Investigating the role of oxidative stress and insulin resistance in NAFLD progression with advanced MR techniques and stable-isotope tracers

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Background

Non-alcoholic fatty liver disease (NAFLD) is associated with obesity, insulin resistance and diabetes and is estimated to affect up to a third of adults in Western countries (1). However, only a minority of these individuals develop progressive liver disease. Insulin resistance and oxidative stress are suggested as key pathogenic factors in the progression of NAFLD from fatty liver (simple steatosis) to inflammation (Non-Alcoholic Steatohepatitis; NASH) and fibrosis (2).

Work leading to this application

We have an ongoing collaboration where complementary MR and stable-isotope tracer techniques are utilised to study patients with NAFLD. Using these techniques, we have demonstrated sexual dimorphism in dietary fatty acid oxidation (a marker of mitochondrial function) that may account for the higher prevalence of NAFLD in men (3). We have also shown that non-invasive MR techniques can be used to diagnose NASH and fibrosis in patients with NAFLD (4) and more recently using ^{1}H-MRS for hepatic lipid profiling we have found significant differences in the proportion of saturated fat in patients with NASH and cirrhosis (5).

We have now developed ^{31}Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) at 7Tesla, which can be used to measure the relative abundance of substances reflecting liver energetics (nucleotide di- and tri-phosphates) and oxidative stress reserves (NADPH). The higher magnet strength allows better spectral resolution and higher signal to noise ratio, which may enable us to detect differences between disease categories not identified previously at lower magnet strengths. In preliminary work we have observed significant differences in the ^{31}P-MRS spectra acquired from patients with liver cirrhosis (from varied aetiologies) compared to healthy controls (6).

Although often assumed that mitochondrial function is impaired (based on gene expression data and histological analysis), to our knowledge, no metabolic profiling studies have been undertaken to date to assess *in vivo* fatty acid oxidation, as a marker of mitochondrial function, across the spectrum of NAFLD patients.

Aims

To investigate the role of mitochondrial dysfunction and insulin resistance in the progression of NAFLD from simple steatosis to inflammation with fibrosis and cirrhosis.

Hypothesis

Oxidative stress and mitochondrial dysfunction, assessed using advanced MR techniques and an in vivo postprandial stable-isotope study, will be increased in patients with more advanced forms of NAFLD.

Work to be undertaken

Patients who have undergone a clinically indicated liver biopsy for the assessment of fatty liver disease will be recruited according to disease stage; simple steatosis (n=10); NASH and fibrosis (n=10); NASH and cirrhosis (n=10). Patients will be recruited from the established NAFLD clinical service.

Patients will undergo assessment with advanced MR imaging and spectroscopy techniques including LiverMultiscan (Perspectum Diagnostics, Oxford, UK) for the quantification of liver inflammation / fibrosis, liver fat fraction and liver iron, ^{1}H-MRS for the quantification hepatic lipid composition and glycogen and ^{31}P-MRS at 7T for the assessment of liver energetics.

Patients will then attend a metabolic postprandial study day after consuming "heavy" water ($^{2}H_2O$) to allow the assessment of fasting and post prandial de novo lipogenesis (DNL). After baseline breath and blood samples are taken, patients will be given a mixed test meal containing a [U^{13}C]fatty acid to assess the fate of dietary fatty acids into oxidation pathways (expired CO_2, 3-hydroxybutyrate), along with plasma biochemistry. Mitochondrial dysfunction will also be studied in human hepatocyte models of NAFLD that are developed in the Hodson laboratory.

We expect that results from this work will help us elucidate mechanisms involved in the progression of NAFLD, potentially identifying new therapeutic targets. Furthermore, we expect to demonstrate how non-invasive techniques can be used to study metabolism *in-vivo*, and this can be a powerful tool in research and in studying the effects of therapeutic interventions.

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