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Feasibility and Reproducibility of BOLD and TOLD Measurements in the Liver With Oxygen and Carbogen Gas Challenge in Healthy Volunteers and Patients With Hepatocellular Carcinoma

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Abstract

Purpose—To quantify baseline relaxation rates R_2^* and R_1 in the abdomen, their changes after respiratory challenges, and their reproducibility in healthy volunteers and patients with hepatocellular carcinoma (HCC) at 1.5T and 3.0T.

Materials and Methods— R_2^* measurements were acquired in the liver in 8 volunteers and 27 patients with 34 HCCs using multiecho T_2^* at baseline and after respiratory challenges with 100% oxygen (O_2) and carbogen (CB=95% O_2 /5% CO_2). R_1 was measured at 1.5T in one volunteer and 21 patients with 23 HCCs. Test retest coefficient of variation (CV) was assessed in 10 subjects. Intra and interobserver variability of R_2^* and R_1 measurements was assessed in 12 and 10 patients, respectively. Parameters for HCC, liver, and muscle were compared between baseline and after gas challenges.

Results—We observed that R_2^* and R_1 imaging of HCCs with O_2 and CB is feasible and reproducible (test retest CV $R_2^*<15\%/R_1<5\%$; intra- and interobserver intraclass correlation coefficient $R_2^*>0.88/R_1>0.7$ and CV $R_2^*<7\%/R_1<3\%$ at 1.5T). R_2^* measurements were observed to be less reproducible at 3.0T (CV<35%). There was a statistically significant decrease in R_2^* values in HCC before and after O_2 ($P=0.02$) and increase in R_1 after O_2 ($P=0.004$). CB had no significant effect ($P R_2^*=0.47/R_1=0.278$).

Conclusion— R_2^* measurements in HCC and liver parenchyma are more reproducible at 1.5T than at 3.0T, and with O_2 than with CB challenge. We observed a decrease in R_2^* and an increase

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in R_1 of HCCs from baseline in response to O_2 challenge, as expected with increased tissue and blood oxygenation.

Intratumoral hypoxia has been shown to correlate with tumor invasiveness, progression, and radioresistance in animal models and human carcinomas.¹ Tumor cells have been shown to adapt to hypoxic stress through genome and proteome changes induced via the hypoxia inducible factor 1- α (HIF 1- α) pathway, which promotes tumor progression and malignancy.^{2,3} Moreover, low oxygen content in tumor tissue decreases the effectiveness of radiotherapy and chemotherapy.² Tumor oxygenation has been shown to be influenced by several factors including microvessel density, blood flow, blood volume, blood oxygen saturation, tissue pO_2 , and oxygen consumption rate.⁴ Information on oxygen bioavailability in tumors can have important clinical implications, such as stratification of patients for therapy and monitoring response to systemic and locoregional therapy.^{5,6}

The development of new imaging techniques, such as oxygen-sensitive magnetic resonance imaging (MRI)^{6–9} makes it possible to determine the perfusion and oxygenation state of tumors noninvasively. MRI techniques such as blood oxygen level-dependent (BOLD) and tissue oxygen level-dependent (TOLD)¹⁰ were first developed to study areas of activation in the brain,¹¹ but have since been used for in vivo evaluation of tumor blood supply and oxygenation.^{5–10,12–17}

In BOLD MRI, deoxygenated hemoglobin (deoxy-Hb) acts as an endogenous contrast agent by increasing magnetic susceptibility and shortening the transverse relaxation time (T_2^*) of the bulk magnetization of water protons in nearby tissues. In hypoxic tissues, the proportion of deoxy-Hb is greater, so the transverse relaxation rate R_2^* ($1/T_2^*$) is higher, and is expected to decrease during a hyperoxic respiratory challenge, as deoxy-Hb becomes more saturated with oxygen. Studies in rodents and humans have shown a BOLD signal increase (R_2^* decrease) in various tumors, including hepatocellular carcinoma (HCC), in response to breathing 100% O_2 ^{5,6,9} or carbogen (CB; 95% O_2 / 5% CO_2 or 98% O_2 / 2% CO_2).^{7,8,10,16} Since Hb saturation with O_2 is dependent on the arterial O_2 pressure and oxygen partial pressure in tissue (pO_2), R_2^* can be thought of as an index of oxygenation. However, since other factors, such as blood volume, flow, and vessel geometry affect the concentration of Hb in tissue, R_2^* cannot be used to infer pO_2 .

The TOLD effect has been observed in tumors and healthy tissues as an increase in the longitudinal relaxation rate of tissue magnetization ($R_1=1/T_1$) in response to O_2 .^{13,14} The TOLD effect relies on the paramagnetic properties of free molecular O_2 , which acts as a weak T_1 -shortening contrast agent.¹⁸ Since the concentration of free blood O_2 is substantial only in well-oxygenated tissues (when hemoglobin is over 90% saturated with O_2), changes in R_1 are sensitive to a different range of tissue oxygenation than changes in R_2^* .⁷ A previous study of tumor xenografts in mice showed that BOLD and TOLD can better characterize tumor oxygenation in combination than each alone.⁷ BOLD or TOLD data in human HCC, at baseline or with physiological challenges, is very limited.

The purpose of our preliminary study was to quantify and assess the test–retest reproducibility, as well as the intra- and interobserver reproducibility at 1.5T and 3.0T, of

baseline transverse (R_2^*) and longitudinal (R_1) relaxation rates, and their changes in response to 100% O_2 and CB respiratory challenges in HCC tumors, liver parenchyma, and muscle.

Materials and Methods

Patients

Between June 2013 to September 2014, patients with HCC and healthy volunteers were enrolled in our prospective, single-center, Health Insurance Portability and Accountability Act-compliant study approved by the Institutional Review Board (IRB) at our institution. Subjects with chronic obstructive pulmonary disease were not enrolled in the study, due to risk of respiratory decompensation during the hyperoxic or hyperoxic/hypercapnic respiratory challenges. Written informed consent was obtained from all subjects.

Eight healthy volunteers (M/F 1/7, mean age 36 years, range 23–55 years) were enrolled in the study for protocol optimization. Twenty-nine initial patients underwent multiparametric MRI of the liver with hyperoxic respiratory challenge. Two patients were excluded due to poor overall image quality ($n=1$) or lack of HCC on pathologic examination ($n=1$). Two patients had been treated with prior transarterial chemoembolization.

Finally, 27 patients (M/F 21/6, mean age 59 years, range 30–76 years) with resectable ($n=15$) and unresectable ($n=12$) HCC were included. HCC was diagnosed based on contrast-enhanced clinical MRI ($n=26$) or computed tomography (CT) ($n=1$) according to the American Association for Study of Liver Diseases (AASLD) 2011 criteria.¹⁹

Twenty-six patients had chronic liver diseases of various etiologies (chronic hepatitis C [$n=18$], chronic hepatitis B [$n=6$], NASH [$n=2$]) and one patient had no history of chronic liver disease (in this case, HCC diagnosis was confirmed on pathology).

Ten subjects underwent test–retest MRI examinations (seven patients and one volunteer at 1.5T, two patients at 3.0T) on two visits, up to 2 weeks apart (mean interval 6 days).

MRI

Subjects were imaged on a 1.5T (Aera, Siemens Healthcare, Erlangen, Germany) or a 3.0T (Skyra, Siemens Healthcare) system, each equipped with a 30 channel spine and flexible body array coil for radiofrequency (RF) receiving. All subjects were instructed to fast for 6 hours before the MRI exam. Six volunteers and one patient were imaged on both systems. Liver anatomy and the presence of lesions were evaluated in all subjects using axial and coronal T_2 -weighted imaging (T_2 WI) half-Fourier single-shot turbo spine-echo (HASTE), axial fat-suppressed T_2 WI, axial dual-echo chemical shift imaging, and axial diffusion-weighted (DWI). 3D T_1 -weighted imaging (T_1 WI) pre- and postinjection of a gadolinium-based contrast agent, as well as DCE-MRI, was performed in all patients.

BOLD Imaging

A fat-suppressed, 2D multiecho gradient echo (MGRE) pulse sequence was acquired axially (Table 1) in all subjects, in 15-second breath-holds at end-expiration. At 1.5T, the protocol with five echoes in phase was initially chosen because in-phase conditions yield greater

signal-to-noise ratio (SNR). However, since the MGRE sequence is fat-suppressed, fat-water chemical shift has a less pronounced effect on the signal, so we expanded the protocol to 12 echoes in a wider range of TEs (Table 1; 1.44–40 msec). The last TE (40 msec) was chosen to correspond to the T_2^* of some HCCs. The first TE (1.44 msec) was chosen for greater sensitivity to decreased liver T_2^* values in the presence of iron. R_2^* was measured in 11 patients and 7 volunteers using the five echoes protocol, and in 12 patients using the 12 echoes protocol. The 1.5T protocols were compared in seven subjects for room air respiratory conditions. For patients, slices were centered over the lesions identified on routine sequences. The MGRE sequence was performed before contrast administration at baseline and at the end of O₂ and CB breathing periods.

TOLD Imaging

The TOLD effect was assessed by precontrast quantitative T_1 measurements at baseline and at the end of the gas challenges. A Look-Locker inversion recovery (IR-LL) pulse sequence with SNAPSHOT-FLASH readout²⁰ (Table 1) was used to provide quantitative T_1 mapping in 1–2 slices through each lesion in one 18-second breath-hold.

Respiratory Challenge

Hyperoxic (100% O₂) and hyperoxic-hypercapnic (CB) respiratory challenges were integrated into the multiparametric imaging protocol (Fig. 1). 100% O₂ available in the MRI exam room was administered at 15 L/min for 10–15 minutes, through a Hudson mask (Westmed, Responsive Respiratory, Tucson, AZ) with one-way non-rebreathing valves, covering the subject's nose and mouth. CB (95% O₂ / 5% CO₂, TW Smith, Brooklyn, NY) was administered in the same way after a 2-minute recovery period under room air, during which the subject was breathing without the mask. BOLD and TOLD data were acquired during breath-holds at the end of each respiratory challenge. In all, 18 of 29 enrolled patients and all volunteers received both gas challenges, while 11 patients underwent only the O₂ challenge. CB challenge was not administered in the first three enrolled patients because we wanted to test the tolerance of volunteers to CB before administering it to patients. For subsequent patients, the CB challenge was not administered because of reduced respiratory capacity in one patient with pneumonectomy, patient's refusal to undergo the CB challenge ($n=2$), and unavailability of CB ($n=4$). With both respiratory challenges we defined the failure rate as the percent difference between the number of HCCs imaged under the respective challenge with each technique, and the number of HCCs with interpretable images for the challenge.

Image Analysis

HCCs were identified by a single experienced radiologist (observer 1, C.B., with 5 years of experience) according to the AASLD 2011 criteria¹⁹ on standard imaging sequences and/or prior clinical imaging studies (Figs. (2 and 3)). An image analyst (observer 2, O.B.), a physicist with 6 years of experience in MRI, placed regions of interest (regions of interest [ROIs]: $15.2 \pm 22.9 \text{ cm}^2$, range 0.9–83.2 cm²) on the MGRE and IR-LL images in consensus with observer 1. Images on which more than 50% of the lesion was not clearly visible due to motion artifacts, or obscured by tissue–air susceptibility artifacts, were considered

noninterpretable and excluded from the analysis. Care was taken to contour the lesion cross-section of largest diameter for all respiratory conditions.

Measurements were repeated in a sample of 12 patients for MGRE and 10 patients for IR-LL, by a third observer (M.W., a radiologist with 3 years of experience) to assess interobserver reproducibility. Observer 2 repeated the R_2^* and R_1 measurements in the same sample of 12 and 10 patients, respectively, for intraobserver variability assessment.

R_2^* ANALYSIS—ROIs were delineated using Osirix (Pixmeo SARL, Switzerland) on the MGRE image with the longest TE, in the HCCs (encompassing the whole lesion, excluding necrotic areas, partial volume, and susceptibility artifacts), the right hepatic lobe (2 cm², avoiding artifacts and major blood vessels), and paravertebral muscles in the same slice as the lesion (R_2^* values averaged for two ROIs of 2 cm², one on each side), for all respiratory conditions. As tissue–air susceptibility artifacts are most noticeable at the longest TE, ROIs were delineated on the image with longest TE to exclude all areas affected by artifacts from the ROI contour. Mean signal in the ROI was fitted to a monoexponential decay curve to measure T_2^* , using custom software written in MATLAB R2013b (MathWorks, Natick, MA). For lesions with maximum diameter greater than 6 cm, R_2^* was obtained as the average of ROI-based R_2^* values in two adjacent slices. R_2^* maps were calculated by fitting the signal pixel-by-pixel to the same monoexponential model.

R_1 ANALYSIS— T_1 maps were calculated in MATLAB from pixel-by-pixel fit of signal to the Look-Locker signal recovery equation.²⁰ ROIs were transferred in Osirix from the MGRE images to the IR-LL magnitude images and T_1 maps, with exclusion of partial volume artifact areas. R_1 was calculated as inverse of the mean T_1 value in each ROI. Changes in the transverse and longitudinal relaxation rate between baseline and hyperoxic respiratory challenges were calculated as:

$$\Delta R_{1,2}(\%) = 100 * [(R_{1,2}^{*baseline} - R_{1,2}^{*gas}) / R_{1,2}^{*baseline}]$$

Statistical Analysis

Data are presented as mean $\pm$ standard deviation. Coefficient of variation (%) was used to assess test–retest reproducibility of R_2^* and R_1 in all studied tissues, on both platforms in a subset of 10 subjects. R_2^* values at 1.5T and 3.0T were compared by Mann–Whitney U -test. R_2^* and R_1 values for each tissue at room air and at hyperoxic respiratory challenges were compared using a paired Wilcoxon sign rank test. For assessment of lesion response to gas challenge, we used the CV at baseline to denote changes due to normal physiological variation. Lesions that showed absolute ΔR_2^* or R_1 with O₂ or CB greater than the mean test–retest CV were considered responsive to the increase in oxygen content. Intraobserver and interobserver reproducibility was assessed by measuring CV and intraclass correlation coefficients (ICC); ICC 0–0.2 indicates poor agreement; 0.3–0.4 indicates fair agreement; 0.5–0.6 indicates moderate agreement; 0.7–0.8 indicates strong agreement; and >0.8 indicates excellent agreement.²¹ Relationships between R_2^* , R_1 , and their changes with

respiratory challenges to tumor size were assessed by Spearman correlation. R_2^* , R_1 , ΔR_2^* and R_1 for the different respiratory conditions were compared between lesions of different histopathologic grades by Kruskal–Wallis test, followed by Mann–Whitney U -test. ΔR_2^* and R_1 with O_2 and CB in HCC was compared to that of the liver and muscle by Mann–Whitney U -test.

Results

All subjects tolerated the multiparametric MRI exam, although all volunteers and patients who received CB reported greater difficulty in maintaining a breath-hold at the end of the 10-minute CB challenge. One patient refused to complete the CB challenge, and another refused a repeat of the same challenge in the retest exam. There were no adverse events reported with the use of gas challenges.

Forty-one lesions were identified in the 27 patients. Of the two treated patients, one had a 100% necrotic lesion that was excluded (while liver and muscle were analyzed) and the other one had a partially necrotic lesion (20% necrosis) that was analyzed (Fig. 3). One patient had two lesions, one HCC and a mixed HCC-cholangiocarcinoma on pathology; the latter was excluded from the study. In addition, 5 of 39 HCCs evaluated with R_2 under O_2 challenge were excluded due to motion and/or susceptibility artifacts (failure rate of 12.8%). Four of the 25 HCCs for which R_2^* imaging was attempted with CB were uninterpretable due to motion artifacts and were excluded (failure rate of 16%). Two of the 25 HCCs imaged with R_1 under O_2 did not have interpretable images due to motion artifacts and mismatch of tumor cross-sections between baseline and oxygen challenge (failure rate of 8%). With R_1 under CB, the failure rate was similar (15%), as 3 of 20 HCC exams were not evaluated due to motion artifacts.

Finally, 34 HCCs (mean size 4.3 ± 3.5 cm, range 1.0–13.0 cm) in 27 patients were evaluated for BOLD and 23 HCCs (mean size 4.2 ± 3.1 cm, range 1.4–13.0 cm) in 21 patients for both BOLD and TOLD effects (Table 2).

Liver and paravertebral muscles were analyzed for R_2^* and baseline and with O_2 in all of the 27 patients and in the eight volunteers (Table 2). The numbers of interpretable liver and muscle images with R_2^* under CB were smaller, due to motion artifacts (Table 2). R_1 was evaluated in the liver at baseline and under O_2 in 20 patients and one volunteer, and in the muscles, in 21 patients and one volunteer (Table 2). The number of subjects with interpretable R_1 images for liver and muscle images also decreased under CB due to motion artifacts (Table 2).

Histopathology

Histopathological proof of HCC was available for 18/34 lesions in 17 patients (partial hepatectomy, $n=15$, or biopsy, $n=2$), with the following tumor grades: well differentiated (two lesions), moderately differentiated (10 lesions), poorly differentiated (five lesions), undifferentiated (one lesion). All resected lesions had microvascular invasion.

Test–Retest Reproducibility

At 1.5T, R_2^* measurements for all studied tissues and all respiratory challenges had CV below 15% (Table 3). The test–retest CV for HCC was higher under CB (15%) than at baseline and under O_2 (9%), likely due to respiratory motion artifacts. Test–retest reproducibility was poorer at 3.0T, with mean CV for HCCs under O_2 at 21.5% and under CB at 32%. The two protocols used for quantification of R_2^* were equivalent in the liver, with bias of -1.3% and all values within the limits of agreement of $[-7\%, 4.4\%]$. In the eight HCCs identified in the patients used for protocol comparison, bias between the five echoes and the 12 echoes measurement was -2.9% , and limits of agreement were $[-19.2\%, 13.5\%]$. R_1 (measured only at 1.5T) was highly reproducible, with CV $<5\%$ in all tissues, under all respiratory conditions (Table 3).

Intra- and Interobserver Reproducibility

R_2^* had strong to excellent intra- and interobserver agreement in all tissues for all respiratory conditions (Table 4). R_1 measurements showed excellent intra- and interobserver agreement in all tissues for all respiratory challenges (Table 4).

Response to Hyperoxic Challenges

BOLD—Baseline R_2^* values were statistically lower at 1.5 T vs. 3.0T for HCC, liver, and muscle ($P < 0.0001$). We observed a significant decrease for all HCCs between baseline R_2^* and after O_2 challenge (paired Wilcoxon $P=0.019$), but not between baseline R_2^* and after CB ($P=0.47$) (Table 2). In the liver, we were unable to detect a statistically significant change in R_2^* with O_2 , although there was a trend of decreasing R_2^* with O_2 ($P=0.095$). No significant change in R_2^* was observed with CB ($P=0.71$) in the liver (Table 2). Muscle R_2^* showed a statistically significant increase with CB ($P=0.006$), and no difference for O_2 ($P=0.15$). $\Delta R_2^* O_2$ observed in HCC was significantly greater than that observed in muscle, but not significantly greater than that observed in liver. There were no significant differences between HCC, liver, and muscle for $\Delta R_2^* CB$ (Table 2).

Of note, the changes in R_2^* in HCC with O_2 and CB were 28 lesions studied with O_2 (baseline test–retest CV=9.1%) showed a decrease in R_2^* from baseline (mean $\Delta R_2^* O_2=16.8\%$), one showed an increase ($\Delta R_2^* O_2=-27.7\%$), and the majority (23 out of 28, $\Delta R_2^* O_2=4.3\%$) were considered nonresponders (Fig. 4). With CB (Fig. 4), 4 out of 18 lesions showed a decrease in R_2^* ($\Delta R_2^* CB=17.6\%$), five showed an increase ($\Delta R_2^* CB=-20.3\%$), and nine were considered nonresponders ($\Delta R_2^* CB=5.1\%$). Of the eight lesions studied at 3.0T with O_2 , four showed a decrease in R_2^* ($\Delta R_2^* O_2=18\%$), one showed an increase ($\Delta R_2^* O_2=-17.54\%$), and three were nonresponders ($\Delta R_2^* O_2=5.4\%$). With CB at 3.0T, two lesions showed a decrease in R_2^* ($\Delta R_2^* CB=-25.3\%$), and one showed no response ($\Delta R_2^* CB=2.8\%$).

There was no correlation between baseline R_2^* in HCCs and tumor size (Spearman's $r=-0.174$, $P=0.33$), while there was a borderline significant correlation between $R_2 O_2$ and tumor size (Spearman's $r=-0.32$, $P=0.06$), and a moderate significant correlation between

$\Delta R_2^* O_2$ and tumor size (Spearman's $r=0.54$, $P=0.0001$). For R_2^* under CB challenge, borderline significant Spearman correlations with lesion size were obtained ($R_2^* CB$ Spearman's $r=-0.44$, $P=0.057$; $\Delta R_2^* CB$ Spearman's $r=0.44$, $P=0.059$).

TOLD— R_1 in the HCCs showed a significant increase with O_2 (paired Wilcoxon $P < 0.001$), which is expected with the increase of free O_2 concentration in the tissue (Table 2). The same increase in R_1 was not reproduced with CB ($P=0.278$). No statistically significant changes in R_1 were observed for the liver (O_2/CB $P=0.79/0.89$). A statistically significant increase in R_1 was observed for muscle with O_2 ($P=0.0495$), but not with CB ($P=0.28$). $R_1 O_2$ for all HCCs (Table 2) was statistically greater than $R_1 O_2$ in the liver (Mann–Whitney $P=0.0039$), and $R_1 O_2$ in muscle ($P=0.024$).

An increase in R_1 with O_2 administration (Fig. 5) was observed in 10 out of 23 lesions ($R_1 O_2=-8.5\%$), while a decrease was observed in one lesion (undifferentiated HCC) ($R_1 O_2=10\%$), while 12 lesions showed no difference ($R_1 O_2=1.2\%$).

Administration of CB resulted in no R_1 response in 14 out of 16 lesions ($R_1 CB=2.1\%$), while an increase or decrease in R_1 were observed in one and one lesion, respectively.

Significant negative correlations were observed between R_1 at baseline (Spearman's $r=-0.52$, $P=0.01$), under O_2 (Spearman's $r=-0.48$, $P=0.022$), and under CB (Spearman's $r=-0.50$, $P=0.046$) with lesion size. No statistically significant correlations were observed between $R_1 O_2$ or $R_1 CB$ and HCC size (Spearman's $r=0.22$, $P=0.312$; Spearman's $r=0.22$, $P=0.31$, respectively).

Combined BOLD and TOLD Findings

Five out of 22 lesions which had both R_2^* and R_1 measurements (Fig. 6) showed a substantial decrease in R_2^* (above test–retest CV) and modest response in R_1 (below test–retest CV) with O_2 . The same response pattern was reproduced with CB in one of the five tumors (lesion 11-1). Only three lesions showed a substantial increase in R_1 and modest decrease in R_2^* with O_2 (Fig. 6).

Correlation With Histopathology

None of the R_2^* or R_1 measurements were significantly different between different grades of tumor (Kruskal–Wallis test P -value range 0.117–0.857).

Discussion

BOLD and TOLD have emerged as noninvasive alternatives to characterize tumor oxygenation.^{5,9,11,22–24} Although their noninvasive nature and wide availability make BOLD and TOLD attractive for clinical use, proof of reproducibility is necessary before their use as imaging biomarkers. Our study expands the scope of previous work on BOLD and/or TOLD in liver tumors^{8,12,14,24} by examining both effects in human HCC with O_2 and CB challenge. Our experience shows that R_2^* measurements with respiratory challenge are feasible in human HCC and more reproducible at 1.5T than at 3.0T, and that R_1

measurement is highly reproducible at 1.5T. Although we observed a significant decrease in R_2^* and increase in R_1 of HCC with O_2 challenge, R_2^* and R_1 responses to O_2 and CB challenges were highly heterogeneous, possibly due to variability in blood flow / blood volume and capacity for vasodilation among tumors.

R_2^* measurement in the liver is particularly challenging because of artifacts due to respiratory motion, fluctuations of the magnetic field at tissue–air interfaces, chemical shift, and cardiac pulsation.⁷ To avoid the documented effects of increasing glucose levels on blood flow²⁵ and R_2^* in the liver,²⁶ all subjects were imaged in fasting conditions. We obtained higher variability in R_2^* measurements at 3.0T than at 1.5T, which is consistent with greater magnetic field inhomogeneity at 3.0T. To control for the effect of chemical shift on signal, we used fat saturation in both our five in-phase echoes and 12 echoes protocol. R_2^* values from both protocols were found to be equivalent and in accordance with previously published R_2^* values for liver and HCC R_2^* at 1.5T^{7,12} and 3.0T.²⁶ For T_1 measurement in the liver, we sacrificed spatial coverage for more accurate T_1 quantification with a more time-intensive Look-Locker method, which acquires many data points to sample the longitudinal relaxation of signal. We found that T_1 measurement was highly reproducible on test–retest measurements, and repeated observations by two observers. T_1 values obtained in the liver and muscle were within the expected published range.^{13,14} Variable flip angle (VFA) methods can provide full liver coverage in a short time; however, quantification of T_1 is less accurate due to B_1 field inhomogeneities, particularly at higher field strength.^{5,27}

We observed a significant decrease in R_2^* of HCCs from baseline with administration of O_2 , as expected. However, since deoxy-Hb saturation depends on blood volume, flow, and vessel geometry, ΔR_2^* is influenced by both lesion pO_2 and vascularity, with changes in blood volume introducing perturbations in BOLD signal.⁴ Correlation with measures of vascularity, such as DCE-MRI, is needed to differentiate hypoxic from hypovascular tumors.⁴ Contrary to published BOLD data in other tumors,^{5,7–9,16,17} we did not observe a statistically significant decrease in R_2^* with the administration of CB. Some lesions showed a paradoxical response, an increase in R_2^* with administration of O_2 or CB, which has also been observed by Jhaveri et al¹² in their study of human HCC with O_2 challenge and by Guo et al⁸ in a study of the Novikoff hepatoma model in rats, with CB. The increase in R_2^* with CB can be explained by the immature vasculature in some HCCs, which fail to dilate in response to CO_2 .²³ Hypercapnia causes greater vasodilation in the mature vasculature of neighboring liver tissue, which in turn causes vascular steal away from the tumor, and thus poorer oxygen delivery.⁸ Hypercapnia can also decrease Hb affinity for O_2 , thus increasing R_2^* .⁴

We observed a significant increase in R_1 of HCC in response to O_2 . Although an increase in R_1 has been observed in response to O_2 and CB in other body carcinomas,^{5,7} there are scarce data on HCC. The only study of the TOLD effect in 10 patients with abdominal tumors¹⁴ showed a statistically significant increase in R_1 with O_2 , based on multiple measurements in one patient with HCC. A decrease of R_1 with O_2 or CB administration, observed in some HCCs in our study, is possible whenever O_2 delivery to the tumor tissue is

impaired, such that the concentration of free paramagnetic O_2 is smaller. This may be due to vascular steal, or to a local increase in deoxy-Hb concentration due to hypercapnic vasodilation.¹⁰

In 5 out of 22 lesions that had both R_2^* and R_1 measurements with O_2 , and in one of five lesions that had R_2^* and R_1 measurements under CB, a substantial decrease in R_2^* was associated with a modest response in R_1 , a response observed previously in hypoxic tumors.⁷ Only three lesions showed a pattern of response associated with baseline normoxia/mild hypoxia or hyperoxia,⁷ namely, a substantial increase in R_1 and modest decrease in R_2^* with O_2 .

The liver and paravertebral muscle did not show a change in R_2^* in response to O_2 inhalation, in accordance with previous studies.¹³ However, with CB inhalation we were able to reproduce the results of O'Connor et al, showing a significant R_2^* increase in the paravertebral muscles, but we did not reproduce the significant increase in R_2^* with CB in the liver, or the significant increases in R_1 in the liver and muscle with both O_2 and CB.¹³ The discrepancy may be due to different methods for R_2^* and R_1 quantification: O'Connor et al¹³ used an MGRE sequence with two TEs for R_2^* measurement, and a dual flip angle 3D SPGR sequence for T_1 measurement, which may not be as sensitive as our methods.

We administered CB after a 2-minute recovery period, during which the patient was breathing room air. Although other investigators used longer recovery periods (8 minutes),¹³ we judged 2 minutes to be sufficient based on the work of Remmele et al in brain tumors,¹⁰ which showed R_2^* and R_1 to revert to baseline values 100 seconds after the end of a hyperoxic challenge. We found that the CB challenge did not produce a more significant response in the R_2^* or R_1 of HCCs than O_2 . Four out of 19 HCCs imaged with both gases showed the same type of R_2^* response under O_2 and CB (ie, an increase or decrease greater than physiological variation), and in these cases the ΔR_2^* was greater with CB. Furthermore, administration of CB, although tolerated by patients, introduced more respiratory motion artifacts. The artifacts account for the failure rate of imaging with CB in our study (comparable to the R_2^* failure rate observed with CB in nonabdominal human tumors¹⁵, and for higher test-retest variability. A possible solution to the decreased failure rate of MRI with CB challenge would be to use a 98% O_2 / 2% CO_2 mixture instead of a 95% O_2 / 5% CO_2 mixture.¹⁵ More sophisticated physiological monitoring of end-tidal CO_2 pressure during CB challenge would also allow finer calibration of the hyperoxic-hypercapnic challenge and monitoring of physiological response.²⁸

ΔR_2^* under O_2 and CB challenge were found to have modest positive correlation (significant and borderline significant, respectively) with lesion size. The borderline significant correlations for $R_2^* O_2$ and $R_2^* CB$ with lesion size were negative, however. Our finding contradicts a previous study in rat HCC, which found significant negative correlation between $\Delta R_2^* CB$ and lesion size, with ΔR_2^* close to null or even negative for larger lesions. The discrepancy may be explained by the larger number of tumors ($n=34$) with greater

variation in etiology in our human study, versus the small number of lesions ($n=14$) of controlled etiology in the rodent study.⁸

We found significant negative correlations between R_1 at baseline, R_1 O_2 and R_1 CB, and lesion size. Lower R_1 at baseline and in response to O_2 and CB observed with increasing lesion size suggests poor oxygenation at baseline⁷ in larger HCCs, possibly due to impaired O_2 delivery.

Our study had several limitations. The results summarize our initial experience with BOLD, TOLD, O_2 , and CB challenge in a small series of subjects recruited in the first year of a 5-year prospective study. To assess the BOLD and TOLD effects, we used an imaging protocol with breathhold exams, similar to protocols used by other investigators in the abdomen.¹² Use of a breath-hold at end expiration may modify oxygen demand and availability. Breath-holds after CB breathing also decreased subjects' comfort and cooperation, and caused a large number of motion artifacts on the images. Furthermore, the voxel size and number of slices was not identical between the BOLD and TOLD sequences, which precluded exact registration of ROIs between the BOLD and TOLD series. A single reader identified the HCCs based on clinical and research imaging exams. Since not all lesions studied were resectable, we had limited pathology confirmation of HCCs (available in 17/27 patients).

A future study could correlate R_2^* and R_1 results with DCE-MRI metrics of perfusion and with histopathology markers of vascularity and hypoxia, such as microvessel density and HIF 1- α expression.^{7,29}

We conclude that R_2^* and R_1 measurements in the liver and HCCs with O_2 and CB challenge are feasible, with better test–retest reproducibility of R_2^* measurements in HCC at 1.5T, and with O_2 compared to CB challenge. Our preliminary experience with BOLD and TOLD MRI demonstrates the variable response of HCC to O_2 and CB challenges, which should be correlated with DCE-MRI findings to decouple tumor oxygenation from blood flow and volume effects, and with advanced histopathology findings.

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FIGURE 1.

MRI acquisition protocol demonstrating the succession of respiratory challenges for BOLD and TOLD imaging.

FIGURE 2.

A 67-year-old male patient with left hepatic lobe HCC measuring 5.4 cm (arrow on postcontrast T_1 WI), which demonstrates a decrease in R_2^* after O_2 ($\Delta R_2^* O_2 = 18.7\%$) and carbogen ($\Delta R_2^* CB = 20\%$) gas challenges. HCC shows no significant change in R_1 ($R_1 O_2 = -1.7\%$), below the level of physiological variation denoted by test-retest $CV = 3.6\%$. The decrease in R_2^* above the level of physiological variation, accompanied by no significant change in R_1 , is consistent with baseline hypoxia.

FIGURE 3.

A 68-year-old male patient with right hepatic lobe HCC treated with chemoembolization. There is central tumor necrosis on postcontrast T_1 WI (arrow). The viable tumor at the periphery of the lesion demonstrates response to hyperoxia (arrows), with a decrease in R_2^* under O₂ ($\Delta R_2^* \text{O}_2 = 17\%$) and carbogen ($\Delta R_2^* \text{CB} = 23.7\%$). The tumor also shows overall increase in R_1 under O₂ ($\Delta R_1 \text{O}_2 = -8.5\%$). This lesion may be normoxic or mildly hypoxic, since the R_1 increase with O₂ challenge is also substantial.

FIGURE 4.

Histogram distribution of changes in R_2^* observed in HCC lesions at 3.0T (patients 1–5) and 1.5T (patients 4–27) with oxygen (O_2) and carbogen (CB) gas challenges. The horizontal lines mark the threshold for response $\pm 9\%$ (1.5T CV R_2^* baseline = $\pm 9.1\%$, 3.0T CV R_2^* baseline = $\pm 8.8\%$). $\Delta R_2^* > 0$ denotes decrease in R_2^* from baseline with respiratory challenge ($\Delta R_2^* (\%) = 100 \times [(R_2^* \text{ baseline} - R_2^* \text{ gas}) / R_2^* \text{ baseline}]$). Lesions are indexed by patient number and ordered by superior-to-inferior anatomical location. The changes in R_2^* with O_2 are highly variable between individual lesions. With CB, 6/18 lesions at 1.5T and 2/3 lesions at 3T have paradoxical response (increase in R_2^* , $\Delta R_2^* < 0$).

FIGURE 5.

Histogram distribution of changes in R_1 observed in HCC lesions at 1.5T, with oxygen (O_2) and carbogen (CB) gas challenges. The horizontal lines mark the threshold for response, CV R_1 baseline=3.6%; $R_1 < 0$ denotes increase in R_1 from baseline with respiratory challenge ($R_1 (\%) = 100 * [(R_{1\text{baseline}} - R_{1\text{gas}}) / R_{1\text{baseline}}]$). Lesions are indexed by patient number and ordered by superior-to-inferior anatomical location. The majority of lesions show the expected response with O_2 , increase in R_1 ($R_1 < 0$), while response with CB is variable.

FIGURE 6.

Histogram distribution comparing R_2^* and R_1 response in HCCs to oxygen (O_2) challenge in 22 HCCs. Some tumors show substantial decrease in R_2^* with oxygen from baseline, and modest/no increase in R_1 ; these are possibly hypoxic (thin arrows). Other tumors show a modest decrease in R_2^* and substantial increase in R_1 (thick arrows); these are possibly normoxic/mildly hypoxic/hyperoxic tumors.

TABLE 1

BOLD Imaging Protocol With a Multiecho Gradient-Echo (MGRE) Sequence and TOLD Imaging Protocol With an Inversion-Recovery Look-Locker (IR-LL) Sequence at 1.5T and 3.0T

	2D MGRE 5 echoes IP	2D MGRE 12 echoes	2D MGRE 7 echoes IP	2D IR -LL
Acquisition plane	Axial	Axial	Axial	Axial
Field	1.5T	1.5T	3.0T	1.5T
TR (msec)	242		165	2.26
TE (msec)	4.76, 9.53, 14.29, 19.06, 23.82	1.66, 2.92, 4.25, 5.58, 6.91, 10, 15, 20, 25, 30, 35, 40	2.46, 4.92, 7.38, 9.84, 12.3, 14.76, 17.22,	1.04
FA	35°	20°	35°	8°
TI (ms)	—	—	—	42-1576.5 32 TI's every 48 msec
FOV read (mm)	340	340	340	420
FOV phase (%)	75	75	75	68.75
Slice thickness (mm)	7	7	7	8
Acquisition matrix	192 × 115	192 × 115		128 × 88
Slices	4-5	4-5	4-5	1-2
Parallel imaging factor	2	2	2	
Acquisition time (sec)	15	15	15	20

R₂^{*} and R₁ Values at Baseline and During Respiratory Challenges, With Percentage Changes From Baseline

TABLE 2

HCC			Liver			Muscle			
<i>n</i> ^a	<i>R</i> ₂ [*]	<i>R</i> ₂ [*]	<i>n</i>	<i>R</i> ₂ [*]	<i>R</i> ₂ [*]	<i>n</i>	<i>R</i> ₂ [*]	<i>R</i> ₂ [*]	
All	Air	34	32.2±17.8	35	42.1±17.5	35	36.2±4.2		
	O ₂	34	<i>b</i> _{29.5} ±10.6	35	40.9±19.4	35	36.6±4.2	21.4±8.7	
	CB	19	31.7±16.28	24	40.8±16.1	0.03±13.3	21	<i>b</i> _{38.8} ±6.1	212.1±18.5
<i>n</i> ^a	<i>R</i> ₂ [*]	<i>R</i> ₂ [*]	<i>n</i>	<i>R</i> ₂ [*]	<i>R</i> ₂ [*]	<i>n</i>	<i>R</i> ₂ [*]	<i>R</i> ₂ [*]	
1.5T	Air	28	28.4±8.6	30	38.8±15.1	30	35.6±3.7		
	O ₂	28	28.3±10.9	30	37.5±16.1	2.6±14.6	30	35.8±3.8	-0.8±5.7
	CB	18	29.6±13.5	19	38.2±15.0	-2.03±13.3	20	38.2±6.0 ^a	-11.7±19.3
<i>n</i> ^a	<i>R</i> ₂ [*]	<i>R</i> ₂ [*]	<i>n</i>	<i>R</i> ₂ [*]	<i>R</i> ₂ [*]	<i>n</i>	<i>R</i> ₂ [*]	<i>R</i> ₂ [*]	
3.0T	Air	8	45.7±14.6	12	61.2±14.9	12	40.9±3.8		
	O ₂	8	42.8±16.3	12	61.3±19.5	1.28±10.6	12	40.7±3.5	-0.3±13.5
	CB	3	62.5±11.4	9	60.2±18.9	-2.24±20.3	8	40.5±17.6	-12.3±26.9
<i>n</i> ^a	<i>R</i> ₁	<i>R</i> ₁	<i>n</i>	<i>R</i> ₁	<i>R</i> ₁	<i>n</i>	<i>R</i> ₁	<i>R</i> ₁	
1.5T	Air	23	1.34±0.2	21	1.76±0.2	22	1.38±0.1		
	O ₂	23	1.39±0.2 ^a	21	1.7±60.2	-0.13±4.4	22	1.39±0.1 ^a	1.39±0.1
	CB	16	1.37±0.2	13	1.77±0.3	-0.74±4.7	14	1.41±0.1	1.41±0.1

n^a, number of lesions; *n*: number of patients;
Δ*R*₂^{*} O₂ all HCCs vs. Δ*R*₂^{*} O₂ liver, *P*= 0.884 (Mann-Whitney test).
Δ*R*₂^{*} CB all HCCs vs. Δ*R*₂^{*} CB liver, *P*= 0.452 (Mann-Whitney test).
Δ*R*₂^{*} O₂ all HCCs vs. Δ*R*₂^{*} O₂ muscle, *P*= 0.013 (Mann-Whitney test).
Δ*R*₂^{*} CB all HCCs vs. Δ*R*₂^{*} CB muscle, *P* = 0.538 (Mann-Whitney test).

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R1 O2 all HCCs vs. R1 O2 liver, $P=0.0039$ (Mann-Whitney test).
R1 CB all HCCs vs. R1 CB liver, $P=0.369$ (Mann-Whitney test).
R1 O2 all HCCs vs. R1 O2 muscle, $P=0.024$ (Mann-Whitney test).
R1 CB all HCCs vs. R1 CB muscle, $P=0.921$ (Mann-Whitney test).
^a significant, paired Wilcoxon test ($P < 0.05$), for data grouped by field strength;
^b significant, paired Wilcoxon test ($P < 0.05$), for combined data at both field strengths.

TABLE 3

Mean Coefficients of Variation (%) for Test-Retest Variability Measurements of R_2^* and R_1 Values at Baseline and During Respiratory Challenges

		HCC		Liver		Muscle	
1.5T	R_2^*	n^a	Mean CV (%)	n	Mean CV (%)	n	Mean CV (%)
		Air	7 9.1	8 11.64	8 7.6		
		O ₂	7 9.43	8 12.09	8 12.9		
		CB	2 15	4 8.7	4 5.9		
	R_1	n^a	Mean CV	n	Mean CV	n	Mean CV
		Air	8 3.67	7 4.12	7 1.61		
		O ₂	8 4.23	7 4.06	7 1.1		
		CB	2 3.58	2 4.37	3 3.23		
	R_2^*	n^a	Mean CV	n	Mean CV	n	Mean CV
		Air	4 8.8	2 9.1	2 8.1		
		O ₂	4 21.5	2 8.2	2 3.58		
		CB	1 32.4	1 12	— —		

n^a : number of lesions assessed on test-retest; n : number of patients assessed on test-retest.

TABLE 4Intra- and Interobserver Agreement for R_2^* and R_1 Measurements

R_2^*	HCC ($n^a = 16$)			Liver ($n=12$)			Muscle ($n=12$)			
	Air	O ₂	CB	Air	O ₂	CB	Air	O ₂	CB	
Intraobserver	CV (%)	2.55	2.25	3.92	3.18	2.72	11.15	1.91	1.08	3.05
	ICC	0.97	0.98	0.98	0.92	0.97	0.7	0.69	0.98	0.79
	<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0049	0.0048	<0.0001	<0.0001
Interobserver	CV (%)	4.86	3.66	6.19	3.62	6.06	10.31	0.86	1.93	2.66
	ICC	0.88	0.96	0.94	0.96	0.88	0.76	0.99	0.96	0.83
	<i>P</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0016	<0.0001	<0.0001	<0.0001
R_1	HCC ($n^a = 12$)			Liver ($n=10$)			Muscle ($n=10$)			
	Air	O ₂	CB	Air	O ₂	CB	Air	O ₂	CB	
Intraobserver	CV (%)	1.75	1.89	2.35	1.33	1.61	1.99	1.87	1.44	2.14
	ICC	0.97	0.96	0.71	0.98	0.97	0.96	0.84	0.87	0.88
	<i>P</i>	<0.0001	<0.0001	0.009	<0.0001	<0.0001	<0.0001	0.0008	0.0002	0.0011
Interobserver	CV (%)	1.77	2.37	2.35	2.97	2.88	3.27	1.87	2.56	3.18
	ICC	0.96	0.95	0.85	0.84	0.87	0.91	0.79	0.8	0.77
	<i>P</i>	<0.0001	<0.0001	<0.001	0.0003	0.0003	0.0004	0.0025	0.0015	0.0079

 n^a : number of lesions assessed; n : number of patients assessed.