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Kidney Int. Author manuscript; available in PMC 2010 August 10.

Published in final edited form as:
Kidney Int. 1999 January ; 55(1): 294–298. doi:10.1046/j.1523-1755.1999.00237.x.

Changes in renal medullary pO_2 during water diuresis as evaluated by blood oxygenation level–dependent magnetic resonance imaging: Effects of aging and cyclooxygenase inhibition

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Abstract

Background—Hypoxia of the renal medulla has been implicated in the development of renal injury, particularly acute renal failure, and its regulation in humans may therefore be relevant to certain renal disorders. Changes in oxygenation of the renal medulla can now be monitored noninvasively with blood oxygenation level–dependent (BOLD) magnetic resonance imaging (MRI). Using this method, water diuresis has been shown to improve medullary oxygenation in young persons. Urinary excretion of prostaglandin E_2 (PGE_2) likewise increases during water diuresis in younger but not in older people. We used BOLD MRI to measure the effects of aging and of inhibiting prostaglandin synthetase on the renal response to water diuresis in healthy human subjects.

Methods—Nine younger (25 to 31 years) and nine older (59 to 79 years) female volunteers were studied with BOLD MRI during antidiuresis in the postabsorptive state and during water diuresis. Simultaneously, urinary excretion of PGE_2 was determined. PG synthetase was inhibited by administering ibuprofen.

Results—Renal medullary oxygenation, initially low, greatly improved during diuresis in younger subjects, whereas PGE_2 excretion increased. In older women, however, water diuresis elicited no change in oxygenation of renal medulla or PGE_2 excretion. Ibuprofen inhibited excretion of PGE_2 and blocked the increase in medullary oxygenation normally produced by water diuresis in the young.

Conclusions—The increase in oxygenation of the renal medulla accompanying water diuresis depends on PGE_2 synthesis. Attenuation of renal PGE_2 synthesis in older people is probably responsible, at least in part, for the loss of the ability to improve medullary oxygenation that younger subjects possess. Inability to improve renal medullary oxygenation might predispose to hypoxic renal injury in older patients.

Keywords

radiology; hypoxia; renal injury; acute renal failure; medullary oxygenation; BOLD-MRI

The medulla of the mammalian kidney normally operates in a hypoxic environment maintained by the countercurrent arrangement of vessels and tubules necessary to conserve

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water [1]. Renal medullary hypoxia is relieved by loop diuretics that reduce the work and oxygen consumption of medullary tubules or by vasodilating agents that increase medullary blood flow [2]. Intrarenal oxygenation can be monitored noninvasively in humans by the technique of blood oxygenation level–dependent (BOLD) magnetic resonance imaging (MRI) [3], which depends on the principle that variations in the oxygen saturation of hemoglobin result in changes in local magnetic susceptibility and hence in the signal intensity of T_2*–weighted magnetic resonance images. We have used this method to examine the way in which the response of the renal medulla to water diuresis is affected by normal aging and by inhibition of prostaglandin (PG) synthesis.

Methods

Study plan

To compare responses to water diuresis in younger and older subjects, we recruited nine healthy, young female volunteers 25 to 31 (mean 28) years of age and nine healthy older women 59 to 79 (mean 69) years of age. These studies were repeated during cyclooxygenase inhibition in six younger volunteers. In all subjects, the medical history and a preliminary physical examination were essentially negative. Blood pressure was below 150/90 mm Hg, and complete blood count and urinalysis were normal. To avoid contamination of the urine with PGs produced in the male reproductive tract, the study was limited to women. The study was approved by the Beth Israel Hospital Committee on Clinical Investigation, and all volunteers gave written informed consent prior to their participation in the study.

All subjects were asked to take nothing by mouth after 8:00 p.m. on the night before the study. The next morning, at 8:00 a.m., they reported to the Clinical Research Center, where they were weighed, vital signs were measured, a sample of blood was drawn for blood urea nitrogen (BUN) and serum creatinine, and a timed sample of urine was obtained by spontaneous voiding. They were then taken to the MRI suite, where baseline MRI images were obtained in the supine position. The subjects were taken out of the magnet and were asked to void again for a second urine collection. They then drank 20 ml of flavored water per kg of body weight within 15 minutes in order to induce a water diuresis. Urinary output was measured every 15 minutes. After it exceeded 5 ml per minute, the subject returned to the magnet, where a second set of BOLD MRI measurements was obtained. Immediately following this, a final sample of urine was obtained for measurement of osmolality, creatinine, and PGE_2.

In the studies of cyclooxygenase inhibition, ibuprofen, 600 mg, was taken by mouth three times daily with meals for two days prior to the test and at 8 a.m. the morning of the study. These results were compared with a control study in each subject of water diuresis without ibuprofen, performed at least a week before or after ibuprofen was given.

Laboratory methods

Urinary osmolality was determined using freezing point depression with an Osmette osmometer (Precision Instruments, Sudbury, MA, USA). Urinary creatinine concentration was determined using a rate-dependent modification of the Jaffe reaction (SMAC II; Technicon Instruments, Tarrytown, NY, USA).

Urinary samples for determination of PGE_2 were immediately aliquoted into containers acidified with 2 n HCl. Samples were then adjusted to pH 3 to 4. PGE_2 was assayed on diluted urine samples using a standard double antibody radioimmunoassay with reagents obtained from New England Nuclear (DuPont, Boston, MA, USA). Interassay and intra-assay coefficients of variation are 20% and 5.1%, respectively. All samples were assayed in duplicate.

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MRI methods

Magnetic resonance imaging takes advantage of the energy emitted as radio waves by water molecules, when their alignment in a strong magnetic field is changed. The BOLD MRI technique depends on the principle that hemoglobin (because of its iron content) changes its magnetic qualities depending on whether the hemoglobin is in the oxygenated or deoxygenated form. This change in the magnetic property of hemoglobin in turn influences the MRI signal from the neighboring water molecules in a predictable way. Because the ratio of oxyhemoglobin to deoxyhemoglobin is related to the pO_2 of blood and because the pO_2 of capillary blood is thought to be in equilibrium with the surrounding tissue, changes estimated by BOLD MRI can be interpreted as changes in tissue pO_2 (Fig. 1). All measurements were performed on a 1.5T whole body scanner (Vision; Siemens Medical Systems, Erlangen, Germany). We used R_2^* (= $1/T_2^*$) as the parameter to reflect relative oxygenation status [3]. We used a multiple gradient echo sequence (TR/TE/Flip angle = 60 ms/3–48 ms/40°) to acquire 16 T_2^*–weighted images within a single breath-hold of less than 15 seconds, which permits calculation of R_2^* maps [4]. For studies with and without ibuprofen, a modified sequence involving selective water excitation pulses was used. This increased the range of echo times slightly, 6 to 51 ms. We have shown that both of these sequences give equivalent results as far as R_2^* calculations are concerned [4]. The advantage of the selective water excitation is better delineation between renal medulla and intrarenal fat on R_2^* maps. A decrease in R_2^* implies an increase in tissue pO_2 (Fig. 1).

Statistical methods

Results are expressed as mean ± SEM. Results were analyzed for significance using Student's *t*-test, and the differences were considered not significant if P was more than 0.05.

Results

Effects of age on the response of the medulla to water diuresis

BOLD MRI measurements of medullary oxygenation (R_2^*) indicated that the renal medulla was appreciably less well oxygenated than the cortex in both younger and older subjects (Table 1 and Fig. 2). Water diuresis increased medullary oxygenation in the young (R_2^* = 17.9 ± 0.4 before water was drunk and 12.6 ± 0.5 during water diuresis, $P < 0.01$) as reported in earlier studies [3], whereas cortical oxygenation changed only slightly. By contrast, in older subjects, R_2^* in the renal medulla remained high after drinking water and was essentially unaltered by water diuresis. As noted earlier [5], the minimum urinary osmolality achieved during water diuresis was lower in younger than in older subjects (56 ± 3 vs. 86 ± 6 mOsm/kg, $P < 0.01$).

Previous studies have shown that water diuresis is associated with an increase in the urinary excretion of PGE_2 in younger subjects but not in older subjects [5,6]. In these experiments as well, PGE_2 excretion was more than doubled by water diuresis in younger subjects (228 ± 37 to 571 ± 64 pg/min), but did not change significantly in older subjects. Accordingly, additional experiments were planned to study the effects of inhibiting PGE_2 synthesis on medullary oxygenation in younger individuals during water diuresis.

Effects of ibuprofen on the response of the medulla to water diuresis

In six younger women 25 to 31 years of age, the effects of water diuresis were studied again after taking 600 mg of ibuprofen, an inhibitor of PG synthesis, one hour before water loading on the day of the test and three times daily with meals for the previous two days (Table 2 and Fig. 3). As expected from the two-hour half-life of ibuprofen in circulating blood, baseline values for PGE_2 excretion were unaffected during antidiuresis (measured 12 hr after the previous day's supper dose of ibuprofen), but urinary excretion of PGE_2 was

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greatly inhibited during the water diuresis generated immediately following ibuprofen ingestion on the day of the test. Peak diuresis after water ingestion was slightly but not significantly diminished by ibuprofen, and the minimum urinary osmolality was slightly higher after the drug in five of the six subjects.

The main finding in the studies with ibuprofen is that this drug suppressed the improvement in medullary oxygenation otherwise seen in younger subjects during water diuresis (Fig. 3). Water diuresis without ibuprofen produced a fall in medullary R_2* from 17.9 ± 0.45 to 13.2 ± 0.56, whereas after ibuprofen, there was virtually no change in R_2* of renal medulla (18.45 ± 0.71 to 17.6 ± 0.36).

Discussion

The marked and habitual hypoxia of the renal medulla, as compared with the renal cortex and with most other body tissues, has spurred speculation about its implications for human disease [1]. In particular, it has been postulated that anoxic injury to medullary structures initiates the phenomenon of acute renal failure [7]. If so, the intrinsic ability of the kidneys to improve medullary oxygenation after a variety of physiological and pathological stimuli might be correlated with susceptibility to acute renal injury.

Experiments in animals suggest that PGE_2 is a paracrine factor that importantly influences medullary oxygenation. Produced by medullary collecting ducts and interstitial cells, PGE_2 inhibits active transport by the medullary thick limb [8], inhibits Na,K-ATPase activity in renal cells [9,10], and reduces their oxygen consumption [11] while enhancing medullary blood flow [12]. Receptors for PGE_2 are clustered in high density in the vasa recta and the renal tubular cells lining the thick ascending limb within the outer medulla [13]. In intact rats, medullary pO_2 is reduced by indomethacin, which blocks PG synthetase [14]. Pretreatment with indomethacin greatly increases susceptibility to experimental radiocontrast nephropathy and predisposes to acute renal failure in humans [15]. On the other hand, when urinary excretion of PGE_2 is increased in rats by dietary manipulation, hypoxic damage to their perfused kidneys is substantially reduced [16]. Therefore, there are strong reasons to believe that renal PGs might influence medullary oxygenation in human kidneys.

These experiments provide direct evidence in support of this hypothesis. Inhibition of PG synthesis by ibuprofen completely abolished the increase in medullary pO_2 that normally accompanies water diuresis in healthy, younger subjects. Urinary excretion of PGE_2, which is thought to be derived exclusively from PGs synthesized in the renal medulla [17], fell to low levels during water diuresis after ibuprofen was given to younger subjects, whereas without ibuprofen, urinary PGE_2 excretion was increased by water diuresis. Of note, the dose of ibuprofen that was given did not substantially impair the excretion of a water load or significantly alter the minimum urinary osmolality achieved after water was imbibed. The marked effect of ibuprofen on medullary pO_2 cannot therefore be ascribed to an inhibition of diuresis *per se*, but probably reflects the action of locally synthesized PGs and their inhibition on medullary blood flow during water diuresis.

In the light of these results, the difference we noted between younger and older subjects in the response of renal medullary pO_2 to water diuresis is not unexpected. Although urinary excretion of PGE_2 (and, presumably, renal medullary synthesis of PGE_2) is stimulated by water diuresis in the young [5,6], this response is known to be markedly reduced with age [5]. It seems reasonable to assume that this is responsible, at least in part, for the defect in medullary oxygenation seen with BOLD MRI during diuresis in older people. The action of other local endogenous vasodilators in the renal medulla, such as nitric oxide [18] or

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dopamine [5], may also become attenuated with age. These experiments suggest that a feature of normal aging is the loss of the ability to improve renal medullary pO_2 rapidly during water diuresis. An analogous inability to improve medullary oxygenation in response to physiological or pathological stimuli might help to explain the increased susceptibility of older people to hypoxic renal damage and acute renal failure following circulatory, septic, and toxic insults [19–22]. The physiological basis of this hypothesis can now be investigated noninvasively in human subjects using BOLD MRI.

Acknowledgments

This work was supported in part by grants from the American Heart Association and the National Institutes of Health (DK 53221) to P.V.P. and by the Kidney Foundation of Massachusetts and Rhode Island and the National Institutes of Health (DK 18078) to F.H.E. The authors thank Dr. Wei Li and Ms. Katherine Spokes for their technical support, and the BIDMC NIH General Clinical Research Center (M01-RRO-1032), which assisted in carrying out these experiments.

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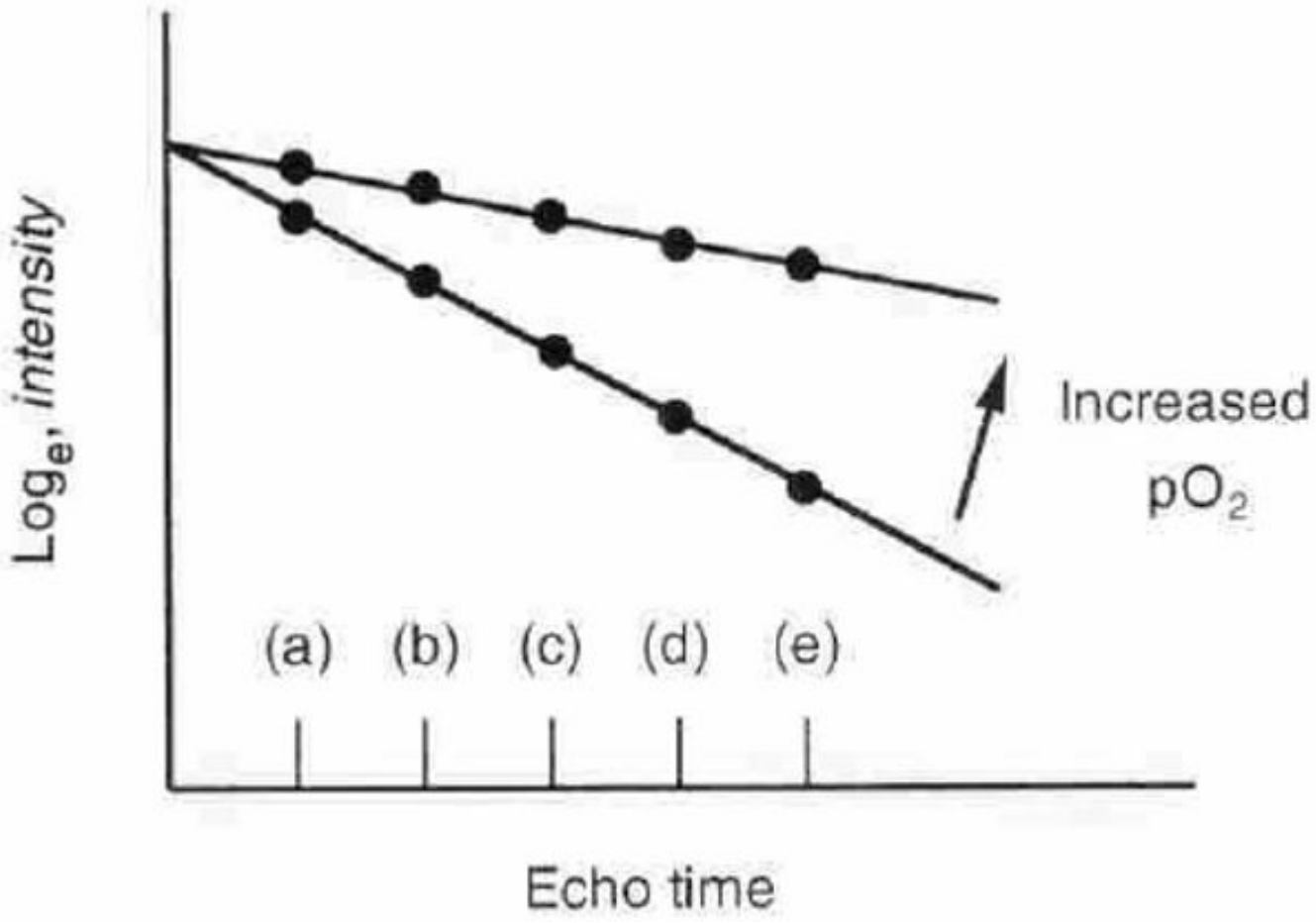

Fig. 1. Correlation of blood oxygenation level-dependent (BOLD) MRI with pO_2

R_2* = slope ~ conc[deoxyHB] ~ blood pO_2 ~ tissue pO_2. The deoxygenation of hemoglobin changes its magnetic characteristics, leading to changes in a parameter of magnetic resonance called R_2* (apparent spin-spin relaxation rate). R_2* can be estimated from signal intensity measurements made at several different echo times (a through e). The slope of $\log_e$ (intensity) vs. echo time determines R_2* and is directly related to the amount of deoxygenated blood. A decrease in the slope implies an increase in the pO_2 of blood. Because blood pO_2 is thought to be in rapid equilibrium with tissue pO_2, changes in BOLD signal intensity or R_2* should reflect changes in the pO_2 of the tissue.

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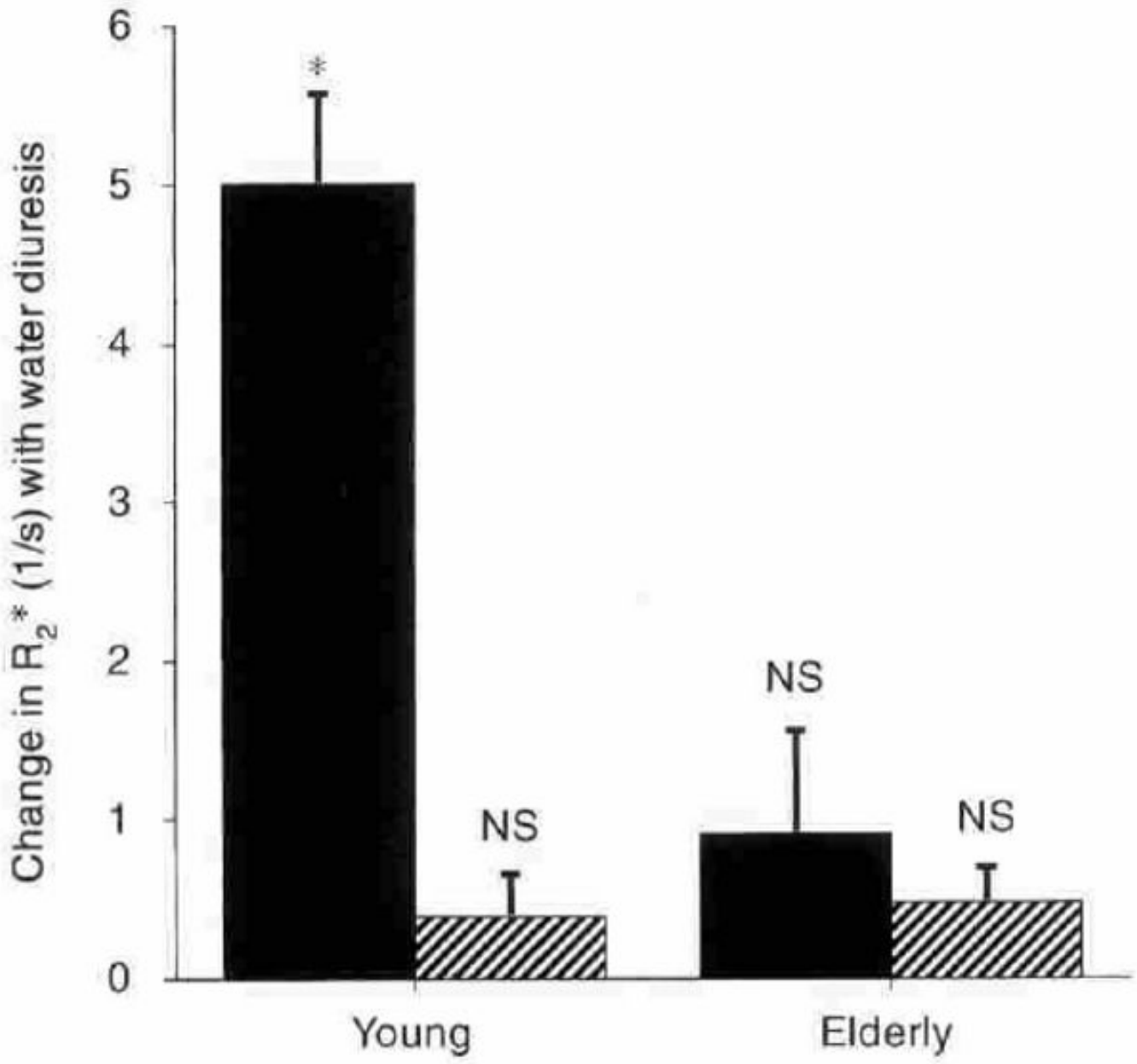

Fig. 2. Comparison of changes in R_2^* (1/s) in response to waterload in 9 young and 9 elderly subjects

Symbols are: (■) medulla; (▨) cortex. Columns are mean ± SEM. NS implies not significant. * implies $P < 0.01$.

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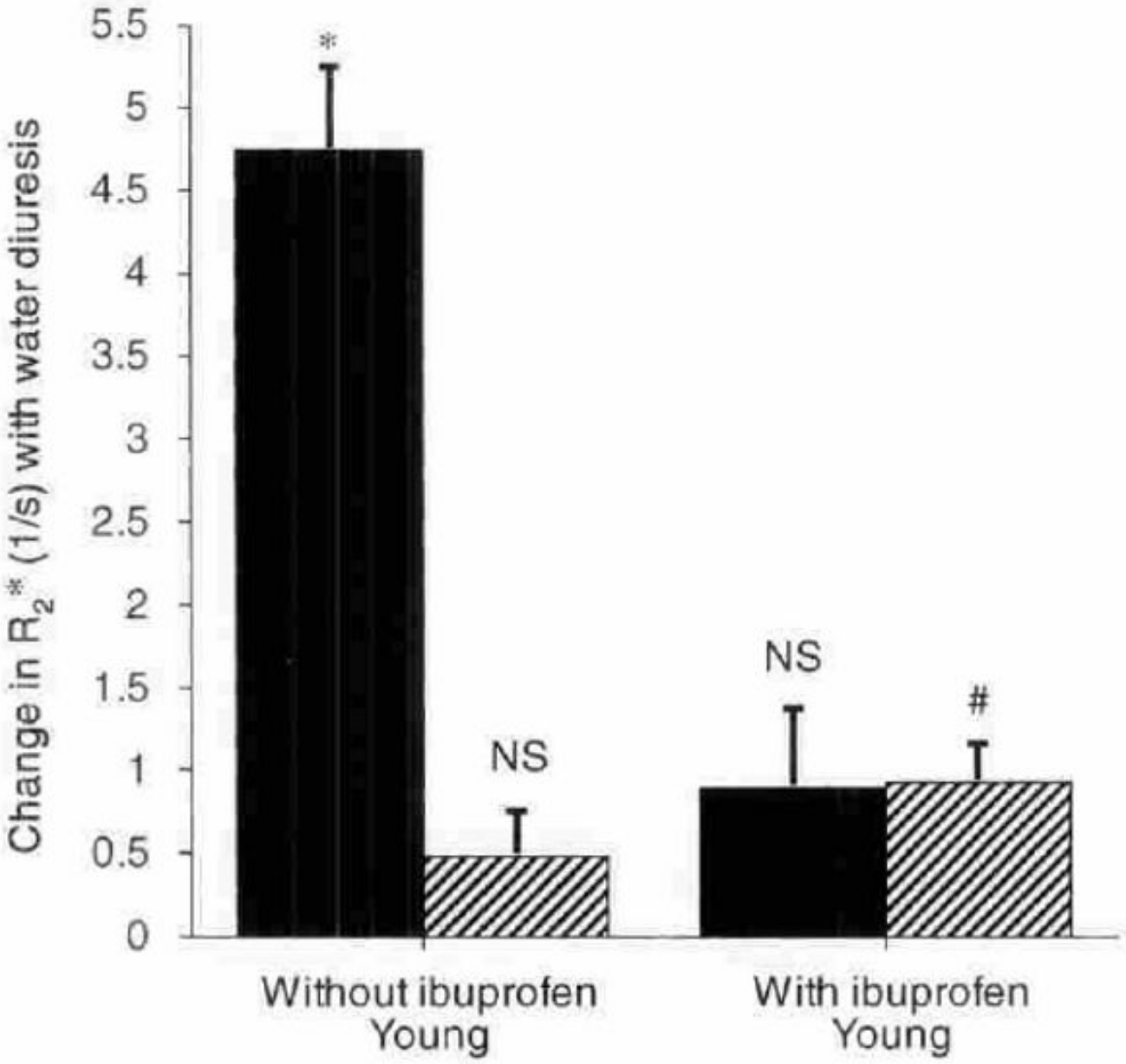

Fig. 3. Comparison of changes in R_2^* (1/s) in response to waterload in 6 young subjects with and without cyclooxygenase inhibition with ibuprofen

Symbols are: (■) medulla; (▨) cortex. Columns are mean ± SEM. NS implies not significant. * implies $P < 0.01$; # implies $P < 0.02$.

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Table 1

Effect of water diuresis in young and elderly subjects

	Antidiuresis						Diuresis					
	R_2* medulla	**R_2* cortex**	**PGE_2 excretion *pg/min***	**V *ml/min***	**U_{Osm} *mOsm/kg***	**C_{Cr} *ml/min***	**R_2* medulla**	**R_2* cortex**	**PGE_2 excretion *pg/min***	**V *ml/min***	**U_{Osm} *mOsm/kg***
Young (N = 9)	17.9 ± 0.4	12.8 ± 0.4	228 ± 37	0.44 ± 0.07	810 ± 55	115 ± 0.5	12.6^{a} ± 0.5	11.7^{c} ± 0.3	571^{b} ± 64	10.9^{a} ± 0.6	56^{a} ± 3
Elderly (N = 9)	18.2 ± 0.4	12.3 ± 0.4	208 ± 50	0.60 ± 0.08	664^{g} ± 28	81^{af} ± 6.3	17.1^{de} ± 0.8	11.9^{d} ± 0.3	122^{de} ± 16	6.9^{ae} ± 63	86^{ae} ± 6

^{a}Different from antidiuresis ($P < 0.01$)

^{b}Different from antidiuresis ($P < 0.02$)

^{c}Different from antidiuresis ($P < 0.05$)

^{d}Not significantly different from antidiuresis ($P > 0.05$)

^{e}Different from young ($P < 0.01$)

^{f}Different from young ($P < 0.02$)

^{g}Different from young ($P < 0.05$)

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Table 2

Effects of ibuprofen on the kidney during water diuresis in young subjects

	Antidiuresis						Diuresis				
	R_2* medulla	**R_2* cortex**	**PGE_2 excretion *pg/min***	**V *ml/min***	**U_{Osm} *mOsm/kg***	**C_{Cr} *ml/min***	**R_2* medulla**	**R_2* cortex**	**PGE_2 excretion *pg/min***	**V *ml/min***	**U_{Osm} *mOsm/kg***
No ibuprofen (6)	17.9 ± 0.45	11.9 ± 0.4	237 ± 62	0.44 ± 0.12	839 ± 80	116 ± 16	13.2^{a} ± 0.56	11.4 ± 0.35	478^{a} ± 21	11.1^{a} ± 0.9	55^{a} ± 4
Ibuprofen (6)	18.45 ± 0.71	12.13 ± 0.34	213 ± 44	0.49 ± 0.14	846 ± 113	121 ± 16	17.6^{b} ± 0.36	11.2 ± 0.28	89^{ab} ± 11	9.1 ± 0.7	64 ± 6.4

[a] Significantly different from antidiuresis, ($P < 0.01$)

[b] Significantly different from no ibuprofen, ($P < 0.01$)

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