Furosemide and water load challenges in renal BOLD imaging

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Purpose

Goal:
To assess the impact of oral water and intravenous furosemide challenges on BOLD signals in the kidney and to examine the R2 contribution to R2* changes.

Introduction

The renal medulla in mammals functions in a state of relative hypoxia. The steep corticomedullary gradient in the partial pressure of oxygen (pO₂) results from the countercurrent blood flow through the medullary vasa recta and the high rate of oxygen consumption, which is required for active reabsorption of sodium in the medullary thick ascending limb of the loop of Henle. Medullary pO₂ is in the range of 10 to 20 mm Hg [1] whereas mean venous blood pO₂ is about 40 mm Hg and cortical oxygenation is about 50 mm Hg.

An increase in medullary hypoxia has been implicated in the development of acute renal failure and also plays a role in the pathophysiology of hypertension and diabetic nephropathy. Renal oxygenation is therefore a useful marker for renal function and for the assessment of residual renal function in patients with various diseases.

Blood oxygenation level-dependent (BOLD) magnetic resonance imaging (MRI) has the potential to assess intrarenal oxygenation noninvasively. BOLD MRI exploits the paramagnetic properties of deoxyhemoglobin. As the renal medulla is naturally hypoxic, it falls in the linear portion of the hemoglobin oxygen desaturation curve, making BOLD MRI a sensitive marker of pO₂ changes [2], especially at high field strengths. The presence of deoxyhemoglobin in the blood creates differences in magnetic susceptibility between the capillaries and surrounding extravascular tissue, causing dephasing among spins and increasing the transverse relaxation rate R2*.

However, other factors such as B0 shimming, the rate of renal blood flow, water content, and magnetic susceptibility effects unrelated to deoxyhemoglobin also affect R2* measurements. For this reason most of the renal BOLD imaging focused on changes in R2* (#R2*). The landmark work of Prasad et al. [2] demonstrated that an oral water load or an IV furosemide injection provide a highly informative physiologic challenges. Both challenges significantly increase the medullary pO₂.

A further consideration in the use of R2* as a surrogate marker for tissue pO₂ is the fact that R2* is also affected by variations in R2, due for example to changes in water
content. It has long been known that the kidney undergoes substantial changes in water content in response to physiological and pharmacological interventions. To eliminate the influence of water content, one must simultaneously measure R2* and R2 and calculate their difference,

$$R2' = R2^* - R2$$

The resulting parameter R2' is directly sensitive to deoxyhemoglobin concentration, without being affected by the confounding effects of water content. In spite of its importance, the impact of R2 has not been extensively studied. To our knowledge, only one small human study at 1.5T [2] has simultaneously evaluated R2* and R2 after water load and furosemide. Despite observing that 24% of medulla $R2^*$ (-6.43 sec$^{-1}$) after water load was related to medulla $R2$ (-1.53 sec$^{-1}$) the authors concluded that the impact of $R2$ on $R2^*$ was negligible. This conclusion was contraindicated by recent animal studies [3,4].
Methods and Materials

Study design

Ten young healthy volunteers were enrolled. This HIPAA-compliant study was approved by our Institutional Review Board, and written informed consent was obtained from all subjects.

The volunteers were imaged on two different visits with a time interval not exceeding 21 days. They were asked to fast overnight. At each visit, the baseline images were followed by a diuretic challenge:

- oral water load for the first visit
- furosemide injection for the second

**First visit:** volunteers were asked to drink 20 mL of water per kilogram of body weight within 15 minutes in order to induce water diuresis. MR imaging started 45 minutes after the beginning of the water load, and subjects were scanned over a period of at least 30 minutes (Fig. 1).

**Second visit:** 20 mg of furosemide was injected intravenously over 10 seconds and flushed with 10 mL of saline. MR imaging started 5 minutes thereafter and continued for a period of at least 30 minutes.
**Fig. 1**: Study timeline. Each cycle of measurements (corresponding to one time point) was repeated continuously during 30 minutes after the diuretic challenges. mGRE: multiple gradient-echo sequence; mSE: multiple spin-echo sequence; T2-W: T2-weighted fast spin-echo sequence.

**References**: Radiology, Rouen University Hospital - Rouen/FR

**MR Imaging**

MR imaging was performed at 3 T:

- **R2* measurements** were made using a 2D multiple gradient echo (mGRE) sequence with a water-selective excitation pulse (TR (msec)/TE (msec)/flip angle/BW (Hz/pixel), 70/4.3-42.7/30°/300) to acquire 12 images with echo spacing of 3.5 msec. The acquisition was performed within a 15-second breath-hold. A single coronal slice was acquired in the long axis of both kidneys. The field of view was 420 x 336 mm, with an acquisition matrix of 320 x 272, and slice thickness of 7 mm.

- **R2 measurements** were made using a 2D multiple spin-echo (mSE) sequence was performed to acquire 7 images with echo spacing of 22 msec. The parameters of this sequence were (TR (msec)/TE (msec)/flip angle/BW (Hz/pixel)/parallel acquisition/turbo factor 700 /22-153/180°/521 /GRAPPA 2/ 4). The acquisition was performed within a 22-second breath hold. The imaging plane and spatial resolution were matched to the mGRE sequence.

- **Urine output measurements**: axial 2D T2-weighted (T2W) acquisitions were repeatedly performed through the bladder (TR (msec)/TE (msec) /flip angle/echo train length BW (Hz/pixel)/FOV/matrix/slice thickness (mm) 5700 /91 /180°/11/210 /280 x 400/162x256/3). These acquisitions were performed before and after each set of mGRE and mSE acquisitions.

Two baseline measurements were performed at each visit to assess repeatability. mGRE and mSE sequences were each run twice (baseline 1) and then the volunteer was taken out of the magnet. After 5 minutes the subject returned into the magnet and the same acquisitions (baseline 2) were repeated with a completely new calibration. In total, 4 mGRE and 4 mSE acquisitions were performed before any diuretic challenge each day.

Over the 30-minute period following the diuretic challenge, 4 or 5 time points were acquired, where each time point consisted of two mGRE acquisitions, two mSE acquisitions, and two acquisitions over the bladder bracketing the other 4 acquisitions to measure the urinary output.
Each time point therefore consisted of six acquisitions with a total duration of 5 to 7 minutes. The volunteers were allowed to get out of the scanner at any time if they felt a need to void. After voiding, they were returned to the scanner and the study was resumed.

**Data analysis**

Images were analyzed offline using customized software routines in Matlab (The MathWorks, Inc, Natick, MA). R2 and R2* maps were generated by fitting the intensity data in each pixel as a function of TE to a monoexponential decay using a nonlinear least-squares Levenberg-Marquardt algorithm. In cases of rapid signal decay, the data were truncated by ignoring samples with intensity below twice the noise level.

Two independent readers processed all the data. Observers drew regions of interest (ROI) over left and right cortex and medulla on anatomic images (Fig. 2). The R2* and R2 values of each pixel within each ROI were averaged to obtain a single representative mean value of R2* and R2 per subject for each region (i.e., medulla or cortex) and each time point.
Fig. 2: ROIs drawn on T2* and T2 images with their corresponding R2* and R2 maps on cropped images over the left kidney. Note that the medullary R2* values decrease post-water load and post-furosemide as compared to the baseline states.

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R2' values were calculated using the equation:

$$R2' = R2^* - R2$$
where $R^*_2$, $R^2$, and $R^'_2$ were expressed in sec$^{-1}$. $#R^*_2$, $#R^2$ and $#R^'_2$ were defined as the changes (post diuretic challenge - baseline) in $R^*_2$, $R^2$ and $R^'_2$ respectively.

As all three $R^*_2$, $R^2$, and $R^'_2$ values are expected to decrease after a diuretic challenge, we computed the contribution of $#R^2$ to $#R^*_2$ for each time point as:

$$(#R^2 / #R^*_2) \times 100\%.$$ 

To measure the urine output during the diuretic challenges, one observer segmented the bladder areas on the T2W images with a validated semi-automatic segmentation tool and calculated bladder volumes based using a modified Simpson's rule. The urine output was calculated from the bladder volumes as:

$$\text{Urine output} = \frac{\text{Volume}(t_{i+1}) - \text{Volume}(t_i)}{(t_{i+1} - t_i)}$$

where $t_i$ and $t_{i+1}$ are successive timepoints.

**Statistical analysis**

Mixed model analysis of variance was used to compare $R^2$ and $R^*_2$ measures with respect to reproducibility as represented by the inter-observer coefficient of variation (CV).

A linear regression analysis was performed to correlate maximal $#R^*_2$, $#R^2$ and $#R^'_2$ values with urinary output.

Paired sample t tests were used to assess the differences in:

- Pre- and post-diuretic $R^*_2$, $R^2'$ and $R^2$ values;
- Post-water load and post-furosemide maximum $#R^*_2$, $#R^2$ and $#R^'_2$ values;
- Time points of maximum urinary outputs for both challenges;
- Time points of maximum $#R^*_2$, $#R^2$ in cortex and medulla;
- Time points of maximum $#R^*_2$ and $#R^2$ for each challenge;
- Maximum urinary outputs after both challenges.
Fig. 1: Study timeline. Each cycle of measurements (corresponding to one time point) was repeated continuously during 30 minutes after the diuretic challenges. mGRE: multiple gradient-echo sequence; mSE: multiple spin-echo sequence; T2-W: T2-weighted fast spin-echo sequence.

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Fig. 2: ROIs drawn on T2* and T2 images with their corresponding R2* and R2 maps on cropped images over the left kidney. Note that the medullary R2* values decrease post-water load and post-furosemide as compared to the baseline states.

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Results

All ten subjects successfully completed the imaging protocol. All of them had difficulties in drinking the full amount of water, all felt moderately uncomfortable immediately after the oral water load.

For one volunteer, severe susceptibility artifacts on all R2* maps were noted, related to the presence of air in colonic flexures that prevented any measurements for either kidney. Data for this volunteer were excluded from the analysis. Hence, nine individuals, including five males and four females, were analyzed. The mean age was 25.9 years (age range, 22-32 years).

A representative plot of changes in R2* and R2 in response to water and furosemide challenge over time is shown in Figure 3.

Fig. 3: Representative changes in R2* and R2 in response to water and furosemide challenge in one volunteer. The time 0 corresponds to the beginning of the diuretic challenge. The long time interval between the fifth and sixth measurements after furosemide injection was due to the need to void.

References: Radiology, Rouen University Hospital - Rouen/FR

The maximum urinary output during the study was consistently greater after furosemide injection (average maximum output of 21.0 mL/min, range 14-31 mL/min) than after water load (average maximum output of 12.4 mL/min, range 9.4-15.6 mL/min) \( p<5 \times 10^{-4} \). The peak time of urine output was consistently earlier with furosemide (mean 21.9 min
following injection) compared to water hydration (mean 69 min after the start of hydration) (Fig. 4).

R2* and R2 changes were synchronous and reached their maximum decrease at the same average time. The renal medulla and cortex showed the same kinetic pattern in response to hydration. After furosemide injection, however, the maximum decrease in R2* appeared significantly (p<0.008) earlier (by 8.9 min) in the medulla than in the cortex.

The results for #R2*, #R2 and #R2' are displayed graphically in Figure 5.

![Graph showing #R2*, #R2 and #R2' (post challenge - pre challenge) after water load and furosemide in the cortex and in the medulla. *: Significant. NS: Non significant.](image)

**Fig. 5**: #R2*, #R2 and #R2' (post challenge - pre challenge) after water load and furosemide in the cortex and in the medulla. *: Significant. NS: Non significant.

**References**: Radiology, Rouen University Hospital - Rouen/FR

R2*, R2 and R2' all decreased significantly after water load and furosemide injection. The changes were also significantly greater with furosemide than with water load in the medulla, although not in the cortex. The responses varied substantially among subjects; in particular, two subjects showed no decrease in medullary R2* following water load.

The relative contribution of medullary #R2 to #R2* with furosemide and water challenge (Fig. 6) was low but not negligible (16%) for both water load and furosemide. This implies that medullary #R2* was predominantly explained by #R2' (84%). In contrast, the contribution of #R2 to cortical #R2* was substantial, averaging 50%.
Fig. 6: Box-and-whisker plots displaying the relative contribution of changes in R2 to changes in R2* after furosemide and water load challenges. The median contribution of medullary #R2 to medullary #R2* was 16% after both challenges. The median contribution of cortical #R2 to cortical #R2* was 48% and 58% after water load and furosemide injection respectively. Each box contains the middle 50% of the distribution (interquartile range), with the inner horizontal line corresponding to the median value. The ends of the tails correspond to the extreme values.

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R2 and R2* baseline values were not significantly different between the two visits. The standard deviations were greater for R2* than for R2.

The standard deviation of R2* differences between observers was substantial, with a 95% confidence interval of -5.00 - 5.44 sec\(^{-1}\).
Measurements of R2 were substantially less variable than R2* in terms of repeatability and reproducibility.

The mixed model analysis estimated the between-reader (within-scan) component of variance to have a CV of 2.0% for R2 in the cortex, 6.4% for R2* in the cortex, 3.3% R2 in the medulla and 10.5% R2* in the medulla. Relative to R2*, the R2 measure was seen to have significantly better reproducibility in the sense of having a significantly lower between-reader CV in the cortex (p<0.0001) and the medulla (p<0.0001).

No significant correlation was found between maximal #R2*, #R2 and #R2' values and urinary output.

**Discussion**

We observed that R2* decreased significantly for both challenges, and a substantial component of the R2* change results from changes in water content, as reflected by R2. Since fluid management is one of the primary functions of the kidney, it is not surprising that changes in R2* reflect changes not only in R2' but also in R2.

We showed that 16% of the median medullary #R2* was explained by #R2 for both water load and furosemide. In the cortex this percentage was much higher, viz. 48% after oral water load and 58% and furosemide injection (Fig. 6). Most likely this reflects the lower oxygen consumption in the cortex. Thus, using #R2* as a surrogate for #R2' introduces errors for both the cortex and the medulla.

Urinary output variation showed for the first time that the maximal #R2* and #R2 preceded the maximal urinary output by an average 10-14 min. This time shift may plausibly explain the absence of significant correlation between the urinary output and maximal #R2*, #R2 and #R2' values.

Our study has several limitations. In one case, we were unable to use the BOLD data because of bowel gas artifacts. It may be helpful to consider preparatory steps in advance of renal BOLD to overcome this limitation. Also, our reproducibility analysis showed that R2* values had a poor precision despite the use of an average of two measurements (two acquisitions) per time point. The precision could be improved by increasing the number of 2D acquisitions, or by using a 3D acquisition. However, 3D acquisitions have usually a lower spatial resolution than 2D acquisitions for a similar breath hold duration.
**Fig. 3:** Representative changes in R2* and R2 in response to water and furosemide challenge in one volunteer. The time 0 corresponds to the beginning of the diuretic challenge. The long time interval between the fifth and sixth measurements after furosemide injection was due to the need to void.

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<table>
<thead>
<tr>
<th></th>
<th>Time of peak urine output [minutes]</th>
<th>Times of maximal ΔR2* [minutes]</th>
<th>Times of maximal ΔR2 [minutes]</th>
<th>p</th>
</tr>
</thead>
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<tr>
<td><strong>Hydration</strong></td>
<td>69 (57.6-77.7)</td>
<td>Medulla 54.8 (45-66)</td>
<td>Medulla 55.7 (45-66)</td>
<td>0.39</td>
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<tr>
<td></td>
<td></td>
<td>Cortex 55.7 (45-66)</td>
<td>Cortex 55.7 (45-65)</td>
<td>0.14</td>
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<td></td>
<td></td>
<td>p 0.29</td>
<td>p 0.5</td>
<td></td>
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<tr>
<td><strong>Furosemide</strong></td>
<td>21.9 (10.5-29.8)</td>
<td>Medulla 7.6 (5-15)</td>
<td>Medulla 11.2 (5-22)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cortex 16.4 (5-37)</td>
<td>Cortex 10.7 (5-22)</td>
<td>0.09</td>
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<tr>
<td></td>
<td></td>
<td>p &lt;0.008</td>
<td>p 0.38</td>
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**Fig. 4:** Times of maximum urine output and maximum R2 and R2* response. Mean times are provided, as well as minimum and maximum times between brackets. Times are in minutes, with the starting point corresponding to the beginning of the diuretic challenge.

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Fig. 5: #R2*, #R2 and #R2' (post challenge - pre challenge) after water load and furosemide in the cortex and in the medulla. *: Significant. NS: Non significant.

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Fig. 6: Box-and-whisker plots displaying the relative contribution of changes in R2 to changes in $R2^*$ after furosemide and water load challenges. The median contribution of medullary #R2 to medullary $R2^*$ was 16% after both challenges. The median contribution of cortical #R2 to cortical $R2^*$ was 48% and 58% after water load and furosemide injection respectively. Each box contains the middle 50% of the distribution (interquartile range), with the inner horizontal line corresponding to the median value. The ends of the tails correspond to the extreme values.

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Conclusion

Furosemide is a better diuretic challenge than oral water load, since it is better tolerated, takes effect more quickly and produces a larger $R_2^*$ response in both cortex and medulla.

Using $R_2^*$ as a surrogate for renal $R_2'$ ignores changes in $R_2$ and yields inaccurate results, particularly in the cortex. To assess renal $R_2'$, $R_2$ measurements must be performed in addition to conventional $R_2^*$ measurements.

Further studies with improved $R_2$ and $R_2^*$ precision will be needed to determine whether there exists any relationship between $R_2^*$ and $R_2$ that could exploited to estimate $R_2'$ changes without measuring $R_2$ in healthy subjects and patients.
References


