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/m²] underwent $^{31}$P MRS before and 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 T₁ 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 $^1$H MRS (Philips, Best, the Netherlands). Transverse and coronal images were acquired to properly localize a 6x6x5 cm³ 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 publication². 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 $T_R$ delay times.

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 (KH₂PO₄)] and an external reference [methylphosphonic acid (MPA)] in order to correct for $T_1$ relaxation time, non-uniform excitation pulse profile, coil loading, $B_0$ 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).

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