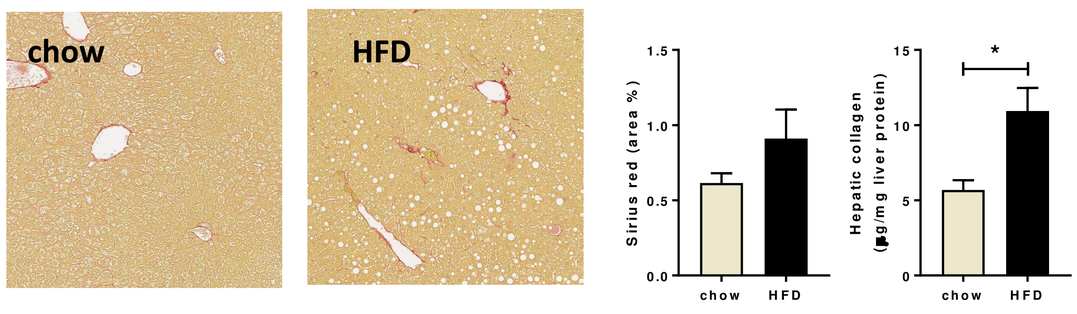
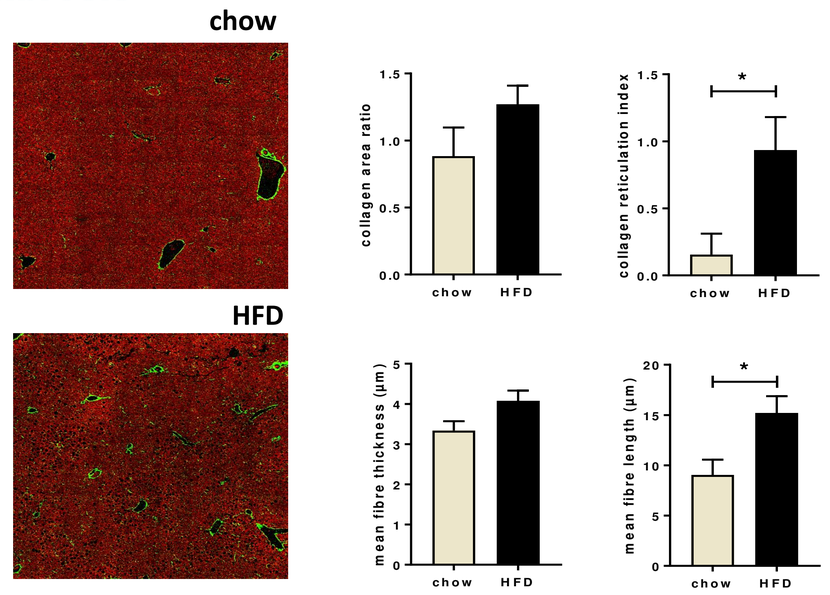


Figure 1*: Hepatic collagen in HFD- and chow fed animals after 16 weeks. Collagen was analyzed histologically by Picro Sirius Red staining and biochemically by total collagen assay.*

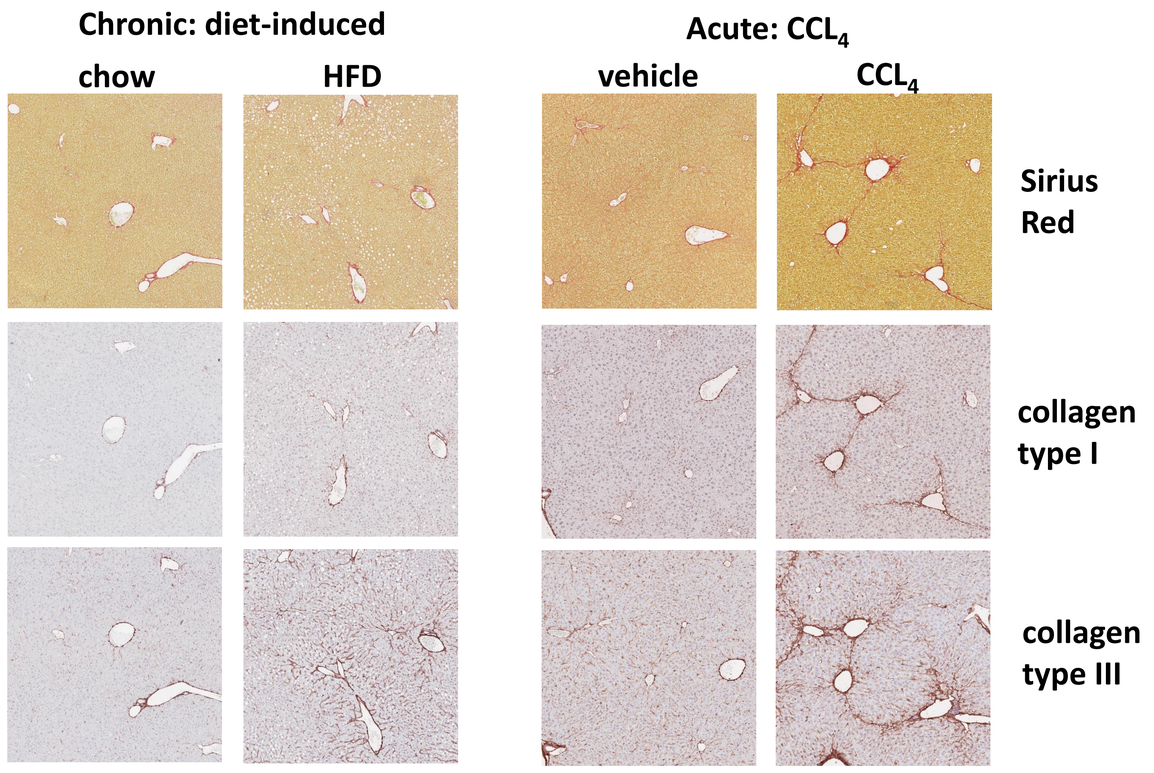
Already after 16 weeks of diet induction, liver fibrosis was observable. Significant induction hepatic fibrosis was only observed using the biochemical total collagen assay.

SHG/multiphoton microscopy reveals various aspects of early fibrosis



In early liver fibrosis morphometric differences could be observed using multiphoton & SHG imaging. Collagen reticulation index and fiber thickness showed significant induction.

Patterns of type I and III collagen expression differ both in and between chronic and acute hepatic fibrosis



Type I and type III collagen show a very different distribution. Type I collagen – which colocalizes with Sirius red staining – is largely absent in healthy liver (besides blood vessel content), while type III is more ubiquitously expressed.

Induction of liver fibrosis (both diet-induced and acute) is most pronounced on type III collagen, indicating that this may provide a good histological readout for early fibrosis.

Fibrosis morphometry differs strongly between diet-induced chronic fibrosis and chemically induced acute fibrosis.

CONCLUSIONS

- Accurate quantification of collagen in liver fibrosis requires hydroxyproline-based assays; since only a part of the collagen can be solubilized. This is also the most sensitive method for quantification in early fibrosis.
- Multiphoton & SHG imaging is a sensitive method for early detection of changes in hepatic collagen though the biological interpretation of some of the parameters requires further study.
- In early liver fibrosis, type III collagen is more strongly increased than type I collagen. Type I collagen and Sirius red staining show a similar pattern of distribution and sensitivity.
- The morphometry of collagen distribution in acute models differs from that observed in diet-induced models and in NASH patients.