

FLUID RESUSCITATION OF ACUTE
HEMORRHAGE IN UNINEPHRECTOMIZED
RABBITS: EFFECTS ON THE EARLY
FUNCTION OF THE REMNANT KIDNEY

ELIF A AKPEK¹, HALE TUFAN², PETEK KORKUSUZ⁵,
SELIM CANDAN¹, ESIN AŞAN³, SERDAR ARDA³,
AYŞEGÜL HABERALAND^{1,4} GÜLNAZ ARSLAN¹

Abstract

The aim of this study was to determine the effects of fluid resuscitation of acute hemorrhage on the early function and histopathology of the remnant kidney in uninephrectomized rabbits. Thirty-nine adult rabbits were studied in four groups. Group 1 (n = 8) included healthy controls; Group 2 (n = 10) healthy, bled animals; Group 3 (n = 10) uninephrectomized, non-bled animals; and Group 4 (n = 11) uninephrectomized, bled animals. In the hemorrhage groups, 8 mL kg⁻¹ of blood was drawn, and replaced with lactated Ringer's solution three times the volume of shed blood. Urine and blood samples were collected after 120-minutes of observation.

None of the animals experienced hypotension during the study period. Serum and urinary electrolytes were similar between the Groups (p > 0.05). Urine output was lower in Groups 3 and 4 than in Group 1 (p – 0.001, both). Urinary microalbumin, NAG, fractional sodium

From Başkent University Faculty of Medicine, 06490 Ankara, Turkey. Departments of

1 - Anaesthesiology.

2 - Pharmacology.

3 - Pediatric Surgery.

4 - Biochemistry.

From Hacettepe University Faculty of Medicine. 06100 Ankara, Turkey.

5 - Department of Histology.

Correspondence: Elif Arslan Akpek, Mesa Koru Sitesi, Akkavak sok. No. 12, Çayyolu 06530, Ankara, Turkey. Tel: (90) 3122408244, Fax: (90) 3122237333, E-mail: elifakpek@baskent-ank.edu.tr.

excretion and creatinine clearance were similar in all four Groups. Light microscopic evaluation revealed only slight enlargement of the proximal tubule lumen in the renal medulla of the rabbits that were both uninephrectomized and bled.

We observed no deleterious effects of well resuscitated hemorrhage on early function and histopathology of the remnant kidney in uninephrectomized rabbits.

Keywords: Uninephrectomy; Acute hemorrhage; Renal function.

Introduction

Progress in transplantation technology and the outstanding results with outcome reports lead to an increasing numbers of people undergoing voluntary uninephrectomy for living-related renal transplantation. These individuals in their future life, may possibly face with an emergent situation that may further threaten the function of their sole kidney. The effects of any resuscitative efforts in acute hemorrhagic conditions and the limits of management strategies still remain to be unclear in these individuals. Thus, preservation of the function of healthy remnant kidney may require special consideration in specific settings.

The aim of the current experimental study was to determine the effects of fluid resuscitation of acute hemorrhage on the early function and histopathology of the remnant kidney late in uninephrectomized rabbits as an initial step towards mimicking the human clinical setting.

Methods & Materials

After receiving Animal Care Committee approval for our experiment protocol, 39 adult New Zealand rabbits weighing between 2000-3500g were studied in four Groups. Twenty-one of the animals underwent uninephrectomy two months before the experiment to constitute the two single-kidney groups. In each of these procedures, anesthesia was given with an intramuscular injection of 40 mg kg⁻¹

ketamine and 6 mg kg⁻¹ xylazine. A repeat injection of anesthetic equal to one-third of original dose combination was given as needed, and prophylactic antibiotics were also administered. Left kidney was removed in all 21 rabbits. The animals then were housed in a controlled laboratory environment for two months where they received standard rabbit chow and water, and were kept at a room temperature of 20 ± 2°C and humidity of %50 ± 10.

For the current experiment, each animal was fasted for 12 hours prior to the study, but received continued free access to water during this time. Three to four animals were studied per day. Animals were anesthetized with the same combined intramuscular injection described above and anesthesia was maintained with a 30 mg h⁻¹ ketamine infusion during the experiment. The animals were kept under normothermic conditions and spontaneous respiration with oxygen through nasal cannula. A cut-down was performed in the neck region using a dissection microscope (Zeiss ®), and placed a 22G intravascular catheter in the carotid artery and a 24G catheter in the jugular vein. The arterial line was connected to a multichannel physiologic recorder (Biopac-MP100A ®) that continuously displayed blood pressure and heart rate. Heparinized saline was infused through the arterial line, and the intravenous line was kept patent with a 4 ml kg⁻¹ h⁻¹ maintenance infusion of lactated Ringer's solution. A catheter was placed in the urinary bladder of the rabbits via percutaneous route to collect urine during the observation period.

Once the vascular and urinary catheterizations were completed, the experiment was initiated (time 0). Animals were studied in four Groups based on their kidney status and application of hemorrhage. Group 1 (n = 8) included healthy control animals; Group 2 (n = 10) healthy, bled animals; Group 3 (n = 10) single-kidney, non-bled animals; and Group 4 (n = 11) single-kidney, bled animals. Animals in Group 1 (healthy controls) and 3 underwent vascular cannulations only and nothing was applied other than the maintenance infusion during observation period. Animals in Group 2 and 4 were subjected to acute hemorrhage and subsequent fluid resuscitation.

Acute hemorrhage was achieved via removal of 8 ml kg⁻¹ of blood from the arterial line over 15 minutes (between 0 to 15 min). Blood volume was replaced with a bolus of lactated Ringer's solution three times the volume of shed blood, and this volume was injected via the jugular vein. Replacement was started at 5 minutes of blood removal, and the entire bolus was administered over 10 minutes (between time 5 to 15 min). Thereafter, the maintenance fluid infusion was continued for 120 minutes of study period. Hemodynamic were recorded throughout this period.

At the end of observation period, the data measured were; serum levels of sodium, potassium, creatinine and urea as well as urine output and urinary levels of sodium, potassium, creatinine, microalbumin and NAG. Two-hour creatinine clearance was also calculated. Finally, animals were sacrificed with a lethal injection of 100 mg/kg sodium thiopental and right kidneys were removed for light microscopic examination.

Histological Method

All specimens were immersed in a solution of 10 per cent buffered formalin following decapsulation. Then they were dehydrated in a graded series of ethanol and embedded in paraffin. Five-micrometer thick sections of the specimens were prepared and stained with Hematoxylin Eosin (HE), and Periodic Acid Schiff (PAS) in order to demonstrate the basement membranes. Photomicrographs were obtained by an Olympus BH-7 light microscope to demonstrate parenchymal histopathological changes.

Statistics

Data are expressed as mean \pm SD. Statistical analyses were done using the Friedman and Wilcoxon tests for comparison of repeated measurements within groups, and the Kruskal Wallis and Mann Whitney-U tests for comparison of variables between groups. All *p* values less than 0.05 was considered significant.

Results

Animals did not differ in respect to their weight among the groups (2837,5 ± 172,6g; 2655,0 ± 27,4g; 2935,0 ± 255,0g and 2827,2 ± 467,0g respectively). Data presenting urinary and blood parameters are shown in Table 1. Urine output in Groups 3 and Group 4 was lower than that in Group 1 (p = 0.001, both). Fractional excretion of sodium did not differ between Groups (p > 0.05). Other urine parameters were similar among the Groups and values were all within normal range.

Table 1
Serum and urinary data in the experimental groups. Mean ± SD.

	Group 1	Group 2	Group 3	Group 4	
Serum sodium (mmol/L)	139±6	142±8	136±6	138±4	NS
Serum potassium (mmol/L)	2.9±0.5	3.3±0.6	4.1±1.1	3.9±0.8	NS
Serum creatinine (mg/dL)	1.0±0.1	0.9±0.5	1.5±0.4	1.4±0.4	NS
BUN (mg/dL)	14.3±2.9	15.6±3.6	17.0±4.6	17.7±4.0	NS
Urinary sodium (mEq/L)	81±34	108±61	125±88	169±67	NS
Urinary potassium (mEq/L)	70±54	23±8	40±44	34±47	NS
Urinary microalbumin (mg/dL)	1.63±2.12	1.21±1.04	4.28±7.16	3.80±2.69	NS
Urinary NAG (U/L)	5.7±3.5	1.9±0.8	1.4±0.6	2.6±3.2	NS
Creatinine clearance (mL/min)	216±22	197±24	118±18	113±10	NS
Urine output (ml/kg/h)	14.4±3.7	10.2±3.8	5.4±2.8*	6.4±3.8	P = 0.001
Urine density	1015.6±9.4	1010.0±2.5	1010.5±3.0	1010.0±3.1	NS

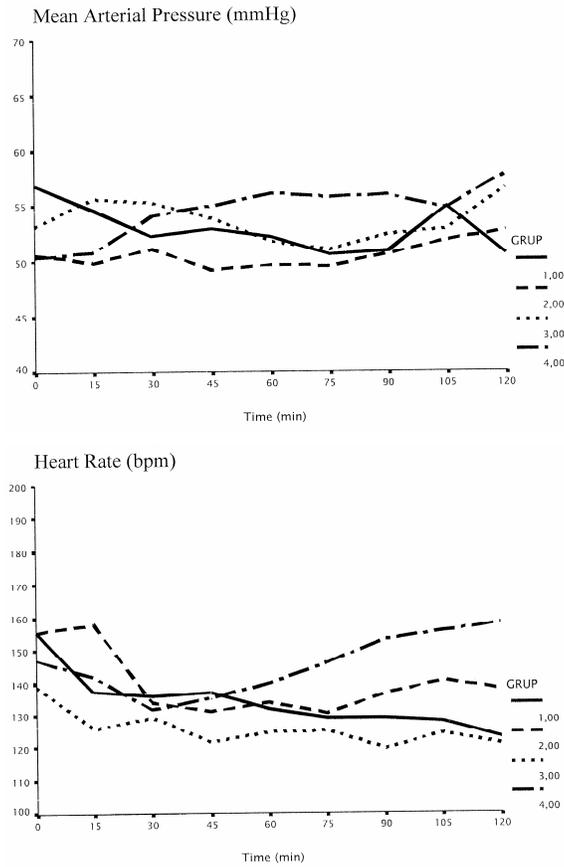
(BUN: Blood Urea Nitrogen; NAG: N-acetyl-B-D-glucosaminidase).

* p < 0.05 compared to group 1.

Hemodynamic measurements revealed no significant changes in

mean arterial pressure over time when findings were compared between and/or within Groups. However, all Groups showed significant changes in heart rate over time. The rates in Groups 1 to 3 declined significantly over time, as compared to baseline levels ($p = 0.047$, $p = 0.012$, $p = 0.048$ respectively). In contrast, heart rates in Group 4 rose significantly compared to those recorded in the first 30 minutes of the experiment ($p = 0.001$) (Figure 1). Comparison of heart rates among the four Groups revealed significantly higher rates in Group 4 compared to Group 3 at 105 and 120 minute time points ($p < 0.01$ both).

Fig. 1
Hemodynamic parameters of the groups



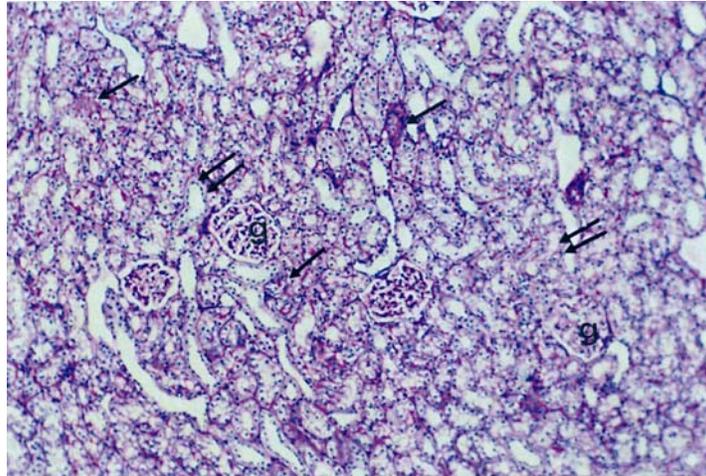
*P<0.05 compared to group 3

There were no significant structural alterations at the different segments of the nephron at the light microscopical level in any of the experimental groups. Necrosis was not present in any of the specimens. Basement membranes of the tubules and glomerules appeared normal with PAS staining. The minor structural alterations that were observed within the different groups were noted as follows;

A slight swelling and vacuolization of the proximal tubule epithelial cells were observed in the cortex of the nephrectomized group. Similar structural alterations were also prominent at the collecting tubules epithelia of the medulla of the nephrectomized groups (Fig. 2a, 2b, 3a, 3b).

Fig. 2a

Renal parenchyma appears normal at the micrograph from the control group. Note the PAS positive reaction at the basement membranes. g: glomerule, arrow: convoluted part of the proximal tubule, double arrow: convoluted part of the distal tubule. 10 x PAS.

*Fig. 2b*

Tubules and the collecting ducts () appear normal at the renal medulla of the control group. 20 x PAS.*

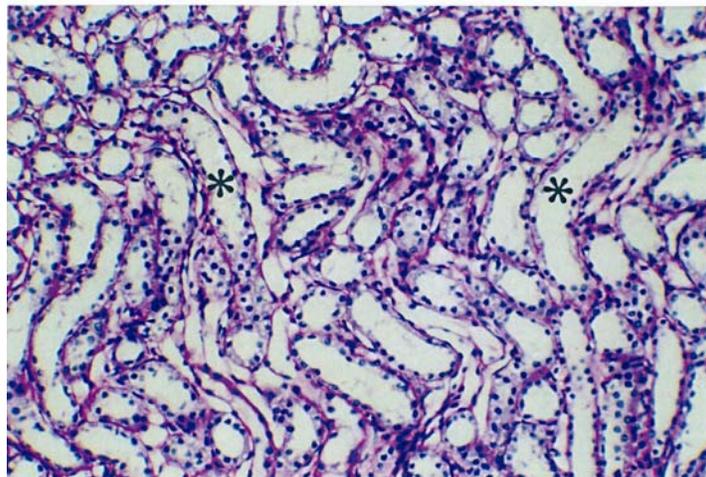


Fig. 3a

Micrograph from the nephrectomized group. Renal corpuscle is observed with its glomerule (g). Note the swelling of the proximal tubule epithelial cells. 20 x PAS.

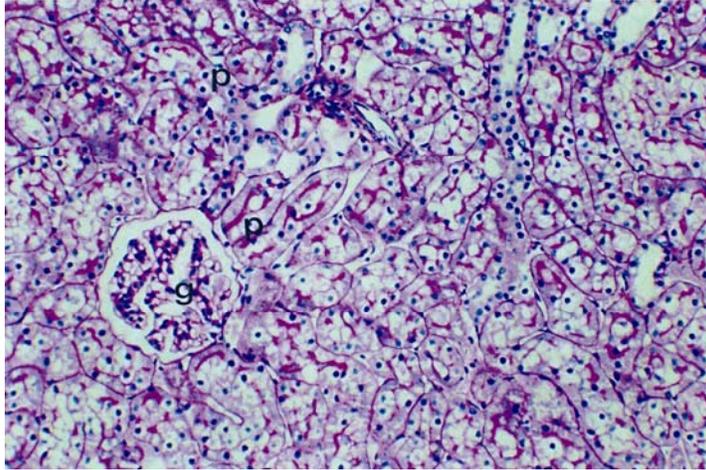
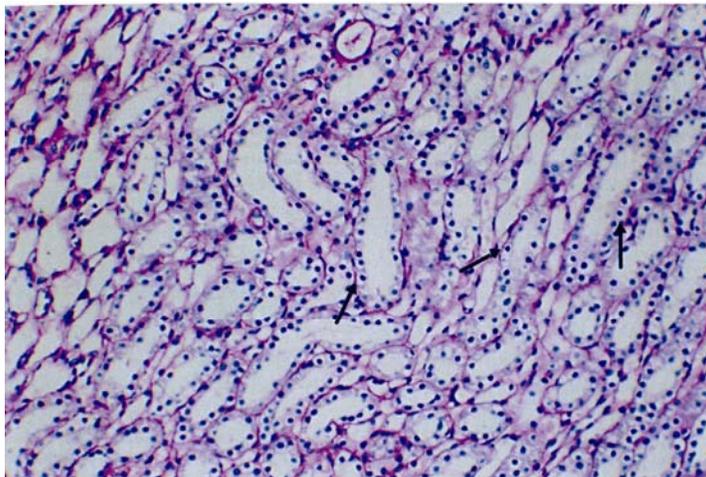


Fig. 3b

Micrograph from the medulla of the nephrectomized group. Swelling of the collecting ducts epithelial cells (arrow) is observed. 20 x PAS.



The cortical structure was well preserved in the hemodilution group. Both the proximal and distal tubules and the glomerules were structurally in accordance in this group when compared to the control group (Fig. 4a). A slight dilatation was present at the lumen of the collecting tubules in the medulla of the hemodilution group; however, cellular alterations were not noted (Fig. 4b).

Fig. 4a

Renal parenchyma appears normal at the micrograph from the hemodilution group. g: glomerule, arrow: proximal tubule, double arrow: distal tubule. 10 x PAS.

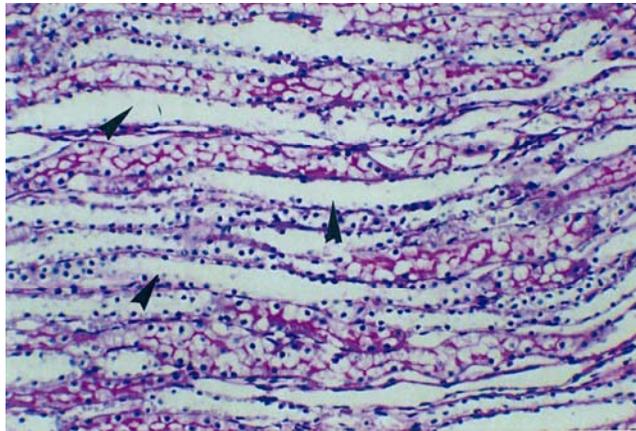
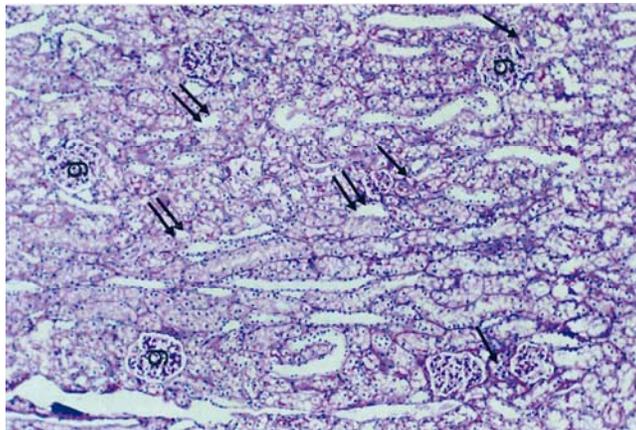


Fig. 4b

Micrograph from the haemodilution group shows the renal medulla. Note the dilation of the collecting ducts lumens (arrow head). 20 x PAS.



When single-kidney, hemorrhage applied group was compared to the sole nephrectomy group, a significant structural difference between could not be observed. Staining properties altered of the convulated proximal epithelial tubules in the single-kidney, hemorrhaged group. Tubules became more significant at some locations with the PAS technique (Fig. 5a). Collecting tubules of the medulla presented no significant alterations. The straight parts of the lumens of proximal tubules were slightly dilated (Fig. 5b).

Fig. 5a

Micrograph from both nephrectomy and hemodilution applied group. Glomerules (g) and tubules appear normal at the cortex. 20 x PAS.

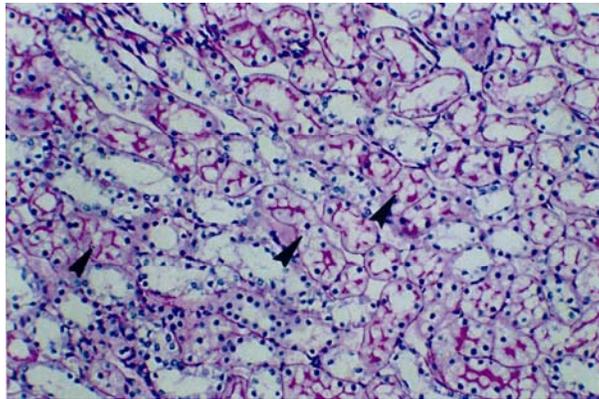
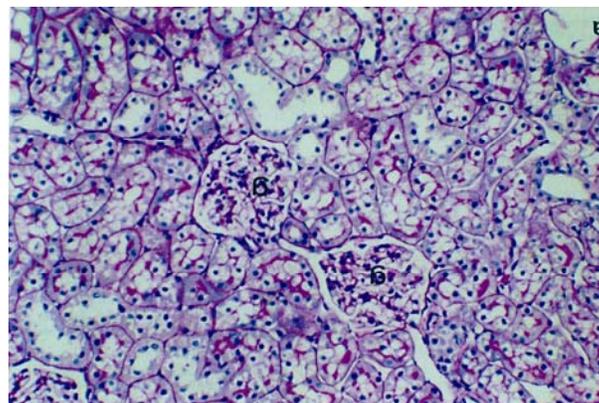


Fig. 5b

Micrograph showing the medulla from both nephrectomy and hemodilution applied group. Tubules and collecting ducts appear normal. Note the local slight dilatation at the lumen of the proximal tubules (arrow head). 20 x PAS.



Discussion

There appears to be an increasing number of individuals with single kidney in the population as the clinical application of living-related renal transplantation increases. This study investigated the early outcome of renal function after acute hemorrhage and subsequent fluid resuscitation in rabbits with single kidney. Background of this study was the concern that whether reflection of this setting to clinical practice would have any impact on the management strategies of cases in which hemorrhage is likely to appear, such as, trauma and/or major surgical procedures of various types.

To date, long-term studies of kidney donors and experimental studies with uninephrectomized animals have shown no adverse effects on the remnant renal function¹⁻⁵. Some authors have reported that basal urinary sodium excretion and urine flow rates are significantly higher in anesthetized uninephrectomized rats^{6,7}. Age at nephrectomy, length of time with a single kidney and gender were shown to have little effect on the remnant renal functions. There was a positive correlation between current mean arterial pressure and serum creatinine⁸.

Hypertension, diabetes and advanced age are also risk factors for deterioration of renal function⁹. Argiles¹⁰ has shown that functional adaptation characterized by an increased glomerular filtration rate and tubular function began in the first two days after nephrectomy. Early in the adaptive response following uninephrectomy in animals, biochemical changes are expressed as an increase in kidney weight and morphologically as an increase in the cross-sectional surface area of the kidney as early as three weeks in the rat¹¹. Assessment of proximal and distal tubular function 30 days after uninephrectomy was suggested to demonstrate the chronic changes of the remnant kidney⁷. Thus two months in this study is considered sufficient for the remnant kidneys to compensate to the level of adaptive renal function. All the animals were adults, uni-nephrectomized groups had single kidneys for the same period of time, so the standardization of the groups is appropriate. Baseline preoperative values were not obtained because each of the hemorrhage

and nephrectomized groups had an appropriate non-hemorrhage and non-nephrectomized control groups of each, rather than acting as their own controls. Moreover, the comparison of histopathological status could only be achieved by separate control groups. Histopathological examination did not demonstrate any significant changes indicating early signs of renal injury in any of the groups.

Although the various surgical procedures and/or anesthetic management of these patients later in their life are not rare, the extreme situations encountered as hemorrhage and subsequent fluid resuscitation being one of them, have not been investigated to date. In the animal model of this study, biochemical markers of glomerular and tubular function, as well as histological examination were used for assessment of renal function. Functional alteration in the glomeruli and tubules is indicated by various aspects of proteinuria; specifically, microalbuminuria for glomerular function and low molecular weight proteins and enzymes for tubular function^{12,13}. No significant differences were found between the groups in terms of blood chemistry, urinary microalbumin and NAG levels, or creatinine clearance. The tendency to higher urinary sodium concentration in animals other than those in healthy controls (Group 1) was correlated with the decreases in urinary output. Thus the urinary sodium excretion did not increase in either of the groups and were similar between the groups. Urine output for all groups was appropriate for observation time when it was calculated as volume per body weight. Thus lower urinary flow rates in Groups 3 and 4 can only explain the higher urine sodium concentrations but create no deleterious limits. Lower urinary output, increased urinary sodium concentration, as well as no increases in urinary sodium excretion and no decreases in serum creatinine and BUN levels secondary to hemodilution in groups other than healthy controls might reasonably reflect slightly lower volume status in these groups. However, none of the animals developed hypotension to suggest hypovolemia enough to constitute a risk against organ protection and the increasing heart rates in Groups 2 and 4 are the results of hemodilution. These findings possibly indicate a limited hemodilution similar to that is used in clinical practice with an acceptable volume status.

Hemodilution is often used intentionally in anesthetic practice for specific situations. The technique of blood removal based on a fixed volume per body weight and subsequent replacement with crystalloid and/or colloids, is extensively used for preservation of autologous blood and to minimize the risks of heterologous blood transfusion. It has been demonstrated that hemodilution to a hematocrit 20% does not impair hepatorenal function in healthy people¹⁴. It reduces renal vascular resistance, resulting in higher flow to the outer renal cortex and consequently, enhanced urine flow. Increased urinary output points out the necessity for proper adjustment of crystalloid infusion to maintain normal intravascular volume and to avoid hypovolemia and the associated risk of tissue hypoxia¹⁴. This study can be criticized on this point as the findings do not apply to a definite degree of hemodilution. Clearly, the level of hemodilution that can be reached is finite in terms of its effects on the body. At some point, oxygen delivery is compromised and anaerobic metabolism begins. However, hematocrit values and central venous pressures were not recorded in this study because the objective in hemodynamic monitoring was to prevent hypotension and its deleterious effects mimicking an emergency setting, rather than to investigate the effects of certain degree of hemodilution or to strictly meet the criteria relevant to the term of acute normovolemic hemodilution. Obviously, studies to determine the critical level of hemodilution in patients with single kidney may further suggest the elective use of hemodilution technique for these patients.

In conclusion, no deterioration of early renal function and histopathology were observed with acute hemorrhage and subsequent fluid resuscitation in uninephrectomized rabbits. It may be valuable to further test higher degrees of hemodilution by systematically following hematocrit, and thus determine the critical level.

References

1. NARKUN-BURGESS DM, NOLAN CR, NORMAN JE, ET AL: Forty-five year follow-up after uninephrectomy. *Kidney Int*; 43(5):1110-5, 1993.
2. TALSETH T, FAUCHALD P, SKREDE S, ET AL: Long-term blood pressure and renal function in kidney donors. *Kidney Int*; 29(5):1072-6, 1986.
3. MATHILLAS O, ATTMAN PO, AURELL M, ET AL: Proteinuria and renal function in kidney transplant donors 10-18 years after donor uninephrectomy. *Ups J Med Sci*; 90(1):37-42, 1985.
4. MATHILLAS O, ATTMAN PO, AURELL M, ET AL: Long-term outcome of renal function and proteinuria in kidney transplant donors. *Proc Eur Dial Transplant Assoc Eur Ren Assoc*; 21:574-9, 1985.
5. VINCENTI F, AMEND WJ, KAYSEN G, ET AL: Long-term renal function in kidney donors. Sustained compensatory hyperfiltration with no adverse effects. *Transplantation*; 36(6):626-9, 1983.
6. MULRONEY SE, WODA C, JOHNSON M, PESCE C: Gender differences in renal growth and function after uninephrectomy in adult rats. *Kidney Int*; 56(3):944-53, 1999.
7. SHIRLEY DG, WALTER SJ: Acute and chronic changes in renal function following unilateral nephrectomy. *Kidney Int*; 40(1):62-8, 1991.
8. OHISHI A, SUZUKI H, NAKAMATO H, ET AL: Status of patients who underwent uninephrectomy in adulthood more than 20 years ago. *Am J Kidney Dis*; 26(6):889-97, 1995.
9. KHADER K, ZIADE J, BANSARD JY, ET AL: Outcome of renal function in 114 patients who underwent uninephrectomy for renal cancer. *Prog Urol*; 8(3):341-6, 1998.
10. ARGILES A, MOURAD G, BASSET N: Acute adaptive changes to unilateral nephrectomy in humans. *Kidney Int*; 32:714-20, 1987.
11. MALT RA: Compensatory growth of the kidney. *N Engl J Med*; 280:1446, 1969.
12. IP-YAM PC, MURPHY S, BAINES M, ET AL: Renal function and proteinuria after cardiopulmonary bypass: the effects of temperature and mannitol. *Anesth Analg*; 78:842-7, 1994.
13. PRICE RG: The role of NAG (N-acetyl-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. *Clin Nephrol*; 38:S14-S19, 1992.
14. HABLER O, KLEEN M, HUTTER J, ET AL: Effects of hemodilution on splanchnic perfusion and hepatorenal function. II. Renal perfusion and hepatorenal function. *Eur J Med Res*; 2(10):419-24, 1997.

