Furosemide increases water content in renal tissue

Michael Pedersen,1,2 Zsolt Vajda,3 Hans Stødkilde-Jørgensen,1 Søren Nielsen,3 and Jørgen Frøkiær2,3

1MR Research Centre, Aarhus University Hospital, and 3Institute of Clinical Medicine and 2The Water and Salt Research Center, University of Aarhus, Aarhus, Denmark

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Furosemide increases water content in renal tissue. Am J Physiol Renal Physiol 292: F1645–F1651, 2007. First published January 30, 2007; doi:10.1152/ajprenal.00060.2006.—The present study was designed to evaluate the short-term effects of intravenous administration of furosemide on key functions in the kidney cortex and the outer and inner medulla of rats by using magnetic resonance imaging (MRI). Renal tissue water content, renal tissue oxygenation (in relation to the magnetic resonance spin-spin relaxation rate), the apparent diffusion coefficient (ADC) of water, and volume of renal blood flow were measured. Furosemide administration resulted in an increased water content in all regions of the kidney. In parallel with this, we found a significant reduction in ADC in the cortex (2.7 ± 0.1 × 10−3 to 2.3 ± 0.1 × 10−3 mm²/s; P < 0.01) and in the outer medulla (2.3 ± 0.1 × 10−3 to 2.0 ± 0.1 × 10−3 mm²/s; P < 0.01), indicating that the intra- to extracellular volume fraction of water increased in response to furosemide administration. Furosemide also increased the blood oxygenation in the cortex (49.1 ± 2.9 to 40.9 ± 2.0 s⁻¹; P < 0.01), outer medulla (41.9 ± 2.1 to 32.2 ± 1.6 s⁻¹; P < 0.01) and in the inner medulla (37.1 ± 2.9 to 26.7 ± 1.8 s⁻¹; P < 0.01), indicating an increased amount of oxygenated Hb in the renal tissue. Moreover, renal blood flow decreased in response to furosemide (6.9 ± 0.2 to 4.4 ± 0.2 ml/min; P < 0.001). In conclusion, furosemide administration was associated with increased renal water content, an increase in the intra- to extracellular volume fraction of water, an increased oxygen tension, and a decrease in the renal blood flow. Thus MRI provides an integrated evaluation of changes in renal function, leading to decreased renal water and solute reabsorption in response to furosemide, and, in addition, MRI provides an alternative tool to monitor noninvasively changes at the cellular level.

Address for reprint requests and other correspondence: J. Frøkiær, The Water and Salt Research Center and Institute of Clinical Medicine, Univ. of Aarhus, Aarhus Univ. Hospital, Skekby, 8200 Aarhus N, Denmark (e-mail: jf@ki.au.dk).

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**Innovative Methodology**

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**Material and Methods**

**Experimental procedure.** Male Wistar rats weighing 350–400 g (120–130 days of age) were anaesthetized with Melumal (100 mg/kg body wt; Nycomed, Copenhagen, Denmark). The rats were maintained on a dry pellet diet with free access to water before the experiment. For drug administration, a polyurethane catheter (0.015 in. Tygo; Norton Performance Plastics, Taunton, MA) was implanted in the femoral vein. MRI was performed with a Philips NT 1.5 T system (Philips Medical Systems, Best, The Netherlands) using a small surface coil of 4 cm in diameter. The animal was placed supine with the kidneys placed above the imaging coil. The fully anaesthetized rat was then subjected to an imaging protocol as shown in Fig. 1. After the scout images obtaining and the optimal positions were found, a group of baseline scans including BOLD, ADC, RBF, and T1 measurements was initially acquired, followed by intravenous administration of 0.1 mg/kg body wt furosemide (injected in a volume of 1 ml/kg: Furix; Benzlon Pharma, Copenhagen, Denmark; n = 6) or a similar volume of saline (n = 5). MRI was subsequently performed, ending with a T1 measurement 57 min after the injection. Rectal temperature was monitored throughout the experimental protocol. All animals were cared for according to the local ethical standards of laboratory animals, and approval was obtained before experimental procedures.

**Renal water content.** Accurate and noninvasive measurements of the kidney water content were performed by determining T1. Even though the kidney demonstrates a large blood volume, a linear increase in T1 vs. percent water content from cortex to inner medulla has been found in rats (23) and rabbits (20). The reciprocal of the total water content (W), defined as grams of water per grams of tissue, is linearly dependent on the reciprocal of T1, expressed by 1/W = A + BT1, where A and B are constants depending on field strength and physical conditions (13, 41). The constants A and B were determined using tissue-mimicking gelatine standards of different water content (68–90%), and the results have shown to correlate to the percentage of water within 1% (13). Having established the measurement methodology with the in vitro work, in vivo correlation was performed between the relaxation time and the amount of tissue water present. T1 measurements were performed by a MIX-mode sequence provided by the Philips software. The following time parameters were used: 50–350 ms echo time (TE), a 370 ms inversion recovery delay, a 760-ms repetition time (TR), and a 2,500-ms delay between the excitation pulse in the inversion recovery part and the previous excitation in the spin-echo part. Quantitative T1 maps were calculated from pixel-by-pixel analysis using a nonlinear least-squares fit to the magnitude image data and were then converted to water content maps using determined constants A and B. Other relevant parameters were 13 × 13-cm field-of-view, 128 × 128 matrix, 2-mm slice thickness, and three transients.

**Relative water diffusion (ADC).** To obtain specific information on relative intra- to extracellular water diffusion, diffusion weighted images were acquired using a spin-echo-based echo planar imaging (EPI) sequence that combines the diffusion-sensitizing gradients before and after the 180° pulse with a fast EPI readout. The diffusion-sensitizing gradients were applied in three orthogonal gradients (x, y, and z). Seven different gradient strengths were applied corresponding to diffusion-weighting factors (b values) between 0 and 1,000 s²/mm. With the use of pixel-by-pixel nonlinear least squares fit, the signal intensities were fitted to the exponential expression, S(b) = S(0)exp(−b·ADCdirection), where S(b) is the signal intensity at diffusion-weighting factor b, S(0) is the signal intensity at b = 0, and ADCdirection is the apparent diffusion constant at individual directions. The factor b was given by: b = γ²δ²(Δ - δΔ)/G², where γ is the gyromagnetic constant, δ is the duration of the individual gradient pulses, Δ is the time separation between the leading edges of the two gradient pulses, and G is the strength of the diffusion field gradient. The ADC map was approximated by the mean diffusivity, given by (ADCx + ADCy + ADCz)/3 (38). The size, thickness, number of the slices, and resolution were the same as in the water content measurement. Other parameters were TR = 1,200 ms and TE = 31 ms.

**Renal oxygen changes (BOLD MRI).** BOLD MRI was acquired to obtain specific information on the renal oxygen concentration. BOLD MRI is sensitive to changes in regional oxygenation and is based on the fact that oxyhemoglobin is diamagnetic, whereas deoxyhemoglobin is paramagnetic. Reduction in deoxyhemoglobin concentration therefore causes an increase in the relative spin-spin relaxation rate (R²*), the so-called BOLD contrast. A double-echo gradient-echo sequence was used to determine R²*, which is directly proportional to the tissue content of deoxyhemoglobin (9, 30) and is thereby inversely proportional to the tissue concentration of oxygen. BOLD scans were performed in three coronal slices to image both kidneys. Using TE of 4 and 40 ms in combination with a 40° flip angle and a TR = 100 ms resulted in heavily 1/R²* weighted images. As for ADC scans, the position, size, resolution, and thickness are equal to the diffusion parameter chosen for the measurements of the tissue water content. The slope of fitted Log(intensity) vs. TE was performed to derive a R²* map on a pixel-by-pixel frame.

**RBF.** Volume blood flow was measured using a phase-contrast gradient echo sequence equipped with a flow-sensitizing bipolar gradient in the slice direction. The strengths of the flow-encoding gradients were set according to RBF values from the literature (35). Ten slices of 3 mm thickness were prescribed perpendicular to the renal veins. Each slice had an 11 × 11-cm field of view and a resolution of 256 × 256 pixels to ensure appropriate pixels to find and derive the blood flow in the renal veins. Other parameters were TE = 4 ms, TR = 25 ms, 40° flip angle, and four transients. The phase images were subtracted, and quantitative RBF, in units of milliliters per milliliter, was determined by multiplication with the renal vein area.

**Data analysis.** For each time frame, T1 scan with TE = 50 ms, ADC scan with b = 0, and BOLD scan using TE = 4 ms were used as anatomic templates to facilitate placements of regions of interest (ROI). Slices with clearly visualized cortex, inner medulla, and outer medulla were chosen for our analysis. ROIs were manually inserted to encompass those three regions and were then duplicated to the water map, ADC map, and BOLD map, which were made using Osiris Medical Software (Geneva University Hospital, Geneva, Switzerland) and Photoshop (Adobe Systems, San Jose, CA) graphical software. RBF values were summarized for all slices in which the renal vein was clearly seen. The mean values and SEs were calculated for all measurements. The following statistical analysis was performed: ANOVA of data from one observation time to another for the same parameter was performed by a one-way ANOVA model. Where

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**Fig. 1.** Study design. Investigation of the effect of iv furosemide administration on renal functions in the anaesthetized rat by magnetic resonance imaging (MRI). The time scale shows the different MRI procedures performed before and after injection of furosemide.
ANOVA indicated that statistical significance existed, Dunnett’s multiple comparison was used to determine statistical significance among baseline and treated values. Comparisons between furosemide and saline groups were evaluated by Student’s t-test for unpaired data. Differences were considered statistically significant at the 0.05 level. The saline group was considered as the control.

RESULTS

The gradient echo images used as an anatomical template revealed excellent spatial information of the rat kidney. Only minimal artifacts from respiratory movements were seen, and cortex as well as inner and outer medulla were easily recognized in both kidneys (Fig. 2). Pixel-by-pixel color maps of the effect of furosemide and saline on tissue water content, ADC, R2*, and RBF are presented in Fig. 3. Changes are indicated by color shifts, with red shift indicating a relative decrease and blue shift indicating a relative increase. Data are presented and as relative changes (index) compared with data obtained before intravenous administration of furosemide or saline (Figs. 4 –7).

**Furosemide increases the renal water content.** The renal water content increased significantly in all regions of the kidney following administration of furosemide. In the kidney cortex, the water content increased from 78.5% ± 0.7% at baseline to 81.9% ± 1.0% (P = 0.009) after furosemide. The outer medullary water content increased from 81.8% ± 0.9% at baseline to 85.8% ± 0.8% (P = 0.002) after furosemide, and the inner medullary water content showed a similar increase, from 87.2% ± 0.9% at baseline to 89.4% ± 0.6% (P = 0.007) after furosemide. The renal water content of cortex, outer medulla, and inner medulla did not change significantly in response to saline administration. Thus furosemide induced a marked increase in renal water content. Comparison of the renal water content in the furosemide-treated rats with the saline-treated rats showed that the change was primarily located to the renal outer medulla (P = 0.016) and secondarily to cortex (P = 0.09) and inner medulla (P = 0.22; Fig. 4).

**Furosemide decreases the ADC.** Indexed ADC values are presented in Fig. 5. Furosemide induced an abrupt decrease in the ADC inside the kidney. ANOVA for repeated measurements comparing ADC values from the furosemide group with the saline group showed no significant differences at 8 or 26 min after administration (8 min: P = 0.316; 27 min: P = 0.086). However, at 46 min after furosemide administration, the relative change in the cortical ADC was significantly larger than the relative change after administration of saline (−13 ± 3% vs. 1 ± 5%; P = 0.01), indicating that furosemide induced a shift in the intra- to extracellular volume fraction ratio. Similar reduction in the outer medullary ADC was found in the furosemide group compared with the saline group (−11 ± 6% vs. 1 ± 6%; P = 0.036). The relative change in ADC of the inner medulla remained unchanged during the protocol, and no significant differences between groups were found at any time (P > 0.419).
Furosemide decreases renal energy expenditure. Figure 6 shows absolute and relative BOLD MRI (R2*) data. For all regions, the response to furosemide showed a significant difference between baseline and treated R2* values (P < 0.01). R2* values of control kidneys did not change significantly after administration of saline (7.3 ± 0.4; P > 0.2). However, when comparing the responses to furosemide with the responses to saline at 3 min after the administration, we found no significant differences between relative BOLD values in the cortex (−9 ± 7% vs. 0 ± 5%; P = 0.065) nor in the outer medulla (−15 ± 6% vs. −5 ± 4%; P = 0.116). Inner medullary BOLD values differed significantly between the furosemide group and controls (−18 ± 4% vs. −1 ± 7%; P = 0.023). Repeating the scan at 21 min showed a significant difference between relative BOLD values of furosemide and saline groups in the outer medulla (−26 ± 7% vs. −7 ± 10%; P = 0.029) and in the inner medulla (−20 ± 8% vs. 0 ± 4%; P = 0.019). However, relative cortical values remained unchanged at 21 min (−13 ± 8% vs. 0 ± 5%; P = 0.1). At 39 min after administration, a marked reduction in the relative BOLD values of the furosemide group was observed compared with the saline group, and statistics showed a significant difference for cortex (−20 ± 5% vs. −2 ± 3%; P < 0.001), outer medulla (−25 ± 5% vs. −1% ± 3%; P < 0.001), and inner medulla (−32 ± 6% vs. −11 ± 8%; P = 0.007).

Furosemide decreases RBF. Relative changes in RBF in response to furosemide are shown in Fig. 7. RBF decreased significantly after administration of furosemide (−30 ± 14%; P < 0.001), whereas RBF was unchanged in the saline-treated rats (14 ± 9%; P = 0.385). Relative RBF did not differ between furosemide and saline treatment at 16 min (−31 ± 6% vs. −13 ± 13%; P = 0.086) or at 34 min (−32 ± 5% vs. −20 ± 9%; P = 0.095). However, at 52 min, the relative RBF was significantly reduced in the furosemide group (−36 ± 4% vs. −16 ± 5%; P = 0.021).

DISCUSSION

During the present study, changes in renal tissue water content, molecular water diffusion, intrarenal oxygenation, and RBF were measured in response to a furosemide bolus using MRI. The main finding was that furosemide induced a significant increase in the renal water content, predominantly located to the cortex and the outer medulla. In parallel, a significant reduction in the diffusion of the tissue water molecules was demonstrated in the cortex and in the outer medulla, indicating an increase in the intra- to extracellular water compartment ratio. BOLD MRI revealed that blood oxygenation in the outer and inner medulla was increased following administration of furosemide, indicating increased levels of blood oxygenated Hb, i.e., reduced oxygen uptake by the renal tissue. Furthermore, furosemide administration was associated with an immediate reduction in RBF.

Renal water content increases in response to furosemide. Furosemide induced a marked increase in the overall kidney water content. From the MRI approach used in the present

![Graph](http://ajprenal.physiology.org/)

Fig. 4. Effect of furosemide or saline on the renal tissue water content in cortex, outer medulla, and inner medulla. Data reflect the percentage of change between baseline and treated kidneys; *P < 0.05. The outer medullary water content increased significantly after administration of furosemide.

![Graph](http://ajprenal.physiology.org/)

Fig. 5. Effect of furosemide or saline on ADC in cortex (A), outer medulla (B), and inner medulla (C). Data are indexed values relative to baseline values; *P < 0.05. ANOVA for repeated measurements showed a significant difference between baseline and treated values for cortex (at 44 min) and outer medulla (at 44 min) but not in the inner medulla. Decreased ADC can be interpreted as an increase in the intra- to extracellular volume fraction.
Study, tissue water content can be derived by measuring the spin-lattice relaxation rate, a constant that corresponds to the percentage of water in the tissue. This demonstrated that the renal water content increased in all regions of the kidney after furosemide administration. Thus it may be anticipated that handling of water in the different nephron segments changes dramatically immediately after administration of furosemide. The urinary concentrating process depends on the coordinated function of the loop of Henle and the collecting duct. In the thick ascending limb of the loop of Henle, reabsorption of sodium and chloride powers the countercurrent multiplier process responsible for generation of the corticomedullary osmotic gradient, whereas the collecting ducts, under the control of vasopressin, allow variable degrees of osmotic equilibration, resulting in variable water excretion and a reciprocal relationship between urinary flow and urinary osmolality. Furosemide specifically binds to and inhibits the \( \text{Na}^+ - \text{K}^+ - 2\text{Cl}^- \) apical membrane cotransporter NKCC2 responsible for the active chloride reabsorption in the thick ascending limb of the loop of Henle in the mammalian renal tubule (10). Thus the direct effect of this inhibitor is a reduction in the active reabsorption of chloride in this segment, resulting in a decreased driving force. It is well established that this rapidly leads to an increased diuresis. The increased renal water content after administration of furosemide may therefore primarily represent an increase in the amount of water present in the lumen of the distal tubule and collecting ducts. Characteristically, in a previous study, immunohistochemical localization of rat kidney NKCC2 was restricted to the medullary and cortical thick ascending limb segments (10). Thus the marked increase in renal water content demonstrated in the cortex and outer medulla may reflect that the density of NKCC2 is high in this area. Correspondingly, administration of furosemide leads to an increased amount of water remaining in the renal tubular lumen of the thick ascending limb and collecting duct. Our current MRI system did not allow us to identify which exact cellular segments the increased water content was located to. To perform such examinations, we have to use either a tracer or drug that is characterized by being located specifically to specific segments or by a very high field magnet providing a much higher spatial resolution.

**Renal intra- to extracellular volume fraction increases in response to furosemide.** Furosemide administration was associated with a significant reduction in the ADC index both in the outer medulla and cortex of the kidney. The origin of the ADC reduction is complex as water diffusion in the renal tissue is affected by a heterogeneous intraRBF distribution, glomerular filtration, tubular secretion and reabsorption, and urine flow (42). Anderson et al. (1) have used a simplified tissue model of osmotically driven cell volume changes on ADC in the rat optic nerve. They found that the changes in membrane polarization and cellular water content were not sufficient to induce significant changes in ADC, whereas changes in the cellular volume fraction induced by cell membrane depolarization are sufficient.

The furosemide-induced reduction in ADC may therefore correspond to an increase in the intra- to extracellular water compartment ratio, indicating that the relative intracellular study, tissue water content can be derived by measuring the spin-lattice relaxation rate, a constant that corresponds to the percentage of water in the tissue. This demonstrated that the renal water content increased in all regions of the kidney after furosemide administration. Thus it may be anticipated that handling of water in the different nephron segments changes dramatically immediately after administration of furosemide. The urinary concentrating process depends on the coordinated function of the loop of Henle and the collecting duct. In the thick ascending limb of the loop of Henle, reabsorption of sodium and chloride powers the countercurrent multiplier process responsible for generation of the corticomedullary osmotic gradient, whereas the collecting ducts, under the control of vasopressin, allow variable degrees of osmotic equilibration, resulting in variable water excretion and a reciprocal relationship between urinary flow and urinary osmolality. Furosemide specifically binds to and inhibits the \( \text{Na}^+ - \text{K}^+ - 2\text{Cl}^- \) apical membrane cotransporter NKCC2 responsible for the active chloride reabsorption in the thick ascending limb of the loop of Henle in the mammalian renal tubule (10). Thus the direct effect of this inhibitor is a reduction in the active reabsorption of chloride in this segment, resulting in a decreased driving force. It is well established that this rapidly leads to an increased diuresis. The increased renal water content after administration of furosemide may therefore primarily represent an increase in the amount of water present in the lumen of the distal tubule and collecting ducts. Characteristically, in a previous study, immunohistochemical localization of rat kidney NKCC2 was restricted to the medullary and cortical thick ascending limb segments (10). Thus the marked increase in renal water content demonstrated in the cortex and outer medulla may reflect that the density of NKCC2 is high in this area. Correspondingly, administration of furosemide leads to an increased amount of water remaining in the renal tubular lumen of the thick ascending limb and collecting duct. Our current MRI system did not allow us to identify which exact cellular segments the increased water content was located to. To perform such examinations, we have to use either a tracer or drug that is characterized by being located specifically to specific segments or by a very high field magnet providing a much higher spatial resolution.

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volume of water increases compared with the extracellular volume. Thus the ADC reduction could be a result of an acute intact apical water transport in the principal cells of the cortical and outer medullary collecting ducts increasing the cellular volume in these segments of the kidney. The furosemide-induced impairment of the osmotic gradient would compromise water reabsorption to the interstitial compartment and vasa recta. This finding supports the view that furosemide does not influence short-term regulation of aquaporin 2 in the principal cells. Thus it is anticipated that the trafficking of aquaporin 2 and apical water reabsorption is intact in these segments, whereas the reduced driving force may account for the inhibition of basolateral water transport.

Whether molecules need to cross a membrane or are hindered by different extracellular environments to diffuse in a given direction makes a major difference for ADC measurements (13, 22, 39). In the kidney, the vascular structures supplying the cortex, outer medulla, and inner medulla are different. In the cortex, many glomeruli have long efferent arterioles, which extend to the medulla where they divide, forming vascular bundles, and, in the inner medulla, the cores of these bundles break up into a capillary plexus. Another regional difference concerns the interstitial cells, which become more numerous and larger as one descends from cortex to papilla (40). Changes in ADC should therefore either be a result of hemodynamic changes or be a result of changes in the cellular environment. Because ADC is a scalar obtained from three directions, changes in ADC do not fully reveal anisotropic reorganization of the intracellular compartment. Different experimental approaches have resulted in slightly different ADC values in the rat model, which could be caused by different diffusion-encoding techniques (5, 6, 21, 42). In general, because the structure of the kidney is anisotropic, a full description of the self-diffusion of water inside the kidney should be investigated by more advanced methods, such as application of diffusion tensor imaging (34).

Renal energy expenditure is reduced in response to furosemide. The BOLD technique readily detects the reduction in medullary oxygenation that follows administration of furosemide. We have recently demonstrated that the value of $R_2^*$ correlates inversely with the tissue content of oxygen (27), meaning that a decreased $R_2^*$ implies an increase in the oxyhemoglobin concentration, which subsequently results in a proportional increase in blood and tissue $P_O_2$. The BOLD MRI technique does not allow distinguishing changes in oxygenation produced by alternations in the supply of oxygen from those produced by changes in oxygen consumption (30). The present study showed that furosemide induces an overall decrease in $R_2^*$, especially in the medullary regions. These result values are consistent with previous studies in humans (30) and in rats (32, 33). Similar observations were found using invasive oxygen-sensitive microelectrodes (3), from which furosemide was concluded to enhance medullary oxygen availability. The improved level of medullary oxygen may be explained by the decreased consumption of oxygen necessary to maintain the medullary transport of ions, and therefore the equilibrium from oxy- to deoxyhemoglobin is shifted because furosemide acts by reducing regional tubular oxygen consumption for reabsorptive transport of sodium (30). We demonstrated that $R_2^*$ was mostly decreased in the inner medulla, although one would think that furosemide mainly would act by decreasing the active transport in cells lining the medullary thick ascending limb. This is consistent with previous findings demonstrating that changes induced by furosemide are fully dominated by changes in $R_2^*$ and that the BOLD effect is not dependent on changes in the tissue water content (30).

**RBF decreases in response to furosemide.** A close correlation between the percentage change of relative RBF and the percentage change of ADC has recently been demonstrated (28, 29), suggesting that a decrease in ADC followed by administration of furosemide was mediated by hemodynamic changes. Our data showed that furosemide induced a significant decrease in RBF, supporting the view that furosemide causes renal vasoconstriction (3). These effects may therefore add to the change in ADC, since renal vasoconstriction may be associated with a decrease in the intravascular volume. Furthermore, volume depletion has effects on renal hemodynamics, including a sustained decrease in glomerular filtration rate and the blood perfusion of the kidney (4, 14, 16, 18, 43), which has been shown to depend linearly on RBF (35). Furosemide has in addition been demonstrated to block the tubuloglomerular feedback response, followed by significant decreases in renal interstitial concentrations of ATP (26). Furthermore, furosemide elicited a marked impairment of RBF and glomerular filtration rate autoregulatory efficiency as well as the autoregulation-associated alterations in RVR, as previously reported by other investigators (8, 17). RBF in the sham-operated rats decreased slightly with time, suggesting that anesthesia may reduce the systemic blood flow. However, the decrease was not statistically significant compared with baseline. Importantly, the present study demonstrated that RBF was reduced at all time points following intravenous administration of furosemide.

In conclusion, in vivo magnetic resonance monitoring of kidney tissue water content following administration of furosemide revealed increased cortical and medullary water content. Diffusion-weighted MRI was able to detect a significant decrease in cortical and medullary ADC after administration of furosemide, indicating an increase in the intra- to extracellular volume fraction ratio. Consistent with previous studies, BOLD imaging revealed increased medullary tissue oxygenation. Thus MRI is a noninvasive method providing direct visualization of the kidney and simultaneous estimation of essential renal physiological parameters in vivo.

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Present address for M. Pedersen: MR Research Centre and Institute of Clinical Medicine, Aarhus University Hospital, Skejby, 8200 Aarhus N, Denmark (e-mail: michael@mr.au.dk).

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