Hemodynamic response magnetic resonance imaging: application for renal hemodynamic characterization

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ABSTRACT

Background. The clinical use of iodinated radiocontrast agents or gadolinium for renal perfusion imaging is limited in the presence of renal dysfunction. We have previously demonstrated the feasibility of hemodynamic response imaging (HRI), a functional magnetic resonance imaging (MRI) method combined with hypercapnia and hypercapnic-hyperoxia, for monitoring changes in liver perfusion and hemodynamics. The aim of the present study was to evaluate the utility of HRI for monitoring changes in renal perfusion and oxygenation.

Methods. Renal HRI maps were acquired during graded hypercapnia (95% air + 5% CO\textsubscript{2}) and hypercapnic-hyperoxia (95% O\textsubscript{2} + 5% CO\textsubscript{2}) in control mice. The utility of HRI for monitoring changes in renal perfusion and oxygenation was evaluated using pharmacological inhibition of nitric oxide synthase and cycloxygenase as well as in rhabdomyolysis-induced acute kidney injury (AKI) in mice. HRI results were further interpreted using Doppler ultrasound (US).

Results. Renal HRI maps revealed pronounced signal-intensity changes in response to both hypercapnia and hypercapnic-hyperoxia, reflecting intense vascular reactivity. These changes were significantly attenuated following the pharmacological intervention and during AKI, corresponding with hampered perfusion dynamics, as confirmed by Doppler US.

Conclusions. The applicability of the non-invasive HRI method suggests its potential use for the evaluation of renal perfusion and vascular reactivity, excluding the need for contrast-agent administration.

INTRODUCTION

Intra-renal oxygenation and microcirculation are important determinants in renal pathophysiology. Iodinated and magnetic contrast agents are used for the determination of the spatial distribution of renal perfusion and oxygenation. However, their utilization in patients with evolving or chronic renal impairment is limited due to the increased risks of exacerbating renal dysfunction and systemic adverse effects [1]. Therefore, a non-invasive technique devoid of contrast media administration has a great potential in the setup of renal impairment, where altered vascular reactivity may be both a hallmark for, and a causative parameter in, the pathogenesis.

Blood oxygen level-dependent (BOLD) magnetic resonance imaging (MRI) is a well-established and validated method that forms the basis of functional imaging techniques, based on the paramagnetic properties of deoxyhemoglobin, which affects signal intensity (SI) on \textit{T}\textsubscript{2}\textsuperscript{*}-weighted MRI sequences [2]. The apparent relaxation rate map, denoted as \textit{R}\textsubscript{2}, was shown to provide indirect measures of renal oxygenation \textit{in vivo} [3]. Indeed, BOLD MRI non-invasively reflects intra-renal oxygenation levels of naïve kidneys under normal conditions and during physiological and pharmacological maneuvers [3–5].
Hemodynamic response imaging (HRI) is another BOLD MRI method based on changes in SI on \(T_2^*\)-weighted images during brief exposure to hypercapnia and hypercapnic-hyperoxia. Changes in \(T_2^*\) SI are related to altered oxygen saturation, blood flow and blood volume [6]. BOLD MRI during respiratory challenges have been shown to indicate changes in vascular density, vessel reactivity and tissue perfusion [7], and enables analysis of alterations in vascular architecture, functionality and maturation in intact and malignant tissues [7–10]. Thus, HRI kidneys studies may enable the non-invasive detection of renal hemodynamic changes in the presence of renal impairment without the need of contrast material.

In vivo animal models
Animal experiments were performed in accordance with National Institutes of Health approved guidelines of the Animal Care and Use Institutional Committee (OPRR-A01-5011) on 7–8-week-old CB6F1 mice (Harlan; Jerusalem, Israel). MRI and Doppler ultrasound (US) studies were carried out under anesthesia (Pentobarbital, CTS group, Hod-Hasharon, Israel; 70 mg/kg, i.p.), with breathing of ambient air (normoxia), with subsequent 5 min of hypercapnia (air + 5% CO\(_2\); 4 L/min), followed by 5 min of hypoxia (normoxia), with subsequent 5 min of hypercapnic-hyperoxia (carbogen; 5% CO\(_2\), 95% O\(_2\)). HRI maps were generated as reported previously [7] using a home-built IDL (ITT Visual Information Solutions, Boulder, CO, USA) program. The percentage change in SI (\(\Delta S\)) induced by hypercapnia (\(\Delta S_{CO2}\)) or hypercapnic-hyperoxia (\(\Delta S_{O2}\)) was calculated from the average of six repetitions for each condition, while the first two repetitions were ignored (Figure 1B). The calculations included only pixels with statistical threshold of \(P < 0.05\).

Contrast-enhanced MRI
In order to compare HRI results with a routine method, contrast-enhanced (CE) MRI was performed with \(T_1\)-weighted FLASH sequence (TR/TE = 58.3/5.2 ms; field-of-view = 3.4 cm; in-plane-resolution = 133 \(\mu\)m; slice-thickness = 1 mm; 1 average; 60 repetitions), resulting in a temporal resolution of 7.4 s. Ten repetitions were acquired on baseline, followed by 50 repetitions after Gd-DTPA (Magnevist; Soreq Radiopharmaceuticals, Yavne, Israel; 0.5 M, 100 \(\mu\)L) administration via the tail vein at a dose of 0.1 mmol kg.

US analysis technique
Color velocity and spectral Doppler US measurements were acquired with a 22–55-MHz linear transducer (MS550D) (VisualSonics, Inc., Toronto, Ontario, Canada). Measurements were acquired during normoxia, hypercapnia and hyperoxia (100% oxygen). Arterial renal resistance index (RRI) was calculated as (peak systolic velocity – end-diastolic velocity)/peak systolic velocity [14].

Statistical analysis
Results are expressed as means ± SD. Statistical analyses were performed with the Instat Biostatistics software (GraphPad Software, Inc., San Diego, CA, USA). The differences between groups were analyzed by one-sided Mann–Whitney test (data points <30). \(P\)-value < 0.05 was considered statistically significant.

RESULTS

The effect of hypercapnia and hypercapnic-hyperoxia on renal BOLD MRI
Renal HRI maps (Figure 1A) revealed pronounced SI changes in the kidneys in response to both hypercapnia and hypercapnic-hyperoxia. In control mice, hypercapnia significantly decreased SI in the entire kidney (–27 ± 7%; \(n = 15\)) compared with the moderate effect in the adjacent skeletal muscle (7.6 ± 4.4%). Whereas, hypercapnic-hyperoxia significantly increased SI in the entire kidney (49 ± 19%; \(n = 15\)) compared with the lower effect in the skeletal muscle (12 ± 3%) (Figure 1A and B; Table 1). Prominent SI changes were especially noted in the renal vessels. In contrast, papillary SI usually remained unaffected, conceivably reflecting low regional perfusion. Doppler US confirmed the role of hypercapnia-induced blood-flow changes in HRI maps, showing increased renal arterial velocity by 14 ± 6% compared with baseline, with decreased calculated RRI values by 19 ± 9%

MATERIALS AND METHODS

In vivo animal models
Animal experiments were performed in accordance with National Institutes of Health approved guidelines of the Animal Care and Use Institutional Committee (OPRR-A01-5011) on 7–8-week-old CB6F1 mice (Harlan; Jerusalem, Israel). MRI and Doppler ultrasound (US) studies were carried out under anesthesia (Pentobarbital, CTS group, Hod-Hasharon, Israel; 70 mg/kg, i.p.), with breathing of ambient air (normoxia), with subsequent 5 min of hypercapnia (air + 5% CO\(_2\); 4 L/min), followed by 5 min of hypercapnic-hyperoxia (5% CO\(_2\), 95% O\(_2\), 4 L/min), administered through a face mask. Reagents were purchased from Sigma, St Louis, MO, USA, unless stated otherwise. Pharmacological intervention to reduce vascular reactivity, renal perfusion and oxygenation were achieved by tail vein administration of 
\(\)NAME (10 mg/kg) and indomethacin (10 mg/kg dissolved in PBS; pH 8), inhibitors of nitric oxide (NO) and prostaglandin synthesis, respectively [11–13]. Rhabdomyolysis-induced acute kidney injury (AKI) was induced by intramuscle injection of 50% glycerol (8 mL/kg), to the anterior thigh muscle of hindlimbs. AKI was confirmed on Day 4 with serum urea reaching 433 ± 64 mg/dL.

Magnetic resonance imaging
MRI experiments were performed on a horizontal 4.7 T Biospec spectrometer (Bruker, Ettlingen, Germany) using a 3.5 cm birdcage coil. Renal region of interest was drawn manually by using Analyze-7.0 (BIR, Mayo Clinic, Rochester, MN, USA), based on true-FISP images [true fast imaging with steady-state precession; repetition time/echo time (TR/TE) = 3/1.5 ms], covering the entire kidney. Mean renal HRI values were calculated from the central axial slice of each kidney.

Hemodynamic response imaging
The HRI protocol was implemented as previously described [7], using \(T_2^*\)-weighted gradient-echo images (TR/TE = 147/10 ms; field-of-view = 3.4 cm; in-plane-resolution = 133 \(\mu\)m; slice-thickness = 1 mm) during either normoxia (21% O\(_2\), 79% N\(_2\)), hypercapnia (5% CO\(_2\), 21% O\(_2\), 74% N\(_2\)) and hypercapnic-hyperoxia (carbogen; 5% CO\(_2\), 95% O\(_2\)).
Moreover, during the hypercapnic challenge, we observed the appearance of numerous visible smaller vessels (Figure 1C). This effect was blunted during hypercapnic-hyperoxia (Figure 1C).

**HRI during pharmacological renal hypoperfusion**

Renal vasoconstriction and reduced vascular reactivity induced by NO synthase and cyclooxygenase inhibition was shown to be associated with hypoxia, as reflected by enhanced $R_2^*$. In our HRI experiment, the administration of L-NAME and indomethacin resulted in significant reduction of renal HRI values (ΔS) in response to both hypercapnia and hypercapnic-hyperoxia (Figure 2B), reaching 60% of baseline values by 30 min (Figure 2C; Table 1; n = 5, P < 0.001). In contrast, 30 min following saline administration, only minor changes could be detected (Figure 2A), with an insignificant reduction of 5% in ΔS values (Figure 2C; Table 1). Renal arterial blood flow velocity following L-NAME administration, as determined by Doppler US, markedly declined (Figure 2D; n = 3). The maximal velocity values that were measured in the segmental vessels declined by 3-fold following L-NAME administration, whereas smaller vessels could not be detected at all. In addition, the response to hypercapnia was attenuated following L-NAME administration only minimal changes were observed in renal arterial maximal velocity values (data not shown), in contrast to the hypercapnia-induced velocity...
a potential risk for further renal dysfunction and systemic changes observed at baseline. These results indicate the involvement of hampered blood vessels reactivity.

Renal perfusion attenuation during AKI assessed by HRI

Markedly reduced renal blood flow and hypoxia are known to be involved in the animal model of rhabdomyolysis-induced AKI [15–17]. Renal HRI maps, acquired 4 days after the induction of rhabdomyolysis-induced AKI, revealed significantly attenuated reactivity to hypercapnia and reduced renal oxygenation and perfusion as $\Delta S$ values reached 20% of baseline values (Table 1; $n = 5$, $P < 0.01$; Figure 3A). Indeed, renal arterial blood-flow velocity that was measured by Doppler US was dramatically reduced by 5-fold in AKI mice compared with control mice (Figure 3B). Following hypercapnia, only a slight increase was observed in renal arterial velocity, compared with the hypercapnia-induced increase on control mice (data not shown). Renal perfusion was also evaluated in these mice with CE MRI. As shown in Figure 3C, contrast material in control mice reached maximal level shortly after Gd injection, with subsequent rapid washout, indicating intact perfusion and glomerular filtration, respectively. In contrast, AKI animals displayed delayed kinetics, with delayed renal contrast accumulation and without its clearance from the kidneys during the observation period.

| Table 1. Comparison of hypercapnic and hypercapnic-hyperoxia effects on BOLD MRI changes (HRI) in control mice and AKI mice and following induction of vasoconstriction |
|---------------------------------|---------------------------------|
|                                | $\Delta S_{CO_2}$ (%) | $\Delta S_{O_2}$ (%) |
| Control ($n = 15$)             |                                |
| Kidneys                        | $-27 \pm 7$                 | $49 \pm 19$          |
| Muscle                         | $7.6 \pm 4.4^*$              | $12 \pm 3^*$         |
| L-NAME + indomethacin ($n = 5$)|                                |
| Kidneys                        | $-19 \pm 3^*$                | $27 \pm 13^*$        |
| Saline ($n = 5$)               |                                |
| Kidneys                        | $-29 \pm 6$                  | $46 \pm 18$          |
| AKI-Day4 ($n = 5$)             |                                |
| Kidneys                        | $-3 \pm 2.1^*$               | $9 \pm 3.9^*$        |

The figures illustrate absolute HRI values, reflecting the difference between SI during air breathing and hypercapnia/hypercapnic-hyperoxia ($\Delta S_{CO_2}$ and $\Delta S_{O_2}$, respectively). Results are presented as mean ± SD.

$^*P < 0.0001$ compared with control kidneys value.

**DISCUSSION**

Currently, assessment of renal perfusion in the setting of impaired renal function is limited to Doppler US, as the use of both iodinated and magnetic contrast agents is associated with a potential risk for further renal dysfunction and systemic responses. Recently, we reported the applicability of HRI for monitoring changes in liver hemodynamics without the need of contrast-agent administration [7, 18]. In the present study, HRI was shown to be sensitive to renal hemodynamic changes during either pharmacological or pathological conditions. Therefore, the addition of gas enrichment to the routine BOLD MRI might enable the simultaneous determination of renal oxygenation and microcirculation reactivity and functionality.

Intra-renal oxygenation reflects changes in local perfusion, local oxygen consumption and arterial-to-venous oxygen shunting. Non-invasive techniques for the assessment of renal oxygenation or microcirculation, such as BOLD MRI and Doppler US, respectively, when applied alone, provide only partial information regarding either oxygenation or microcirculation. Therefore, the comprehensive information provided by adding hypercapnic challenge to the basic BOLD MRI routine, as done in HRI, makes it a useful tool for the assessment of renal perfusion and oxygenation as well as vascular reactivity during either pathological or pathological conditions.

Our results demonstrate that hypercapnia, induced by 5% CO$_2$ inhalation, causes a marked decline in HRI maps (30% reduction of $\Delta S$), probably as a result of two main factors: (i) Bohr effect—the increased concentrations of CO$_2$ reduces the oxygen affinity of hemoglobin, thus leading to increased deoxyhemoglobin in the venous blood; (ii) CO$_2$-induced vasodilatation—which promotes tissue flushing with deoxygenated hemoglobin, related to the Bohr effect [19]. These findings resemble studies in the liver, showing decline HRI maps during hypercapnia, in parallel with enhanced portal blood flow [18]. Indeed, our findings are further supported using Doppler US by demonstrating enhanced blood flow and vasodilatation in response to hypercapnia. Other confounding factors that might influence BOLD signals in our studies are hyperventilation with a subsequent increase in hemoglobin saturation and changes in renal blood volume and systemic hemodynamics. However, in our experiments, seemingly this was not the case, as hypercapnia under pentobarbital anesthesia caused unexpected hypoventilation (reduction of respiratory rate by 13 ± 6%, with unaltered tidal volume). Moreover, kidney size remained unchanged, possibly suggesting no change in the renal blood content. Additionally, in parallel experiments in pentobarbital-anesthetized rats, we found that hypercapnia does not affect mean arterial blood pressure.

It was previously shown that hyperoxia enhances blood oxygenation and increases renal SI in $T_2$-weighted images by only 3–4% [20]. In contrast, in this study, hypercapnic-hyperoxia following hypercapnia caused a 50% increase in renal $T_2$-weighted SI, markedly enhancing the sensitivity of this method. The feasibility of 5% CO$_2$ enrichment for human use has been previously reported [21, 22], highlighting the practical value of this method.

The sensitivity of HRI to assess renal perfusion changes was evaluated in the setting of renal vasoconstriction and reduced vascular reactivity. Renal $\Delta S$ during changes in inspired gases were markedly diminished following L-NAME and indomethacin, as well as in rhabdomyolysis-induced AKI, and paralleled the decline in renal arterial blood flow velocity with diminished vasodilatation as shown with Doppler US.
**FIGURE 2:** The effect of L-NAME and indomethacin on renal perfusion and vascular reactivity. (A and B) Representative HRI maps of the left kidney obtained before (top) and 30 min after (bottom) i.v. administration of saline (A) or L-NAME and indomethacin (B), demonstrating the attenuated effects on HRI maps following the administration of the vasoconstrictor drugs (scale bar = 0.5 cm). (C) Mean percent change of renal DSO2 values due to L-NAME and indomethacin, or saline administration (n = 5 mice/group) *P < 0.001. (D) Representative color Doppler US images acquired before (left) and 30 min after L-NAME administration (right), showing the reduced blood flow in the kidney following L-NAME administration (the kidney is demarcated with a striped line).

**FIGURE 3:** Renal perfusion and vascular reactivity during AKI as assessed by BOLD MRI. (A) Representative HRI maps (ΔSCO2 and ΔSO2) during rhabdomyolysis-induced AKI (right; Day 4 post-glycerol injection) compared with baseline (left), showing the attenuated response to both gases (scale bar = 0.5 cm). (B) Representative power Doppler US images during rhabdomyolysis-induced AKI (Day 4 post-glycerol injection) compared with control, showing the reduced blood flow during AKI (the kidney is demarcated with a striped line). (C) Representative normalized renal CE MRI SI time curves measured at baseline and 4 days after the induction of AKI. The injection time is indicated on the graph. Renal SI at baseline picks immediately following Gd injection and declines rapidly, reflecting intact microcirculation and glomerular filtration, respectively. In contrast, following AKI, SI rise is delayed, and signal washout is absent throughout the observation period.
This reduced HRI response indicates that indeed renal HRI might serve as a sensitive marker for altered renal vascular reactivity. HRI maps were found to be much more sensitive for altered renal hemodynamics, underscoring their potential advantage in the assessment of renal impairment.

Finally, as shown in Figure 1A, HRI has the potential to provide excellent resolution capacity, enabling clear distinction of dynamic changes in cortical vessels, probably interlobar and interlobular arteries and/or veins, in the tiny mice kidney.

There are several limitations in this study. First, our high (0.1 mm) resolution emphasized intense changes noted in BOLD signals at the cortico-medullary junction, which conceivably reflects changes in oxygenated hemoglobin within arcuate vessels, rather than changes in blood oxygenation within the renal parenchymal microcirculation. Since we could not, for certain, ignore these signals as non-parenchymal, we decided to assess BOLD changes within the entire kidney. Second, the experiments were performed on anesthetized mice, and further research is required to establish clinical applicability of this technique in conscious humans. Most importantly, with the interpretational complexity of our results during hypercapnia, discussed above, we cannot unequivocally exclude other confounding determinants, including systemic circulatory and neuro-humoral state, that might be better controlled for in larger animals and in human studies. Finally, BOLD MRI SI changes inherently depend on both $T_1$ and $T_2^*$ alterations. Since blood flow could influence $T_1$, any associated changes with hypercapnia on $T_1$ could also influence the SI that we are measuring. Indeed, the reported renal HRI results were sensitive to changes in blood flow, since we were using short-TR value (147 ms) and also relatively short-TE value (10 ms). These results were supported by Doppler US demonstrating enhanced blood flow and vasodilatation in response to hypercapnia and also in agreement with the previously reported results for liver-HRI [18]. Thus, seemingly, combined $T_1$ and $T_2^*$ data are an excellent tool for the assessment of tissue oxygenation and perfusion, compared with $T_2^*$ alone.

In conclusion, HRI was shown to be sensitive to changes in renal perfusion and vascular reactivity providing non-invasive dynamic and spatial indices of renal hemodynamics and renal oxygen availability, without using potentially toxic contrast agents.

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**CONFLICT OF INTEREST STATEMENT**

None declared.

**REFERENCES**


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Pretreatment with paricalcitol attenuates inflammation in ischemia–reperfusion injury via the up-regulation of cyclooxygenase-2 and prostaglandin E2

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ABSTRACT

Background. The effect of paricalcitol on renal ischemia–reperfusion injury (IRI) has not been investigated. We examined whether paricalcitol is effective in preventing inflammation in a mouse model of IRI, and evaluated the cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2) pathways as a protective mechanism of paricalcitol.

Methods. Paricalcitol (0.3 μg/kg) was administered to male C57BL/6 mice 24 h before IRI. Bilateral kidneys were subjected to 23 min of ischemia, and mice were killed 72 h after IRI. The effects of paricalcitol on renal IRI were evaluated in terms of