Quantification of Prenatal Liver and Spleen Iron in a Sheep Model and Assessment of Iron Stores in a Human Neonate with Neonatal Hemochromatosis using R2* Mapping

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Purpose: We evaluated the feasibility of prenatal quantification of liver and spleen iron by magnetic resonance imaging (MRI) gradient recalled echo (GRE) measurements of transverse relaxation time (R2*) (MRI-GRE-R2*) in a fetal sheep model and applied the method to a human neonate with suspected neonatal hemochromatosis.

Methods: We subjected 13 fetal sheep to MRI at 1.5 Tesla using a breath-triggered (ewe) multi-echo sequence to determine the transverse relaxation rate (R2*) of the liver and spleen. In the human neonate, we measured the R2* of the liver, spleen, and pancreas on the 30th postgestational day.

Results: The median R2* of the fetal sheep liver was 25.6 s⁻¹ (range 20 to 114 s⁻¹) and of the spleen, 40.2 s⁻¹ (range 20 to 70 s⁻¹), and the corresponding median iron concentration in the liver was 0.85 mg/g dry weight (d.w.) and in the spleen, 1.22 mg/g d.w. R2* rates in the human neonate liver were elevated between 67 s⁻¹ (average), which corresponded with an iron concentration of 1.9 mg Fe/g d.w., and 126 s⁻¹ (regional maximum), which corresponded with 3.4 mg Fe/g d.w. The average pancreatic R2* (72.4 s⁻¹) was significantly above normal values, which indicated iron overload.

Conclusion: We demonstrated the feasibility of prenatal quantification of tissue iron by fetal MRI in this sheep model and successfully quantified iron, including that in the pancreas, in a human neonate to confirm the diagnosis of neonatal hemochromatosis. Transferring the successful approach of quantifying iron in intrauterine tissue in fetal sheep to human pregnancies with suspected fetal siderosis could aid early diagnosis.

Keywords: fetal sheep, iron quantification, MRI-R2*, neonatal hemochromatosis

Introduction

Magnetic resonance imaging (MRI) using R2 or R2* (T₂ or T₂*) sequences has emerged as a non-invasive tool for the accurate estimation of iron concentration in organs.1 Quantification of iron in the liver is most often applied in patients with transfusion-related siderosis and is established as the primary measurement of outcome in iron chelator therapy.2,3 Iron quantification is mandatory to adjust chelator drug therapy and prevent toxic side effects by drug overdosage.4 In addition, as the accumulation of iron in tissue has been demonstrated to predict organ dysfunction even in the absence of clinical symptoms, the increasing application of
noninvasive iron assessment by MRI has improved patient outcomes.\textsuperscript{5,6}

Physically, iron induces local magnetic field inhomogeneities that lead to higher transverse relaxation rates, R2 and R2*, with decreasing MR signal intensities of the affected tissues.\textsuperscript{7} Multi-echo sequences reveal exponential signal decay with increasing echo times that correspond with local tissue iron content.\textsuperscript{8} Iron measurements by MRI-R2 or -R2* were calibrated by chemical iron quantification in dry-weight liver biopsies.\textsuperscript{9,10}

MRI-R2* is established for the noninvasive quantification of iron in such tissues as the liver and heart in adolescent and adult patients. Single nonquantitative approaches in fetuses and neonates have been performed to evaluate tissue signal intensity ratios in conventional T2-weighted images.\textsuperscript{11-14} Application of the more sophisticated multi-echo R2 or R2* method was reported only recently.\textsuperscript{15}

The possibility of noninvasive quantification of organ iron in the fetal and neonatal period may be of clinical interest in that such fetal pathologies as neonatal hemochromatosis (NH) are associated with intra- and extrahepatic iron accumulation.\textsuperscript{16,17} Precise noninvasive methods for quantifying iron by MRI remain challenging with the physiological increase in liver iron in the third trimester but will be a necessary prerequisite for diagnosing iron overload in prenatal iron overload disorders.\textsuperscript{18}

We evaluated the feasibility of prenatal quantification of iron in the liver and spleen by MRI in a fetal sheep model for the first time, and we present a case of successful quantification of organ iron by the MRI-R2* method in a human neonate with suspected NH.

Materials and Methods

Animals: Our local animal protection authorities approved the study. We subjected 13 pregnant ewes carrying singleton fetuses of 112 to 125 days' gestation (gestation is normally 150 days in sheep) to MRI. Fetal ultrasound before MR examination revealed no abnormalities. Prior to examination, the ewes were prepared with a venous catheter and intubated after intravenous administration of 50 mg of Diazepam (Diazepam-ratiopharm\textsuperscript{®}, ratiopharm GmbH, Ulm, Germany). Subsequent anesthesia was performed by artificial ventilation using 1.5% isoflurane (Forene\textsuperscript{®} 100%, Abbott GmbH and Co., Wiesbaden, Germany) with pure O\textsubscript{2} (2 L/min). The ewes were placed in lateral position in the MR imager, and an experienced veterinarian monitored anesthesia and respiratory function throughout the examination.

MRI protocol: All animals were examined using a 1.5 Tesla whole-body MR imager (Achieva, Philips Medical Systems, Best, The Netherlands) with a 16-channel phased-array coil. Initially, for anatomical orientation, a T\textsubscript{2}-weighted multislice turbo-spin-echo sequence (echo time [TE], 90 ms; repetition time [TR], 5000 ms; field of vision [FOV], 430 mm × 363 mm; voxel size, 1.68 × 2.48 × 5.0 mm\textsuperscript{3}, 20 slices with no gap) was performed in transverse, sagittal, and coronal views (referring to the fetus). Scan duration was 54 s.

For functional evaluation, a respiratory-triggered (ewe) gradient recalled echo (GRE) multi-echo sequence in transverse orientation was performed to include the fetal liver and spleen using: TE, 4.6 to 54.1 ms (12 echoes, Δ = 4.5 ms); one slice; 5.5-mm slice thickness; 20\textdegree flip angle; and pixel bandwidth, 283 Hz/pixel. The average TR was 578 ms and the average scan time, 17 s.

Quantitative R2* analysis and statistics: To quantify iron in the fetal liver and spleen, we determined the transverse relaxation rate, R2*, (± standard error [SE]) by a monoexponential fit to the echo-time-dependent signal intensities (SI) averaged over the liver and spleen slice. A constant signal level offset, SI\textsubscript{LO}, was added according to Eq. [1] to account for potential fit distortions by iron-sparse regions with short relaxation rates (R2*), as proposed by Wood and Ghugre\textsuperscript{19}:

\[ SI(TE) = SI(0) \cdot \exp(-TE \cdot R2*) + SI_{LO}. \]  \[ \text{[1]} \]

Calibration of dry-weight (d.w.) concentrations of liver and spleen iron (mg Fe/g d.w.) versus R2* values was adopted from the measurements of human liver iron by Wood and associates.\textsuperscript{9}

We analyzed images on a personal computer using CMRtools (Cardiovascular Imaging Solutions Ltd., London, UK) to determine the echo time-dependent signal intensities of the fetal liver and spleen. We placed regions of interest (ROI) to cover the whole parenchyma of the respective organs in a representative transverse slice and excluded structures that might produce artifacts, such as blood vessels. We used a nonlinear fit algorithm to fit Eq. [1] to the signal intensity data (SlideWritePlus, Advanced Graphics Software Inc., Encinitas, CA, USA). The SI\textsubscript{LO} was determined from the air space free of breathing artifacts (plus one SD to compensate for slowly relaxing contributions like blood) in cases where the 3-parameter fit failed for this parameter.

We used nonparametric statistics as well as a paired Wilcoxon test to calculate median values and interquartile ranges (IQR).
On a 1.5T MR imager (Avanto, Siemens Healthcare, Erlangen, Germany), we assessed R2* measurements in an intubated and ventilated term human neonate (2990 g, 47 cm) with severe acute liver failure and clinical suspicion of NH. We acquired a breath-hold GRE multi-echo sequence (TE, 1.4 to 25.7 ms; 12 echoes; TR, 244 ms; 5-mm slice thickness; 20° flip angle; pixel bandwidth, 283 Hz/pixel) to measure iron in the liver, spleen, and pancreas while pausing ventilation for 11 s.

Results

All images were of diagnostic quality with no significant artifacts. The fetal liver and spleen were easily identified in all 13 examined fetal sheep and in the human neonate. The pancreas tail was reliably delineated in the human neonate.

The liver R2* of the fetal sheep ranged from 19.5 to 113.5 s⁻¹, and the corresponding liver iron concentration (LIC), calculated from R2* values, ranged from 0.7 to 3.1 mg/gd.w. with a median LIC of 0.85 mg/gd.w. (IQR 0.26, median r² = 0.998). The spleen R2* of the fetal sheep ranged from 20.4 to 70 s⁻¹, and the corresponding spleen iron concentration (SIC), calculated from R2* values, ranged from 0.7 to 2 mg/gd.w. with a median SIC of 1.22 mg/gd.w. (IQR 0.16, median r² = 0.98) (Table).

Figure 1 shows transverse images of fetuses revealing the lowest (0.7 mg/gd.w.) and highest LIC (3.1 mg/gd.w.) at the level of the fetal liver and spleen at minimum and maximum TE (Fetuses 10 and 13).

Fig. 1. Transverse images of Fetus 10 reveal low liver iron concentration (LIC; 0.7 mg/gd.w.) at minimum (a) and maximum (b) echo time (TE); transverse images of Fetus 13 reveal highest LIC (3.1 mg/gd.w.) at minimum (c) and maximum (d) TE. Liver (L), spleen (S), and placement of regions of interest (ROI; white dots) are indicated.

Table. Summary of fetal liver and spleen relaxation rates (R2*) and calculated individual liver (LIC) and spleen iron concentrations (SIC) with standard error (SE) and median ± median absolute deviation (MAD)

|         | Liver                     | Spleen                    |
|---------|---------------------------|----------------------------|
|         | R2* [s⁻¹]     | SE  | LIC [mg/gd.w.] | SE  | R2* [s⁻¹] | SE  | SIC [mg/gd.w.] | SE  |
| Fetus 1 | 29.3          | 1.9 | 0.9           | 0.06| 40.2       | 2.2 | 1.2           | 0.07|
| Fetus 2 | 25.6          | 1.2 | 0.9           | 0.04| 41.3       | 3.2 | 1.3           | 0.10|
| Fetus 3 | 22           | 0.8 | 0.8           | 0.03| 35.1       | 1.3 | 1.1           | 0.04|
| Fetus 4 | 34.1          | 3.8 | 1.1           | 0.12| 70         | 5.2 | 2.0           | 1.47|
| Fetus 5 | 19.5          | 1.1 | 0.7           | 0.04| 44.4       | 1.8 | 1.3           | 0.05|
| Fetus 6 | 39.6          | 1.6 | 1.2           | 0.05| 26.9       | 1.4 | 0.9           | 0.05|
| Fetus 7 | 19.6          | 0.1 | 0.7           | 0.00| 40.3       | 2.2 | 1.2           | 0.07|
| Fetus 8 | 30.6          | 0.3 | 1.0           | 0.01| 20.4       | 0.7 | 0.7           | 0.02|
| Fetus 9 | 19.9          | 0.6 | 0.7           | 0.02| 36.2       | 1.8 | 1.1           | 0.06|
| Fetus 10| 20.3          | 0.2 | 0.7           | 0.01| 33.7       | 1.6 | 1.1           | 0.05|
| Fetus 11| 22.3          | 0.4 | 0.8           | 0.01| 37.6       | 1.9 | 1.2           | 0.06|
| Fetus 12| 29.9          | 1.2 | 1.0           | 0.04| 41.5       | 2.7 | 1.3           | 0.08|
| Fetus 13| 113.5         | 3.9 | 3.1           | 0.11| 51.5       | 2   | 1.5           | 0.06|

Median ± MAD 0.85 ± 0.13 1.22 ± 0.11
10 and 13). We observed moderate signal decay of the liver in Fetus 10 with increasing echo time and more rapid and more significant signal decay in Fetus 13 due to the higher iron content.

Of the 13 examined fetuses, ten revealed higher iron concentrations in the spleen, and three in the liver. The LIC was about 1 mg/gd.w. in all but one fetus (Fetus 13) that showed remarkably higher iron concentrations in the liver (3.1 mg/gd.w.) and spleen (1.5 mg/gd.w.). Excluding this fetus, there was a significant difference between SIC and LIC ($P = 0.005$, paired Wilcoxon test; see also Fig. 2).

The average R2* of the human neonate liver, 67.1 s⁻¹ (SE ± 1.8), corresponded with an average LIC of 1.9 mg Fe/gd.w. and the regional maximum R2* value of 127.9 s⁻¹ (SE ± 3.2) in the right post-erolateral lobe corresponded with a regional maximum LIC of 3.4 mg Fe/gd.w., reflecting the inhomogeneous distribution of iron. The R2* rate in the pancreas tail, 72.4 s⁻¹ (SE ± 7), was significantly higher than normal (95% range 2 to 28 s⁻¹). Spleen iron of 0.88 mg/gd.w. did not differ significantly from normal concentrations using the LIC (R2*) calibration with splenic R2* of 26.6 s⁻¹ (SE ± 0.9) (Fig. 3).

During clinical care, liver biopsy performed in the human neonate revealed fatty replacement, beginning cirrhosis with moderate siderosis, and cholestasis. Serum ferritin was severely elevated at 3016 µg/L.

Discussion

We demonstrated prenatal quantification of tissue iron by noninvasive MRI for the first time in a fetal sheep model. The fetal LIC was around 0.9 mg/gd.w. in all but one fetus (Fetus 13; 3.1 mg/gd.w.), while in sheep fetuses killed after 94 and 141 days of gestation demonstrated fetal erythropoietic activity...
in the reticuloendothelial system. In contrast to the relatively high concentration of iron in the spleen in fetal sheep, iron concentrations in normal human neonates and those with NH are lower in the spleen (normal, 0.4 to 1.1 mg/g.d.w.) than liver (normal, 0.6 to 1.5 mg/g.d.w.).

The reason for the 3-fold higher liver iron content in one fetus remains unclear and might reflect alterations in fetal iron storage or placental iron transfer. Ultrasound prior to MR examination did not reveal fetal abnormality, and after birth, fetal lambs revealed no clinically pathologic findings. However, liver iron concentrations in human stillborns showed high individual variability, between 0.2 and 3.6 mg/g.d.w., so our results may express the physiological range of iron storage.

The present trial offers the opportunity for noninvasive prenatal assessment of fetal iron storage as well as patterns of distribution. Thus, this method may provide additional information to explain the conflicting observations between gestational age and organ iron content in sheep and humans.

Quantification of tissue iron by R2* measurements proved high sensitivity and accuracy in the liver and myocardium of patients with iron overload disease and is established in clinical routine. The method enables noninvasive assessment of organ iron content to facilitate identification of affected organs and monitoring of chelator drug therapy and even contributes to favorable patient outcome. Therefore, quantitative assessment of intrauterine iron and comparison with control groups might also allow prenatal diagnosis of pathologic organ siderosis and its quantitative analysis. Diagnosis of fetal iron overload cannot be assessed by any other imaging modality. Because no deleterious effect is documented for the fetus, MRI may be also performed in pregnancy.

Rare reports of the noninvasive detection of organ iron accumulation in the prenatal and newborn period using MRI exist. Those studies show lower signal intensity of the fetal liver that suggests hepatic siderosis. All those investigations were based on analysis of T2*-weighted MR images, either by visual analysis of signal intensities of fetal/newborn livers or by calculation and comparison of signal intensity ratios of fetal liver and lung, muscle or fat. Elevated iron concentration in the liver as a result of extramedullary hematopoiesis is a physiological condition in the perinatal period that causes lower signal intensity on T2*-weighted images, which is then difficult to distinguish qualitatively from pathologic organ iron overload. Introducing a more sensitive, quantitative approach to measure iron in the physiologically siderotic fetal liver noninvasively might allow differentiation between physiologically and pathologically elevated concentrations of liver iron. A very recent study investigated healthy human third-trimester fetuses to determine normal T2* values of the liver. In agreement with our study, the MRI-R2* method seems applicable even to prenatal estimation of organ iron content and therefore diagnosis of fetal siderosis.

Noninvasive prenatal iron quantification is of clinical impact in pregnancies with fetuses at risk of GRACILE syndrome (growth retardation, aminoaciduria, cholestasis, iron overload, lactacidosis, and early death) because the syndrome often recurs in subsequent pregnancies. Because no treatment is available to change the fatal course of this syndrome, early diagnosis in women with previously affected pregnancies is of major importance. The GRACILE syndrome targets the liver, and hepatic siderosis is a leading symptom. Until now, prenatal diagnosis could only be performed by genetic analysis, which requires invasive chorion villus biopsy.

The rare gestational condition of NH is characterized by severe and acute liver failure in often premature and small-for-gestational-age neonates. Besides intrahepatic iron accumulation, extrahepatic siderosis is common and typically affects the pancreas, myocardium, and minor salivary glands but excludes the reticuloendothelial system. Diagnosis is generally made in the neonatal period or postmortem based on the characteristic complex of disease symptoms, laboratory parameters, and typical pattern of tissue siderosis assessed by invasive biopsy. Prenatal diagnosis is far more challenging and has not been reliably performed. However, evidence of increased iron concentration in tissues, especially extrahepatic tissues like the pancreas, is a cardinal finding on which diagnosis is based. Thus, fetal MRI in pregnancies previously affected by NH or in suspected cases of pathologic liver siderosis might aid early diagnosis.

MRI-R2* measurements in the human neonate revealed hepatic and pancreatic iron overload but normal iron levels in the spleen, the characteristic finding in NH. Hepatic R2* rates were increased compared to the mean R2* rate (50.8 ± 19.1 s−1), converted from the assessed T2* values measured in healthy human fetuses. The estimated LIC (average 1.9 mg Fe/g.d.w.; maximum 3.4 mg Fe/g.d.w.) was higher than the LIC of 0.25 mg/g.d.w. histochemically assessed in control perinates by Silver and colleagues. However, hepatic iron in NH rarely exceeds 6 mg/g.d.w., which would fit into our MR measurement range.
firmed hepatic siderosis in our case. In the clinical course, severe liver damage required liver transplantation. The possibility to delineate the neonate’s pancreas has to be emphasized because proof of pancreatic siderosis is an important and specific finding in the diagnosis of NH. Even intrauterine identification and assessment of fetal pancreatic iron stores may be possible in third trimester pregnancies.

This study has some limitations. It lacks histological correlation of iron concentrations in fetal sheep liver and spleen, for which we had no consent from the local animal protection authorities. We estimated iron concentrations in the tissues of fetal sheep based on human calibration reference values, but the similarity of iron properties in mediating changes in R2* is also known from animal models. Species-specific differences may affect the magnetic susceptibility-dependent R2* relaxivity of liver iron less than the correlation time-dependent R2. Therefore, we might also apply the R2* calibration of liver iron for humans to sheep. Finally, we could not delineate the pancreas to measure iron in the sheep fetuses, possibly because of the variable anatomical conditions compared to human fetuses. However, demonstrating pancreatic siderosis is important in diagnosing NH and measuring pancreatic iron by MRI-R2* could be reliably performed in the human neonate.

Conclusion

In this feasibility study, we successfully estimated prenatal tissue concentrations of iron in a fetal sheep model. Thus, sheep may be an appropriate model for future neonatal iron studies in animals. Assessment of iron stores of the fetal liver, spleen, and pancreas by noninvasive MR relaxometry offers the opportunity to differentiate NH with its unique pattern of iron distribution from other siderotic disorders. This was emphasized by revelation of the specific pattern of organ iron overload in a human neonate, which confirmed the diagnosis of NH. The MRI-R2* technique has a strong potential for even intrauterine application and prenatal diagnosis of NH in the future.

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