NOTE *Internal Medicine*

Short Term Change of Urinary N-Acetyl-β-D-Glucosaminidase in Reduced Kidney Mass with Renal Artery Ligation

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ABSTRACT. The aim of the present study was to evaluate short term urinary NAG levels in a model of reduced kidney mass. The half and quarter kidney mass were made from ligation of the renal artery. Both groups decreased in the level of excreted NAG on day 1 and 2 after operation. On day 5 after operation, both groups achieved urinary enzyme levels comparable to that of the sham-operated group. The remaining compensated nephrons held normal range of excreted urinary NAG levels, although reduced number of nephrons resulted in a decline in urinary NAG levels.

KEY WORDS: kidney function, renal disorder, urinary enzyme.

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Urinary *N*-acetyl-β-D-glucosaminidase (NAG) levels have been reported to increase after exposure to various toxic substances, such as lead and cadmium [7], solvents [10], contrast media [8], aminoglycoside antibiotics [11], nephrotoxic drugs [4], puromycin aminonucleoside-induced glomerulonephritis [1], and various human glomerular diseases, including diabetic nephropathy [9]. Serum creatinine has limited value in the detection of early renal damage, and thus urinary enzymes such as NAG are commonly used as markers for tubular injury. To date, there have been no studies on urinary NAG levels in reduced kidney mass. The aim of the study was to evaluate short term urinary NAG levels in a model of reduced kidney mass with renal artery ligation.

The experiments were performed on 32 male Sprague-Dawley rats (Charles River Japan, Inc.), with a mean weight of 294 ± 11 g. Each rat was housed in a metabolism cage with controlled temperature (23°C) and relative humidity (52 ± 13%), and a synchronized 12-hr light/dark cycle. The rats were fed food and water *ad libitum*. Dietary intake and body weight were measured once daily.

Prior to laparectomy, the rats were anesthetized with pentobarbital 40 mg/kg. The rats were assigned to sham operation (n=9), ligation of the right renal artery and vein (1/2 kidney; n=11), or ligation of the right renal artery and vein plus the left renal branch artery (1/4 kidney; n=12).

Urine samples were collected for 4 days before operation as baseline samples and at 1–5, 12, and 19 days after operation. Urine volume was measured during each collection period. One m*l* of collected urine was centrifuged at 3,000 rpm for 5 min to obtain the supernatant, which was immediately stored at 4°C and used for measurement within 48 hr. At 3 days before operation and at 5, 12, and 19 days after operation, 1 m*l* of blood was collected from the subclavian vein under light anesthesia. The blood was centrifuged at 3,000 rpm for 5 min to obtain the supernatant, which was frozen and stored.

The activity of urinary NAG was determined colorimetrically as the absorbance at 580 nm wavelength of m-cresol purple released by NAG from the substrate, sodio m-cresolsulfonphthaleinyl-N-acetyl-β-D-glucosaminide (MCP-NAG) (Shionogi & Co., Ltd., Tokyo, Japan). Serum and urine creatinine levels were measured with the Jáffe reaction method using a commercially available kit (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan).

Enzyme excretion (Eext) was calculated from measures of urinary enzyme activity (Eact) and urine volume (Uvol): Eext (U/kg) = Eact (U/*l*) × Uvol (m*l*)/kg body weight.

The data collected at the different time points were analyzed for differences in body weight, water intake, food intake, urine volume, creatinine excretion, sodium excretion, potassium excretion, and NAG excretion by using analysis of variance and the Tukey test. Significance was defined as $P<0.05$.

Body weight was significantly ($P<0.05$) decreased in the 1/4-kidney group compared with the sham-operated group, from day 2 after operation. Food intake was decreased in the 1/4-kidney group compared with the sham-operated group on day 1, 3, 4, and 5 after operation; however, there was no difference between the groups on day 12.

Urine volume and water intake were significantly increased in the 1/4-kidney group compared with the sham-operated and 1/2-kidney groups after operation (Fig. 1). No differences in creatinine excretion or clearance were observed among the study groups. Sodium excretion was significantly ($P<0.05$) increased in the 1/4-kidney group compared with the sham-operated group, from day 3 after operation. Conversely, potassium excretion was significantly ($P<0.05$) decreased in the 1/4-kidney group compared with the sham-operated group on day 1, 2, 3, and 4 after operation. NAG excretion was significantly ($P<0.05$)

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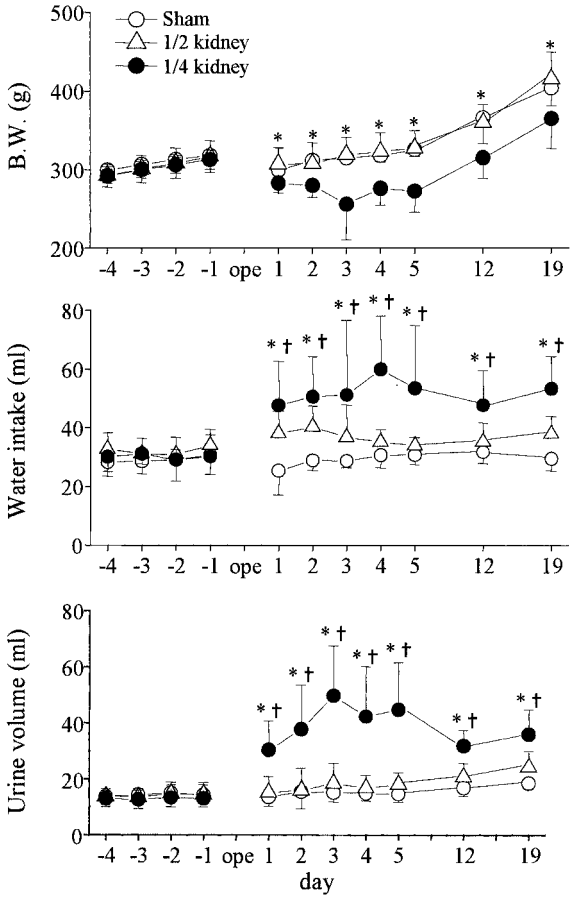

Fig. 1. Changes in body weight (upper), water intake (middle), urine volume (bottom) after surgery for reduced kidney mass. *: P<0.05 (Sham vs. 1/4 kidney). †: P<0.05 (1/2 kidney vs. 1/4 kidney).

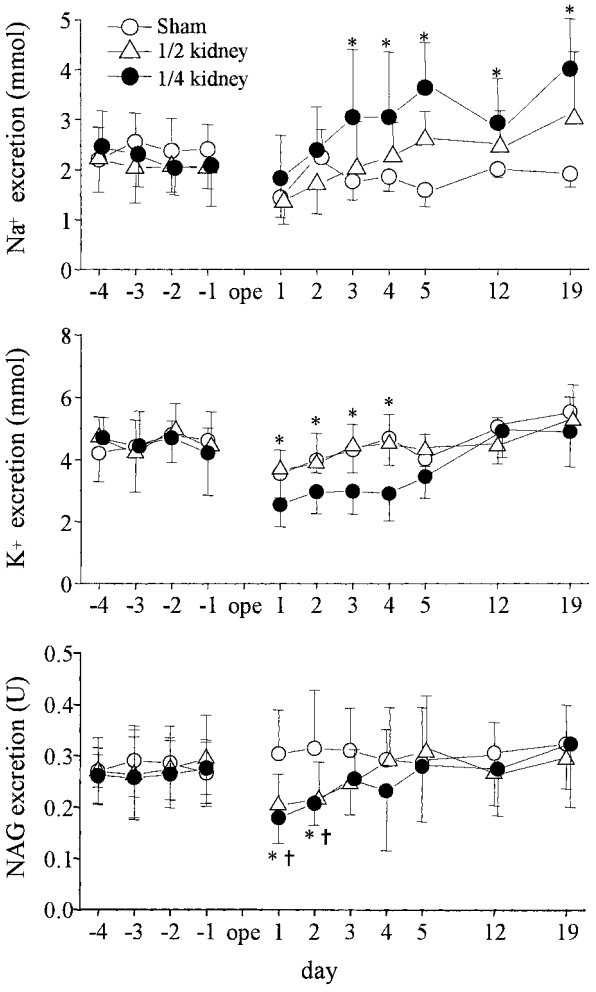

Fig. 2. Changes in Na excretion (upper), K excretion (middle), NAG excretion (bottom) after surgery for reduced kidney mass. *: P<0.05 (Sham vs. 1/4 kidney). †: P<0.05 (Sham vs. 1/2 kidney).

decreased in the 1/4-kidney and 1/2-kidney groups on day 1 and 2 after operation, but there was no difference between the 1/4-kidney and sham-operated groups after day 3 (Fig. 2).

Compared with other urinary enzymes such as gamma-glutamyl transpeptidase and alanine aminopeptidase [2], NAG has a large molecular weight (130–140 kDa) and cannot be filtered through the glomeruli. In healthy patients, serum NAG is not excreted into the urine but rather enters directly into the urine from kidney proximal epithelial cells. Urinary NAG is currently used as an index for renal dysfunction, because the level of NAG escaping from damaged tubular epithelial cells is elevated in patients with tubule-damaging diseases [6, 10].

In the present study, the 1/4-kidney and 1/2-kidney groups demonstrated a decrease in excreted urinary NAG on day 1 and 2 after operation. The decrease in NAG levels appears to be related to the reduction in the number of nephrons. There were no differences in the creatinine clearance or excretion levels between the groups, indicating that the glomerular filtration rate in both groups was approximately equal. The 1/4-kidney group demonstrated an increase in urine volume after operation. Hayslett [5] reported that a reduction in renal mass evoked a compensatory response from the renal tubules. Polyuria resulted from increased ultrafiltration pressure on the remaining functional nephrons (25%) and a decline in the reabsorption rate of increased primary urine by the renal tubules and collecting ducts. In the present study, the 1/4-kidney group demonstrated an increase in water intake; this may be attributable to the triggering of the drinking stimulus in response to a decrease in blood circulation, which may have been caused by the increased urine volume.

On day 5 after operation, urinary enzyme activity in the 1/4-kidney and 1/2-kidney groups, which experienced an acute reduction in the number of nephrons, reached the level of the sham-operated group. These findings may be explained by the compensatory proliferation of epithelial cells (involving anatomical changes of the renal tubules)

and the compensatory hyperfunction of the renal tubules [3, 10]. Thus, despite a decrease in the number of functional nephrons, the level of NAG escaping into the urine remained within the normal range when the renal tubules functioned in a compensatory fashion.

In conclusion, urinary NAG levels appeared within the normal range after the reduction in kidney mass as long as the remaining nephrons functioned in a compensatory fashion; however, a reduction in the number of nephrons resulted in a decline in urinary NAG levels.

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