Fast and Localized [31]P Saturation Transfer Measurement at 7 T Reveals Slower Hepatic Metabolic Rates in Patients with Steatohepatitis

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Abstract. *Invasive liver biopsy is currently the only method used for distinction between non-alcoholic fatty liver (NAFL) and steatohepatitis (NASH). Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) with saturation transfer (ST) technique enables a non-invasive in vivo measurement of hepatic energy metabolism alterations that can indicate liver diseases. Ten suspected NAFL/NASH patients and four healthy volunteers were measured by ^{31}P-MRS 1D-ISIS ST on a 7 T MR system. All patients underwent a liver biopsy for diagnostic purposes. Significantly lower exchange rate constants (p<0.01) were found in NASH patients when compared to healthy volunteers and also to NAFL patients. Strong correlation was also found between the chemical exchange rate and steatosis degree. Thus, we believe that the ^{31}P-MRS ST might provide a clinical tool for future distinction between NAFL and NASH.*

Keywords: ^{31}P-MRS, Saturation Transfer, 7 T, Liver Metabolism, NASH

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a clinical condition characterized by steatosis and increased lipid content with varying degrees of inflammation in individuals consuming <20g of alcohol per day [1]. The histologic spectrum of NAFLD includes benign non-alcoholic fatty liver (NAFL) and potentially progressive steatohepatitis (NASH) [1, 2]. Although originally considered to occur exclusively in obese diabetic women [3], NASH is now known to occur in both genders and even in those who are not obese [4]. Currently, the only established method for distinction between NAFL and NASH is invasive liver biopsy.

It has been recently shown, in type II diabetes patients, that alterations in hepatic energy metabolism are indicative for inflammatory and neoplastic liver diseases [1, 5]. A non-invasive tool dedicated for *in vivo* energy metabolism measurements through concentration assessments of high energy metabolites, e.g., adenosine-tri-phosphate (ATP) or inorganic phosphate (Pi), is called phosphorous magnetic resonance spectroscopy (^{31}P-MRS). This method can be furthermore combined with a saturation transfer (ST) technique and measure the metabolic rate of Pi-to-ATP chemical exchange [6].

Quantitative measurement of resting ATP synthesis in human liver by 1D-ISIS localized ^{31}P-MRS ST has been successfully demonstrated at 3 T [7], but because of low signal-to-noise ratio (SNR), the examination took almost 2 h, what is unpractical for clinical praxis. Recently, it has been shown that going to ultra-high field strengths (7 T) can significantly reduce the measurement time needed for non-localized ST experiments in human skeletal muscle [8] and even for 1D-ISIS localized ST examinations of the liver [9], enabling measurements of chemical exchange rates in clinically feasible scan time.

Therefore, the aim of this study was to test the feasibility of the ^{31}P-MRS ST technique at 7 T for fast non-invasive distinction between NAFL and NASH.

2. Subject and Methods

Ten suspected NAFLD patients (six males, four females; 49.5 ± 13.2 years) and four young healthy male volunteers (25.3 ± 2.9 years) participated in this study. Written, informed consent was obtained from all volunteers and the local ethics committee approved the protocol. All patients underwent an invasive liver biopsy, for diagnostic differentiation of the NAFL and NASH, one day after the MR examinations.

The MR examinations were performed on a 7 T MR system (Siemens Healthcare, Erlangen, Germany) using a double-tuned ($^{31}P/^1H$) surface coil (Rapid Biomedical, Wimpar, Germany), with a 10 cm diameter. Participants were examined in early morning hours after overnight fasting in a lateral position with the right lobe of the liver positioned over the coil, what was further adjusted according to localizer images.

The 30 mm wide ISIS localization slab was placed parallel to the coil through the liver minimizing muscle contamination (see Fig. 1). ST experiment consisted of 1D-ISIS localized liver spectra acquisition with selective saturation, of the γ-ATP frequency (saturation state) and of the frequency mirrored downfield around the Pi (equilibrium state). The apparent longitudinal relaxation time (T_1^{app}) of Pi was measured with an inversion-recovery (IR) sequence with eight inversion times (TI=0.08-3 s) with continuous saturation of the γ-ATP resonance. Unsaturated 1D-ISIS spectra were obtained to estimate the Pi concentration, using the γ-ATP as an internal concentration reference (2.5 mM [1]). The measurement parameters were set as follows: rectangular 400 ms excitation, TE*=0.4 ms, TR=5 s and total acquisition time ~23 min.

Fig. 1. 1D-ISIS localization volume depicted on the localizer image

The chemical exchange rate constant, k, of the basal Pi-to-ATP reaction was calculated from saturation and equilibrium liver spectra according to Eq. 1.

$$k = \left(1 - M_z / M_0\right) \big/ T_1^{app} \qquad (1)$$

where

M_z saturation state magnetization of Pi measured during γ-ATP saturation
M_0 equilibrium magnetization of Pi measured during saturation mirrored around Pi

The resting unidirectional forward exchange flux (F_{ATP}) was then calculated by multiplying the forward rate constant, k, with the estimated resting concentration of Pi.

The MR measured metabolic parameters (k, F_{ATP}) were compared between the subgroups with an unpaired Student's t-test with a significance level cut off at $p < 0.01$. In addition, linear correlation with the histology, regarding the disease status and steatosis degree was investigated by the Pearson correlation coefficient.

3. Results

Summarized data from the ST measurements are given in Table 1, where the patient group is already resolved by the histological diagnosis into NAFL (n=4) and NASH (n=6) subgroups. The NASH patients had significantly lower k values in comparison to NAFL patients ([*], $p < 0.01$) and also to healthy volunteers ([#], $p < 0.01$). There was no overlap in k values between the NASH patients and the other two subgroups. Similar results were found in the
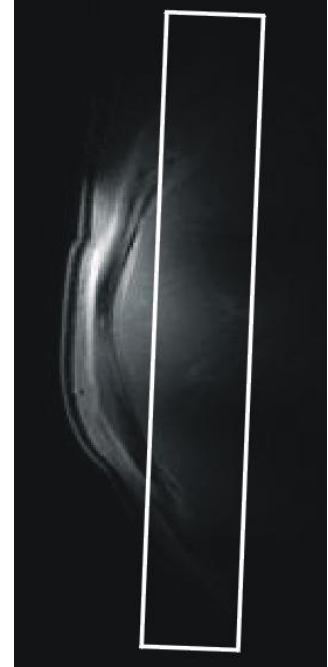
comparison of the uni-directional metabolic fluxes (F_{ATP}) of the NASH patients and the other two groups investigated in this study.

Table 1.　　Hepatic metabolism parameters measured by the ^{31}P-MRS ST technique.
　　　　　　(* - NASH vs. NAFL, p<0.01; $^\#$ - NASH vs.Healthy controls, p<0.01)

	NAFL (n=4)	NASH (n=6)	Healthy controls
[Pi]' (mM)	1.23 ± 0.16	1.09 ± 0.23	1.25 ± 0.04
T_1^{app} (s)	0.80 ± 0.09	0.72 ± 0.19	0.79 ± 0.18
k (s^{-1})	0.34 ± 0.04	$0.18 \pm 0.05^{*\#}$	0.31 ± 0.03
F_{ATP} (mM.s^{-1})	0.41 ± 0.04	$0.20 \pm 0.08^{*\#}$	0.38 ± 0.05

Strong correlation according to Pearson correlation coefficient was found between the forward rate constant of the Pi-to-ATP reaction, *k*, determined by the ^{31}P-MRS ST technique, and the degree of liver steatosis, histologically assessed form the liver biopsy sample. This linear correlation is depicted in Fig. 2.
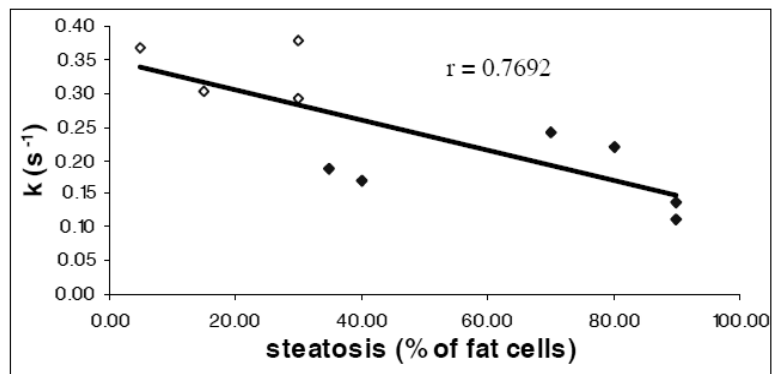

Fig. 2.　Correlation between the exchange rate constant (ST) and steatosis degree (biopsy). Empty diamonds depict NAFL and full NASH patients. Note no overlap between the groups.

4. Discussion

In this study, we investigated the possibility of fast non-invasive differentiation between two states of NAFLD – benign fatty liver and progressive steatohepatitis, by a 1D-ISIS localized ^{31}P-MRS ST technique at 7 T. We were able to show that NASH patients have significantly slower Pi-to-ATP exchange rate and metabolic flux compared to NAFL patients and healthy volunteers. In addition, a strong correlation between the exchange rate constant and steatosis degree, determined by invasive liver biopsy, was found. To the best of our knowledge, no investigation of NAFL and NASH differentiation by ST has been previously reported.

Hepatic ATP synthesis rate constants have been previously measured in healthy volunteers and type II diabetes, with no significant differences between the groups [5, 7]. The values of healthy volunteers (k=0.31 ± 0.03) measured in this study are in good agreement with previous reports (k=0.33 ± 0.12 in [5] and 0.30 ± 0.02 in [7]). As no absolute quantification was performed, no valid comparison regarding the assumed Pi concentrations or calculated uni-directional fluxes can be given.

We report significant differences in basal metabolic rate constants of the Pi-to-ATP reaction between the NASH patients and the NAFL patients as well as between NASH patients and healthy volunteers. No overlap in *k* values between NASH group and the other two groups suggests that *k* values can be used in future for differentiation between NAFL and NASH. These findings might be associated with the mitochondrial abnormalities previously found in NASH patients [2], NASH can be also associated with progressive hepatic fibrosis compromising liver metabolism and resulting into cirrhosis [10].

Furthermore, we report strong linear correlation between the forward rate constant determined by the ST measurement and the histologically determined degree of liver steatosis.

5. Conclusions

Liver Pi-to-ATP chemical reaction, measured *in vivo* by the 1D-ISIS localized ^{31}P-MRS ST technique at 7 T, is decreased in NASH patients in comparison to NAFL patients and healthy controls. This difference is connected to the lower exchange rate constant and might provide a valuable clinical tool for future non-invasive distinction between NASH and NAFL.

Acknowledgements

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