Quantification of phosphoenolpyruvate in the human liver and its application in a meal study employing ^{31}P MRS

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Introduction

Phosphoenolpyruvate (PEP) is an important intermediate in gluconeogenesis. Previously, Changani et al.[1] reported its changes in response to alanine administrations in the rat using high-resolution NMR of liver extracts and showed its importance in glucose metabolism. However, due to severe overlaps with phosphodiester (PDE) compounds in *in vivo* ^{31}P MRS, little is reported about magnetic properties of PEP or its concentrations in human liver. Thus, the goal of this study was to quantify PEP concentrations by ^{31}P MRS and to investigate whether PEP changes in response to a mixed meal in the human liver.

Material and Methods

Subjects: All volunteers were locally recruited and consented to a research protocol, which was approved by the local review board of human studies. Six healthy subjects [age: 27.5 ± 2.5 years, body mass index (BMI): 23 ± 1.5 kg/m^2] underwent ^{31}P MRS before and 140 min after ingestion of a mixed meal containing 55% carbohydrates, 15% protein and 30% fat (652 Kcal; 435 ml). A separate group of 5 healthy subjects (age: 30.2 ± 9.4 years, BMI: 23.8 ± 2.4) were studied to obtain T_1 relaxation times for quantification of PEP.

MRI, ^{31}P MRS and ^{1}H MRS: MR data were acquired on a 3-Tesla MR scanner (Philips Achieva X-series, Best, the Netherlands) using a 14 cm circular ^{31}P receiver/transmitter RF-coil for ^{31}P MRS and a 16 channel SENSE receive coil for liver ^{1}H MRS (Philips, Best, the Netherlands). Transverse and coronal images were acquired to properly localize a 6x6x5 cm^3 voxel of ^{31}P MRS within the liver. The built-in body coil was used for imaging, NOE and proton decoupling. The acquisition sequence parameters were TR/NSA/pulse sequence = 6s/128/ISIS followed by an adiabatic excitation pulse using proton decoupling and NOE (SW = 3000 Hz, data points = 2K). Parameters for proton-decoupling and NOE were employed similar to a previous publication[2]. To assess fat content, ^{1}H MRS was performed using a STEAM sequence (TR/TE = 4s/10ms). T_1 of PEP was assessed using an inversion recovery sequence with 5 different T_{IR} delay times.

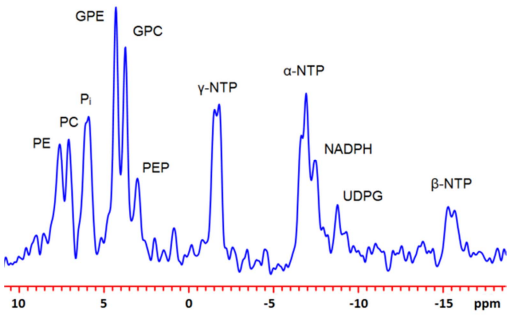

Fig. 1: ^{31}P MRS spectrum of the human liver at 3T

Processing: jMRUI (Java-based Magnetic Resonance User Interface, EC Human Capital and Mobility Networks, France) was used for processing of ^{31}P and ^{1}H MRS using the AMARES algorithm with *a priori* knowledge. The absolute quantification was performed using matching phantoms [ATP and potassium phosphate (KH_2PO_4)] and an external reference [methylphosphonic acid (MPA)] in order to correct for T_1 relaxation time, non-uniform excitation pulse profile, coil loading, B_1 field inhomogeneity and the amount of liver fat by ^{1}H MRS.

Statistics: P values <0.05 were considered statistically significant employing MedCalc (MedCalc software, Belgium).

Results

The hepatic PEP peak is clearly detectable at 2.1 ppm (Fig. 1). The T_1 relaxation time of the PEP peak was calculated to be 0.82 ± 0.16 s. The mean signal to noise ratio was 10. The mean concentration of PEP decreased from 1.14 ± 0.22 mmol/l to 0.86 ± 0.22 mmol/l after ingestion of the mixed meal (p = 0.01, paired t-test; Fig. 2).

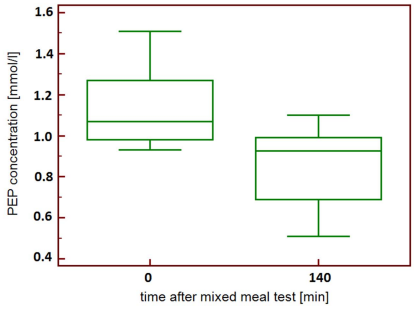

Fig. 2: Box-Whisker plot of PEP concentration [mmol/l] before and 140 min after the mixed meal

Discussion

To our knowledge, this is the first report on molar PEP concentration in human liver measured using ^{31}P MRS at a 3-T scanner. In one previous study, Li et al.[3] reported molar PEP concentrations of 1.4 ± 0.91 mmol/l in human liver on a 1.5-T scanner. These concentrations were markedly higher than in the present study, which might be at least partly due to non-standardized dietary conditions. Of note, our mixed meal study revealed a fall in hepatic PEP concentration by 25% possibly reflecting lower gluconeogenesis. Future studies are needed to investigate the role of changes in PEP for glucose metabolism in humans.

References

1. Changani KK, Barnard ML, Bell JD et al., Biochim Biophys Acta., 1997, 1335(3):290-304. 2. Sevastianova K, Hakkarainen A, Kotronen A et al., Radiology, 2010, 256: 466-473. 3. Li CW, Negendank WG, Murphy-Boesch J et al., NMR in Biomedicine, 1996, 9:141.