Basic Science and Experimental Studies

# Increased Intra-abdominal Pressure Induces Acute Kidney Injury in an Experimental Model of Congestive Heart Failure

NIROZ ABU-SALEH, PhD,[1] DORON ARONSON, MD,[2] MOGHER KHAMAISI, MD, PhD,[3] EMAD E. KHOURY, MSc,[1] HODA AWAD, MSc,[1] AVIVA KABALA, BSc,[1] RAWI RAMADAN, MD,[4] TONY KARRAM, MD,[5] ELI KAKIASHVILI, MD,[6] BISHARA BISHARA, MD,[6] AND ZAID ABASSI, PhD[1,7]

*Haifa, Israel*

**ABSTRACT**

**Background**: Congestive heart failure (CHF) entails a complex interaction between the heart and the kidney that represents a clinical entity called cardiorenal syndrome (CRS). One of the mechanisms underlying CRS includes increased intra-abdominal pressure (IAP). We examined the effect of elevated IAP on kidney function in rats with low- and high-output CHF.

**Methods and Results**: Rats with compensated and decompensated CHF induced by means of aortocaval fistula, rats with myocardial infraction (MI) induced by means of left anterior descending artery ligation, and sham control rats were subjected to either 10 or 14 mm Hg IAP. Urine flow (V), $Na^+$ excretion ($U_{Na}V$), glomerular filtration rate (GFR), and renal plasma flow (RPF) were determined. The effects of pretreatment with tadalafil (10 mg/kg orally for 4 days) on the adverse renal effects of IAP were examined in decompensated CHF and MI. Basal V and GFR were significantly lower in rats with decompensated CHF compared with sham control rats. Decompensated CHF rats and MI rats subjected to 10 and 14 mm Hg IAP exhibited more significant declines in V, $U_{Na}V$, GFR and RPF than compensated and sham controls. Elevated IAP also induced tubular injury, as evidenced by significantly increased absolute urinary excretion of neutrophil gelatinase−associated lipocalin. In addition, in a nonquantitative histologic analysis, elevated IAP was associated with increase in necrosis and cell shedding to the tubule lumens, especially in the decompensated CHF subgroup. Pretreatment of decompensated CHF rats and MI rats with tadalafil ameliorated the adverse renal effects of high IAP.

**Conclusions**: Elevated IAP contributes to kidney dysfunction in high− and low−cardiac output CHF. IAP induces both hemodynamic alterations and renal tubular dysfunction. These deleterious effects are potentially reversible and can be ameliorated with the use of phosphodiesterase-5 inhibition. (*J Cardiac Fail 2019;25:468−478*)

**Key Words:** Cardiorenal syndrome, heart failure, kidney dysfunction, intra-abdominal pressure, NGAL.

The ability to sustain filtration and tubular functions of the kidneys in patients with heart failure is vital to survival and successful alleviation of congestion. The syndrome of acute kidney injury (AKI) complicating acute decompensated heart failure and acute coronary syndromes is defined as acute (type 1) cardiorenal syndrome.[1]

The pathophysiologic mechanisms leading to AKI during heart failure decompensation are not fully clarified. Recently, it has been suggested that the abdominal compartment might contribute significantly to deranged renal function in CHF. The importance of the abdominal compartment is particularly relevant when the capacitance function of the splanchnic vasculature is exceeded, such as in the setting of fluid overload and systemic congestion.[2] Recent studies demonstrated that patients with decompensated CHF have a high prevalence of elevated intra-abdominal pressure (IAP) in the absence of overt abdominal symptoms, and small increases in IAP are associated with impaired renal function.[3]

*From the [1]Department of Physiology, Bruce Rappaport Faculty of Medicine, Technion−Israel Institute of Technology; [2]Department of Cardiology; [3]Department of Diabetes and Metabolism and Internal Medicine, Institute of Endocrinology; [4]Department of Nephrology; [5]Department of Vascular Surgery; [6]Department of General Surgery and [7]Department of Laboratory Medicine, Rambam Health Care Campus, Haifa, Israel.*

Manuscript received July 20, 2018; revised manuscript received March 7, 2019; revised manuscript accepted March 11, 2019.

Reprint requests: Prof Zaid Abassi, Department of Physiology and Biophysics, Faculty of Medicine, Technion, IIT, PO Box 9649, Haifa, 31096, Israel. Tel: 972-4-8295266; Fax: 972-4-8295267. E-mail: abassi@tx.technion.ac.il

See page 477 for disclosure information.

1071-9164/$ - see front matter



https://doi.org/10.1016/j.cardfail.2019.03.008

Decrease in renal perfusion has been suggested as the main factor responsible for development of AKI in this condition.[4] Specifically, renal hypoperfusion might occur during an acute or progressive increase in IAP, due to the reduction of both arterial inflow and venous outflow.[5] However, few data are available regarding the underlying molecular mechanisms.

We have recently shown that rats with decompensated congestive heart failure (CHF) and acute myocardial infarction (MI) were more vulnerable to the adverse renal effects of increased IAP than normal rats or rats with compensated CHF.[6] Rats with decompensated CHF displayed reduced glomerular filtration rate (GFR), renal plasma flow (RPF), and urine flow when exposed to elevated IAP compared with normal control rats or rats with compensated CHF.[6] These hemodynamic alterations were associated with lower urinary excretion of $NO_2$ and $NO_3$, and the administration of phosphodiesterase-5 (PDE5) inhibitors (PDE-I) before increasing IAP mitigated the adverse effects on renal function.[6]

The present study was designed to determine whether increased IAP can induce tubular injury in a rat model of CHF[7] as assessed by urinary neutrophil gelatinase−associated lipocalin (NGAL) excretion in parallel to reduction in kidney function. In addition, we sought to assess whether PDE5 inhibitors can exert early nephroprotecive effects in different CHF rat models, evaluated by kidney function, urinary NGAL secretion, and renal histology.

## Materials and Methods

### Animals

Studies were conducted on male Sprague-Dawley rats weighing 300−350 g. The animals were fed standard rat chow containing 0.5% NaCl and tap water ad libitum. All experiments were performed according to the guidelines of the Committee for the Supervision of Animal Experiments, Technion−Israel Institute of Technology.

### Experimental Model of Pneumoperitoneum

A small incision in the lower third between the xiphoid and pubis was made, through which a regular Veress needle was inserted into the abdominal cavity. Sequential IAPs of 10 and 14 mm Hg were established and maintained with a $CO_2$ gas supply using a special insufflator (Aesculap, Tuttlingen, Germany) connected to the Veress needle. The muscle layer and skin layer of the abdominal wall were closed separately by silk sutures in an airtight manner.

### Experimental Models of Heart Failure

Two experimental models of heart failure were applied: high− and low−cardiac output CHF. High−cardiac output CHF was induced by surgical creation of a fistula between the abdominal aorta and the inferior vena cava as described previously.[8,9] In brief, the abdominal aorta and inferior vena cava were exposed through a midabdominal incision under pentobarbital anesthesia (60 mg/kg intraperitoneally), and an arteriovenous shunt was surgically created between the 2 vessels (side to side, 1.2 mm outer diameter). The animals were housed in metabolic cages for daily monitoring of urine output and sodium excretion. A group of sham-operated rats served as control subjects. Seven days after the operation, rats with an aortocaval fistula (ACF) were divided into 2 subgroups according to their daily absolute rate of sodium excretion ($U_{Na}V$): rats with decompensated CHF ($U_{Na}V$ 200 μEq/24 h) and rats with compensated CHF ($U_{Na}V$ 1200 μEq/24 h).

Low−cardiac output CHF due to MI was induced by left anterior descending artery (LAD) ligation. Rats were anaesthetized with a combination of 87 mg/kg ketamine and 13 mg/kg xylazine, intubated, mechanically ventilated (Small Animal Ventilator Model 683; Harvard Apparatus, Holliston, Massachusetts) at a rate of 80−90 cycles/min with a tidal volume of 1−2 mL/100 g, and the surgical procedures were performed as previously described.[10] The animals were housed in metabolic cages for daily monitoring of urine output and sodium excretion. A group of sham-operated rats served as control subjects. Because all rats with MI displayed reduced $U_{Na}V$ GFR and RPF values similar to rats with decompensated CHF induced by ACF (see results below), the MI group was considered to be equivalent to decompensated CHF.

### Impact of Increased IAP on Clearance Studies

Seven groups of rats were studied: (1) sham-operated control (n = 23); (2) ACF with compensated CHF (n = 8); (3) ACF with decompensated CHF (n = 9); (4) decompensated CHF pretreated with the PDE-I tadalafil (10 $mg \cdot kg^{-1} \cdot d^{-1}$ orally) for 4 days before the experiment (n = 6); (5) MI treated with vehicle (n = 7); (6) MI treated with tadalafil (10 $mg \cdot kg^{-1} \cdot d^{-1}$ orally; n = 11), and (7) sham control group treated with tadalafil (10 $mg \cdot kg^{-1} \cdot d^{-1}$ orally) initiated 4 days after the operation and for an additional 3−4 days (n = 6). On the day of the experiments, animals from the various groups were anaesthetized with the use of inactin (100 mg/kg intraperitoneally) and prepared for clearance studies. After tracheotomy, polyethylene tubes were inserted into the carotid artery for blood pressure monitoring, into the jugular vein for infusion of solutions, and into the urinary bladder for urine collection. A solution of 2% inulin and 0.5% p-aminohippurate (PAH) in 0.9% saline solution was continuously infused at a rate of 1.0%−1.5% of body weight per hour throughout the experiment. Mean arterial pressure (MAP) was continuously monitored with the use of a pressure transducer (model 1050.1; UFI, Morro Bay, California) connected to the carotid arterial line. After a 60-minute equilibration period, 2 baseline 30-minute clearance periods were obtained (IAP = 0). Then rats were subjected to consecutive IAP periods of 10 and 14 mm Hg, for 45 minutes each, followed by a deflation period of 1 hour (recovery); urine was sampled during each IAP. Blood samples were obtained at the end of each clearance period as illustrated in Fig. 1. The values from the 2 baseline collection periods were averaged and combined in all experimental groups.

### Renal Morphology

Rats with heart failure and the sham control rats were anesthetized and their kidneys fixed via right ventricle

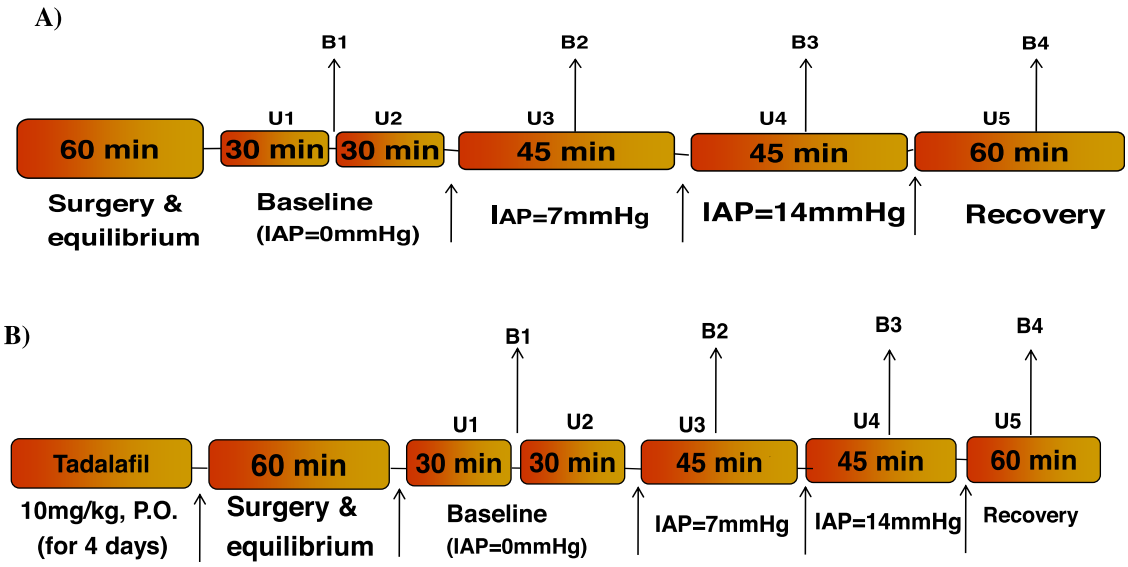


**Fig. 1.** Experimental protocol in (A) rats with compensated and decompensated congestive heart failure (CHF) induced by aortocaval fistula (ACF), rats with myocardial infarction (MI), and sham control rats and (B) rats with decompensated CHF or MI pretreated with tadalafil, a phosphodiesterase-5 inhibitor (10 $mg \cdot kg^{-1} \cdot d^{-1}$) for 4 days before the acute experiment. B1−B4, collection of blood samples; IAP, intra-abdominal pressure; U1−U5, urine collection periods.

perfusion, first with 120 mL phosphate-buffered saline solution (0.01 mol/L PBS, pH 7.4) containing heparin (5 U/mL), then with 220 mL ice-cold 4% paraformaldehyde in 0.01 mol/L PBS, pH 7.4, containing sucrose (4%). Kidneys from the different experimental groups were removed and embedded in 0.01 mol/L PBS, pH 7.4, containing sucrose (4%) and paraformaldehyde (4%). Heart and lung tissues were then progressively dehydrated in graduated alcohol concentrations (70%−100%) and embedded in paraffin. For general histomorphology, 5-μm sections were stained with hematoxylin and eosin (H&E).

### Chemical Analyses

Urine volume was determined gravimetrically. The concentrations of sodium in plasma and urine were determined with the use of a flame photometer (model IL 943; Instrumentation Laboratory, Milan Italy). Concentrations of inulin and PAH in plasma and urine were measured with the use of colorimetric methods. RPF and GFR were estimated as the infusion clearances of PAH ($C_{PAH} = U_{PAH} \times V/P_{PAH}$) and inulin ($C_{in} = U_{in} \times V/P_{in}$), respectively. Filtration fraction (FF) was calculated as the GFR-RPF ratio.

Urinary NGAL ($U_{NGAL}V$) was measured with the use of a commercially available enzyme-linked immunosorbent assay kit (NGAL Rapid ELISA Kit; Bio Porto Diagnostics, Gentofte, Denmark). Urine samples were diluted 1:20,000, added to the ELISA plate, and incubated for 30 minutes with equivalent volume of horseradish peroxidase−conjugated NGAL antibody; 100 μL 3,3′,5,5′-tetramethylbenzidine substrate was added for 15 minutes at room temperature in the dark, the reaction was stopped with the use of 100 μL stop solution, and the plate was read at 450 nm. $U_{NGAL}V$ was corrected to urinary flow and expressed as ng/mL, to $C_{in}$ and expressed as $\mu g/C_{in}$ or to urinary inulin concentration and expressed as ng/mg inulin.

### Statistical Analysis

Data are presented as mean ± SEM. $U_{NGAL}V$ was nonnormally distributed (Shapiro-Wilk *W* test) and was therefore natural logarithm transformed. The relationship between ln NGAL and IAP was analyzed with the use of repeated-measures mixed-effects linear regression models with an unstructured covariance matrix. The model included fixed factors for the experimental setup (eg, control, compensated CHF, decompensated CHF, decompensated CHF + PDE-I, MI, and MI + PDE-I), for IAP (baseline, 10 mm Hg, 14 mm Hg, recovery), and the experimental setup−IAP interaction. The Dunnett test was used for pairwise comparisons of values from different IAP periods with baseline in each group, or by Tukey test for comparison of corresponding values between the groups. A *P* value of <.05 was considered to be statistically significant. Statistical analyses were performed with the use of Stata version 13.1 (Statacorp, College Station, Texas) and Graphpad Prism Software (San Diego California).

## Results

### Effects of Incremental Increases in IAP in Normal Rats (Sham Control)

The effects of elevated IAP on kidney function and renal hemodynamics were evaluated in sham-operated rats. The experiments were carried out in rats 7 days after the operation.

Significant reductions in urine flow (V), $U_{Na}V$, GFR, and RPF were observed following IAP of 10 and 14 mm Hg. V and $U_{Na}V$ decreased from 9.3 ± 0.7 and 0.82 ±

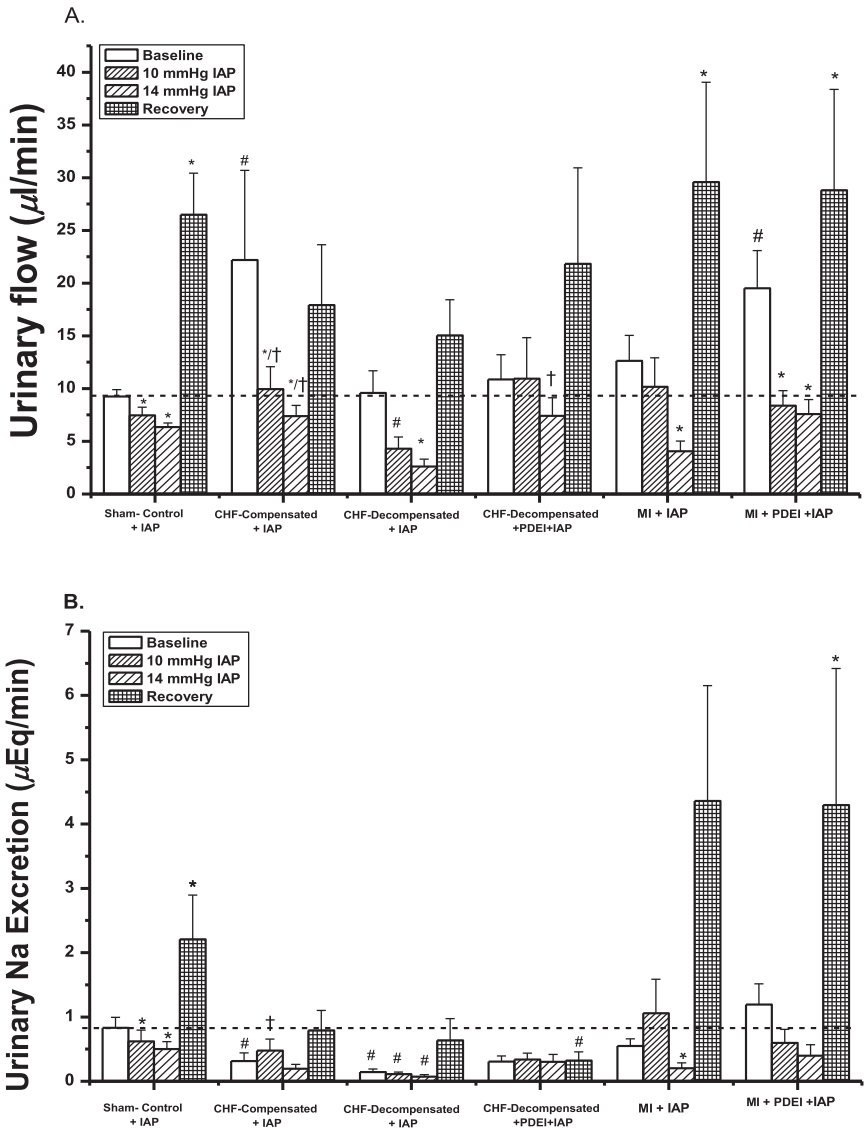


**Fig. 2.** Effects of 10 and 14 mm Hg insufflations on (A) urinary flow and (B) urinary sodium excretion in sham control, compensated and decompensated CHF, decompensated CHF pretreated with tadalafil, and MI untreated and treated with tadalafil. *$P < .05$ vs baseline; #$P < .05$ vs sham; †$P < .05$ vs untreated decompensated CHF; ‡$P < .05$ vs untreated MI + IAP subgroup.

0.17, respectively, to 7.5 ± 0.8 μL/min ($P = .0007$) and 0.62 ± 0.18 μEq/min ($P = .0271$) when IAP of 10 mm Hg was applied. The reduction in both parameters was more profound at 14 mm Hg, where V decreased to 6.4 ± 0.38 μL/min ($P < .0001$) and $U_{Na}V$ decreased to 0.51 ± 0.12 μEq/min ($P = .002$; Fig. 2). Significant declines in GFR were associated with the reduction in excretory functions; GFR decreased from 1.93 ± 0.09 to 1.30 ± 0.22 ($P < .0001$) and 0.94 ± 0.09 mL/min ($P < .0001$), respectively. Changes in GFR were equivalent to −30 ± 12% and −51 ± 5% ($P < .0001$) from baseline. In addition, RPF decreased from 7.3 ± 0.6 mL/min to 4.18 ± 0.5 ($P < .0001$) and to 2.1 ± 0.4 ($P < .0001$): −28 ± 9% and −67 ± 7% ($P < .0001$) from baseline, respectively. All parameters promptly returned to baseline values and sometimes markedly exceeded baseline values after the deflation period of 1 hour (recovery; Fig. 3).

### Experimental Aortocaval Fistula

The effects of elevated IAP on kidney function and renal hemodynamics were evaluated in high−cardiac output condition, namely rats with ACF. The experiments were carried out in these animals 7 days after the placement of fistula.

*Effects of Incremental Increases in IAP on Renal Clearance Parameters and Renal Hemodynamics in Rats With Compensated CHF.* Urinary flow was significantly higher before elevation of IAP in compensated CHF rats (22.18 ± 8.52 μL/min; $P < .0001$) compared with sham-operated rats (9.3 ± 2.2 μL/min), yet it decreased significantly when IAP increased to 10 and 14 mm Hg: to 9.94 ± 2.14 ($P = .0006$) and 7.38 ± 1.07 μL/min ($P < .0001$), respectively. On the other hand, GFR, RPF, and $U_{Na}V$ were lower in compensated rats

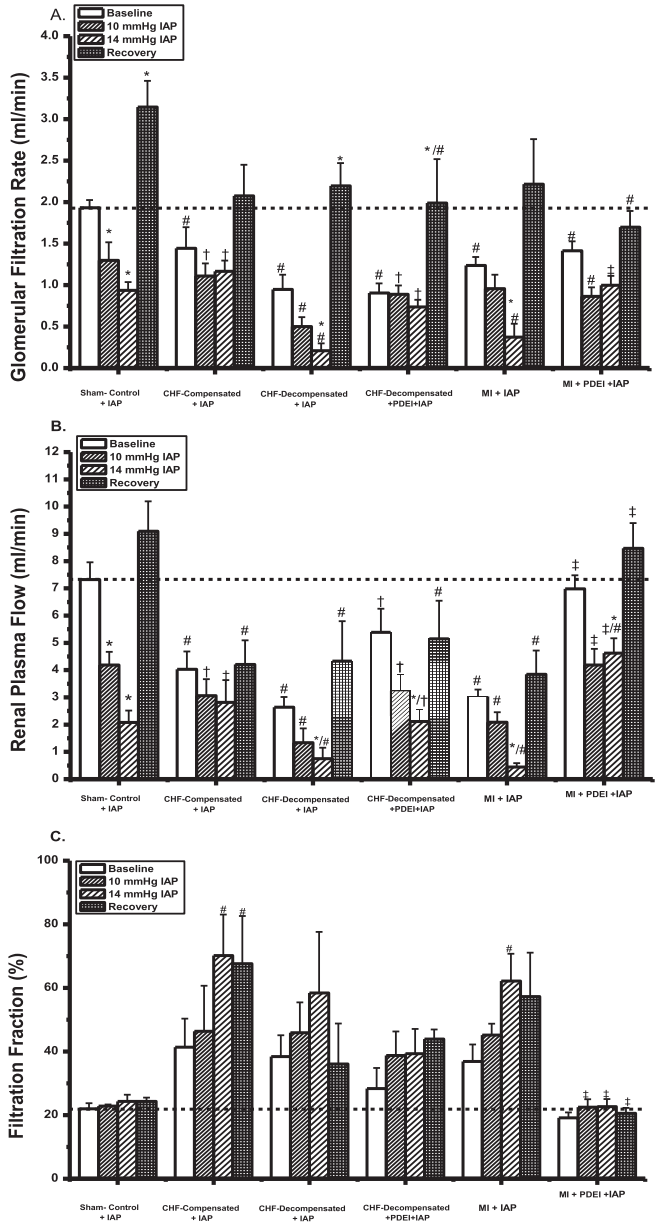


**Fig. 3.** Effects of 10 and 14 mm Hg insufflations on (A) glomerular filtration rate, (B) renal plasma flow, and (C) filtration fraction in sham control, compensated and decompensated CHF, decompensated CHF pretreated with tadalafil, and MI untreated and treated with tadalafil. *$P < .05$ vs baseline; #$P < .05$ vs sham; †$P < .05$ vs untreated decompensated CHF; ‡$P < .05$ vs untreated MI + IAP subgroup.

compared with sham control at IAP 0 (GFR: 1.4 ± 0.25 vs 1.93 ± 0.09 mL/min [$P = .0085$]; RPF: 4.2 ± 0.49 vs 7.3 ± 0.6 mL/min [$P < .0001$], $U_{Na}V$: 0.31 ± 0.12 vs 0.8 ± 0.17 μEq/min [$P = .0059$]). Interestingly, application of IAP of 10 or 14 mm Hg in compensated CHF did not significantly affect either kidney function or renal hemodynamics compared with IAP 0 (Figs. 2 and 3).

*Effects of Incremental Increases in IAP on Renal Clearance Parameters and Renal Hemodynamics in Rats With Decompensated CHF.* Basal $U_{Na}V$, GFR, and RPF were lower in rats with decompensated CHF than in sham control and compensated CHF rats: $U_{Na}V$: 0.11 ± 0.03 μEq/min ($P < .0001$ and $P = .985$, respectively); GFR: 0.95 ± 0.18 mL/min ($P < .0001$ and $P = .003$, respectively); RPF: 2.6 ± 0.5 ($P < .0001$ and $P = .0609$, respectively; Figs. 2 and 3). Application of IAP of 10 or 14 mm Hg in decompensated CHF led to decline in most parameters, which was most pronounced when the IAP was elevated to 14 mm Hg: V decreased to 2.6 ± 0.7 μL/min ($P < .0001$), GFR to 0.21 ± 0.09 ($P < .0001$) mL/min, and RPF to 0.75 ± 0.41 mL/min ($P < .0001$; Figs. 2 and 3). The adverse renal effects of elevated IAP were more profound in decompensated CHF rats than in sham control and compensated CHF rats.

*Effects of Incremental Increases in IAP on Renal Clearance Parameters and Renal Hemodynamics in Rats With Decompensated CHF Receiving Tadalafil.* This protocol

was conducted to investigate the involvement of PDE5/cGMP pathway in the vulnerability of decompensated CHF animals to elevated IAP. Treatment of decompensated CHF rats with the use of the PDE-I attenuated the reduction in V induced by IAP of 10 mm Hg (10.95 ± 3.87 vs 4.3 ± 1.1 μL/min; $P = .0196$) and 14 mm Hg (7.4 ± 1.7 and 2.6 ± 0.7 μL/min; $P = .2007$). Similarly, tadalafil treatment ameliorated the deleterious effects of IAP on $U_{Na}V$ (10 mm Hg: 0.34 ± 0.10 vs 0.11 ± 0.03 μEq/min [$P = .1096$]; 14 mm Hg: 0.30 ± 0.10 vs 0.07 ± 0.03 μEq/min [$P = .0937$]) and revoked V reduction in recovery phase ($P = .0159$; Fig. 2). Tadalafil ameliorated the decline in GFR and RPF in decompensated CHF rats subjected to 10 and 14 mm Hg IAP (GFR: 0.89 ± 0.11 and 0.74 ± 0.08 vs 0.5 ± 0.1 and 0.21 ± 0.09 mL/min ($P = .035$ and $P = .0009$, respectively), RPF: 3.24 ± 0.58 and 2.12 ± 0.44 vs 1.34 ± 0.52 and 0.75 ± 0.41 mL/min ($P = .0023$ and $P = .0675$, respectively; Fig. 3).

### Experimental Myocardial Infarction

The effects of elevated IAP were evaluated in low−cardiac output condition, namely rats with MI. The experiments were carried out in rats 7 days after LAD ligation.

*Effects of Incremental Elevation in IAP on Renal Clearance Parameters and Renal Hemodynamics in Rats With MI.* Basal $U_{Na}V$, GFR, and RPF were lower in rats with MI than in sham-control rats: $U_{Na}V$: 0.54 ± 0.11 vs 0.83 ± 0.16 μEq/min ($P = .9987$); GFR: 1.24 ± 0.10 vs 1.93 ± 0.09 mL/min ($P < .0001$); RPF: 3.03 ± 0.25 vs 7.32 ± 0.63 mL/min ($P < .0001$; Figs. 2 and 3). Increasing IAP to 14 mm Hg significantly decreased the excretory kidney function and renal hemodynamics (Figs. 2 and 3). The most profound adverse renal effect was obtained when IAP of 14 mm Hg was applied, where V decreased to 4.05 ± 0.97 μL/min ($P = .0054$), $U_{Na}V$ to 0.20 ± 0.08 μEq/min ($P < .0001$), GFR to 0.37 ± 0.16 mL/min ($P < .0001$, and RPF to 0.44 ± 0.14 mL/min ($P < .0001$; Figs. 2 and 3). These renal effects of elevated IAP were similar to those observed in rats with decompensated CHF induced by ACF.

*Effects of Incremental Elevations in IAP on Renal Clearance Parameters and Renal Hemodynamics in Rats With MI Receiving Tadalafil.* MI rats receiving tadalafil had higher basal RPF (6.98 ± 0.50 vs 3.03 ± 0.25 mL/min, $P < .0001$) and, to a lesser extent, GFR ($P = .8626$) compared with untreated MI rats. Tadalafil abolished the decline in GFR of MI rats subjected to IAP 14 mm Hg compared with MI rats without tadalafil: 0.99 ± 0.11 vs 0.37 ± 0.16 mL/min ($P < .0001$; Fig. 3). Notably, the PDE-I pretreatment abolished the decline in RPF when these rats were subjected to IAP of 10 and 14 mm Hg: 4.18 ± 0.59 and 4.62 ± 0.55 mL/min vs 2.09 ± 0.36 and 0.44 ± 0.14 ($P < .0001$, $P < .0001$, respectively; Fig. 3). In contrast to the protective effects obtained in decompensated CHF, pretreatment of MI animals with tadalafil for 4 days did not completely abolish the adverse effects of elevated IAP on $U_{Na}V$ and V (Fig. 2).

### Effect of IAP on Filtration Fraction

In the control group, there was no effect of IAP elevation on FF (Fig. 2). Compared with the control group, the overall FF increased with increasing CHF severity (mean FF 41.6% and 44.3% in the compensated and decompensated CHF, respectively, and 45.9% with MI compared with 23.4% in the control group; all $P < .001$). There was a small positive effect of IAP elevation on FF within the compensated CHF group, decompensated CHF and MI rats that was mostly not statistically significant (Fig. 3). Tadalafil pretreatment significantly attenuated the MI-induced FF elevation ($P < .0001$; Fig. 3).

### Effects of Incremental Elevation in IAP on Urinary NGAL Levels

Absolute $U_{NGAL}V$ was higher in compensated ($P = .0001$), decompensated CHF ($P = .08$), and MI ($P < .0001$) subgroups compared with sham-operated rats (Fig. 4). $U_{NGAL}V$ increased significantly in sham-operated rats when IAP increased to 10 and 14 mm Hg ($P = .0145$ and $P < .0001$, respectively). Similarly, $U_{NGAL}V$ significantly increased when these IAPs were applied to compensated rats ($P < .0001$ and $P = .0002$, respectively).

Decompensated CHF rats displayed an attenuated $U_{NGAL}V$ elevation. However, when this subgroup of animals was treated with tadalafil, $U_{NGAL}V$ increased dramatically following IAP elevation, indicating that tadalafil abolished the blunted renal excretion of NGAL characterizing untreated decompensated CHF rats (Fig. 4). $U_{NGAL}V$ levels increased dramatically in response to elevated IAP to 10 and 14 mm Hg in untreated MI rats, in a pressure-dependent manner ($P = .0119$ and $P < .0001$, respectively). Yet tadalafil exerted nephroprotective effect on the kidney in MI rats, where basal $U_{NGAL}V$ was lower than in sham-operated rats and was not affected by IAP. However, all groups subjected to IAP showed a massive increase in $U_{NGAL}V$ in the recovery phase compared with baseline (Fig. 4A). Thus, it seems that $U_{NGAL}V$ correlates with the changes in urinary flow, ie, diuresis-enhanced $U_{NGAL}V$, whereas reduced urine flow decreased the excretion of this biomarker. For this reason, we normalized the urinary excretion of NGAL to either GFR (Fig. 4B) or urinary inulin (Fig. 4C). In general, the same trend of $U_{NGAL}V$ persisted after these corrections in both sham control and compensated and decompensated CHF subgroups. However, the increase in $U_{NGAL}V/C_{in}$ reached statistical significance only in the decompensated CHF subgroup that was subject to IAP of 14 mm Hg (Fig. 2B). These findings suggest that reduced V and GFR are responsible for the lack of IAP-induced NGAL excretion in the decompensated subgroups.

The urinary NGAL levels did not correlate with GFR ($P = 0.85$) or V ($P = 0.23$) but correlated with urinary $C_{in}$ ($P < .001$). Overall, $U_{NGAL}V$ was higher in CHF and MI (main effect of the experimental group: $F(5, 233) = 4.87$; $P = 0.0003$. Compared with the control group, mean $U_{NGAL}V$ was higher in the compensated CHF ($P = 0.005$) and MI ($P = 0.04$) groups. Elevation of IAP was associated with a marked increase in $U_{NGAL}V$ (main effect of IAP: F

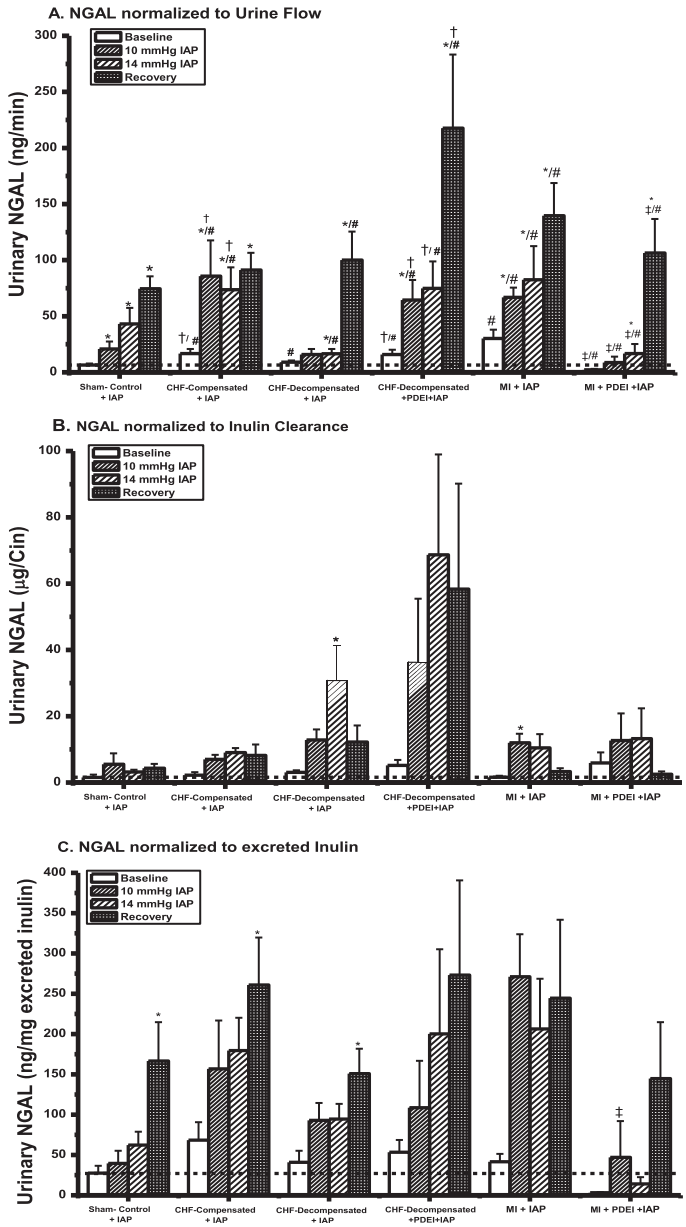


**Fig. 4.** Effects of 10 and 14 mm Hg insufflations on urinary excretion of NGAL in sham control, compensated and decompensated CHF, decompensated CHF pretreated with tadalafil, and MI untreated and treated with tadalafil. uNGAL is normalized to (A) urine flow, (B) GFR, and (C) urinary inulin. *$P < .05$ vs baseline; #$P < .05$ vs sham + IAP; †$P < .05$ vs untreated decompensated CHF; ‡$P < .05$ vs untreated MI + IAP subgroup.

(3, 233) = 6.20; $P = 0.0005$). IAP-induced increase in $U_{NGAL}V$ occurred in a dose-dependent manner in control rats and all other experimental groups (Fig. 4).

### Renal Morphologic Changes in Control Rats and Decompensated CHF Animals With and Without Tadalafil Treatment

Although the applied histologic analysis is nonquantitative, CHF rats that were not subject to elevated IAP exhibited more casts, necrosis, and congestion in the renal cortex and medulla compared with sham control rats. Increased IAP caused renal morphologic changes in both sham control and CHF rats. Specifically, enhanced necrosis and cell shedding to the tubule lumens was observed. Notably, PDE-I pretreatment restored the renal structure to normal in decompensated CHF rats and prevented kidney damage induced by increased IAP (Fig. 5). These findings parallel the obtained alterations in $U_{NGAL}V$, supporting the validity of NGAL as biomarker

## Discussion

The present study provides new insights into the interaction between the deleterious renal effects of CHF and increased IAP, and the potential mechanisms by which increased IAP impairs kidney function in heart failure. The results showed that elevated IAP aggravates renal dysfunction in 2 rat models of CHF. Moreover, pretreatment of rats with decompensated CHF with the use of tadalafil ameliorated the adverse renal effects of

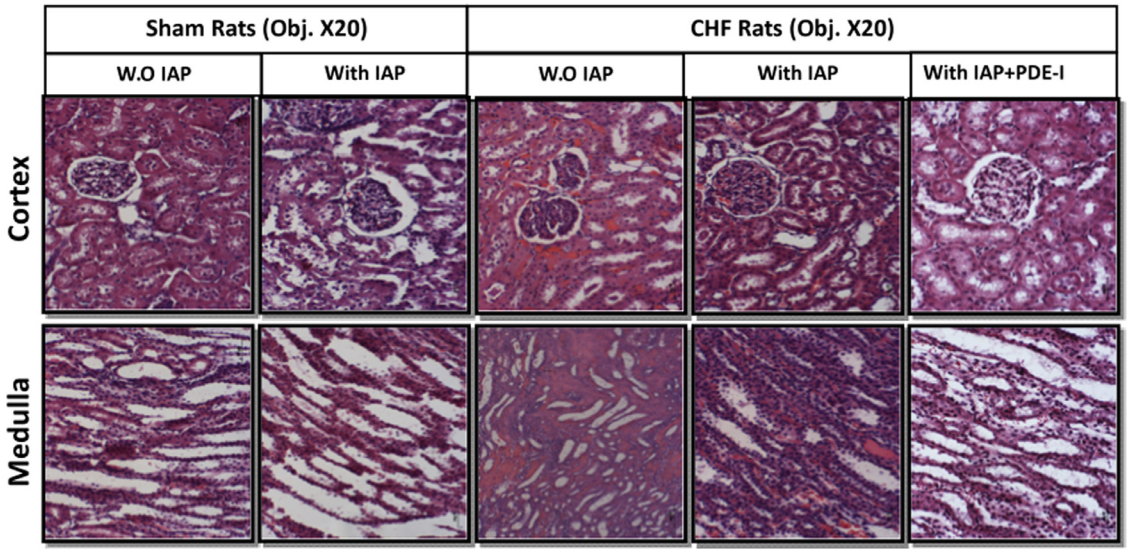


**Fig. 5.** Effects of elevated intra-abdominal pressure (IAP) on renal morphology in sham operated rats and decompensated CHF animals with and without tadalafil (PDE-I) pretreatment.

high IAP. Finally, elevated IAP also induced tubular injury, as evidenced by the increase in necrosis and cell shedding in the tubule lumens and increased urinary excretion of NGAL.

Recent data suggest that the abdominal compartment might contribute significantly to the deranged renal function in CHF.[2] Specifically, increased IAP in the range of 8−12 mm Hg, which accompanies abdominal congestion, is associated with impaired renal function.[3] The contributing mechanisms of renal dysfunction in the setting of elevated IAP are not fully understood. Poor abdominal arterial perfusion, coupled with intrarenal vascular congestion due to elevated renal vein pressure, has been proposed to explain AKI in the setting of intra-abdominal hypertension (IAH).[4,11] IAH decreases the abdominal perfusion pressure, defined as the difference between the mean arterial pressure and the IAP.[11] Previous studies have shown that renal artery blood flow diminishes in a linear fashion with increasing IAP by ~65% at 20 mm Hg, with renal arterial blood flow decreasing more than celiac or superior mesenteric artery flow.[12] Harman et al reported a >500% increase in renal vascular resistance with similar IAP elevations.[13] These observations suggest that the reduction in renal artery blood flow appears to decline disproportionally to the reduction in abdominal perfusion pressure.

One possibility is that vasoconstriction is superimposed on the direct mechanical compression of vascular beds. Specifically, pressure on the renal parenchyma and renal vein together with decreased cardiac output and systemic hormonal effects may lead to AKI. Renal vein pressure is 5 mm Hg in rats, and oliguria is seen during pneumoperitoneum >5 mm Hg.[14] Furthermore, vasoconstriction of the intrarenal vascular network may be enhanced in the setting of CHF owing to generalized neurohormonal activation. In this context, rats treated with tadalafil displayed attenuated renal dysfunction in response to elevated IAP. The observed improvement in RPF with the use of tadalafil probably represents an attenuation of a nitric oxide−mediated intrarenal vasoconstrictive response.[6] The beneficial effects of tadalafil on renal hemodynamic and kidney function was observed in CHF induced by either ACF placement or LAD ligation. Although these surgical procedures produce high and low cardiac output, respectively, both models are characterized by activation of neurohormonal systems, cardiac remodeling, and kidney dysfunction.[15] Moreover, regardless the etiology, low and high cardiac output may progress to advanced stages and eventually lead to increased IAP. Therefore, our findings are of clinical relevance and suggest PDE-5 inhibitors as potential therapeutic approach for CHF of various etiologies.

As expected in CHF,[16] FF increased in rats with CHF and MI, owing to increased efferent arteriolar tone and renin-angiotensin activation, as found in an earlier arteriovenous fistula model.[7] However, in the control group, the IAP-induced reduction in RPF was not accompanied by an increase in FF. This may suggest that no intrarenal compensation occurs in response to the IAP-induced reduction in RPF to protect glomerular filtration. In the setting of IAH, the FF may be affected by several factors. For example, the rise in renal venous pressure[11] is expected to increase FF. In contrast, the increase in proximal tubular pressure and Bowman space[5] may result in a reduction in FF. This pattern is also consistent with afferent arteriolar resistance, rather than increased efferent arteriolar resistance. The lack of compensatory increase in FF contributes to the fall in GFR with IAP-induced reduction in RPF. However, tubular injury is associated with reduced secretion of PAH. Therefore, the IAP-induced tubular damage may adversely affect the accuracy of RPF measurement and subsequently FF.

### Tubular Dysfunction in IAH

NGAL is one of the earliest and most robustly induced genes and proteins in the kidney after ischemic or nephrotoxic

injury.[17] In the 2-compartment model of NGAL trafficking originally proposed by Schmidt-Ott et al,[18,19] $U_{NGAL}V$ is proposed to derive predominantly from local renal synthesis of NGAL in the thick ascending limb of the loop of Henle and the collecting ducts in response to inflammatory and oxidative stress.[18] Under normal conditions, tagged NGAL injected into the circulation would travel to, and be captured by, renal proximal tubular endosomes without appearing in the urine in significant quantities (<0.2%), whereas locally synthesized renal NGAL is excreted into the urine and not efficiently introduced into the circulation.[18,20,21] Expression of NGAL mRNA has been shown to be induced rapidly in renal tubules, especially in distal tubular segments, in response to acute injury.[21,22]

Increased $U_{NGAL}V$ has been reported in patients with chronic[23] and acute heart failure,[24,25] indicating that a large proportion of HF patients may have some form and extent of tubular damage.[26] In the setting of stable CHF, renal tubular injury, expressed by increased levels of $U_{NGAL}V$, may be due to direct tubular damage resulting from ischemic mechanisms and often correlates with the clinical severity of CHF.[26,27] Acute tubular necrosis caused by heart failure–induced hypotension was shown to be associated with higher expression of renal NGAL.[20] The finding that tadalafil therapy mitigated the increase in $U_{NGAL}V$ in the treated MI group, suggests that in IAH tubular injury occurs secondary to renal hemodynamic compromise.

Whereas previous studies emphasized the effect of IAH on renal hemodynamics and GFR, we studied whether IAH can also lead to tubular injury. To the best of our knowledge, this study is the first to evaluate $U_{NGAL}V$ in CHF models subjected to increased IAP. We found that absolute $U_{NGAL}V$ increased significantly when IAP was elevated in sham control rats as well as compensated CHF, decompensated CHF, and MI rats in a pressure-dependent manner. In the decompensated state, absolute $U_{NGAL}V$ increase in response to IAP elevation was significantly attenuated, presumably because of a severe reduction in V and consequent inability of NGAL to be excreted in the urine. Notably, statistical significance was retained in decompensated CHF rats that were subjected to IAP of 14 mm Hg, even after adjustment of $U_{NGAL}V$ to GFR, but not when normalized to urinary inulin (Fig. 4B and C). However, it should be emphasized that even after normalizing $U_{NGAL}V$ to urinary inulin, there is clear stimulatory impact of IAP on NGAL excretion, yet it did not reach statistical significance.

Of note, tadalafil showed mixed results concerning the decrease in $U_{NGAL}V$. Although it reduced $U_{NGAL}V/U_{in}$ in the MI model that was subjected to elevated IAP, in the ACF decompensated CHF rats, tadalafil did not attenuate $U_{NGAL}V$ compared with untreated rats. The lack of $U_{NGAL}V$ attenuation with tadalafil pretreatment in the decompensated CHF rats can be attributed to the stimulatory effect of tadalafil on urinary flow, leading to increased $U_{NGAL}V$ renal washout. As shown in Fig. 4, tadalafil induces profound basal diuresis and increase in both GFR and RPF in rats with MI, but to a lesser extent in rats with decompensated CHF induced by ACF. To avoid interference of kidney function and renal hemodynamics on $U_{NGAL}V$, we expressed the latter in 3 different patterns: absolute urinary excretion rate of NGAL, normalized to urinary creatinine, and normalized to urinary inulin.

Although considered to be a reliable, sensitive, and noninvasive renal ischemia biomarker, NGAL is not specific to AKI.[28] For example, cytokines produced by infiltrated renal neutrophils after pneumoperitoneum-induced ischemia may lead to enhanced NGAL immunoreactivity in the renal tubules.[22,29] Kozan et al[30] recently demonstrated that with incremental pneumoperitoneum pressures achieved by $CO_2$ insufflation there were no differences in serum creatinine levels. However, blood urea nitrogen, cystatin C, NGAL, and interleukin (IL) 18 were significantly increased with high (12 mm Hg) but not with low IAP. Interestingly, they also showed that IL-18, also a biomarker of AKI,[31] was increased along with other inflammatory biomarkers, suggesting that pneumoperitoneum induces inflammation and subsequently higher circulatory and urinary levels of NGAL.

*Urinary NGAL in the Recovery Phase.* The prominent increase in $U_{NGAL}V$ levels at the recovery phase coincided with the increased natriuresis and urinary flow and therefore likely represents enhanced delivery of NGAL that was synthesized in the tubules rather than NGAL that escaped renal proximal tubular reabsorption. The fact that PDE-I provoked a diuretic response in association with increased $U_{NGAL}V$ also supports a "wash-out" phenomenon augmenting urinary excretion of NGAL. Presumably, NGAL may be retained in the tubules in the presence of severe renal dysfunction and therefore its urinary levels increase when renal hemodynamics improves. These findings are relevant to the interpretation of $U_{NGAL}V$ levels after effective relief of IAH via paracentesis.

*Potential Reversibility of IAP-Induced Renal Dysfunction.* We found that the adverse renal effects of elevated IAP in normal, CHF, and MI rats are reversible, as was evident by return to basal or even enhanced kidney function within 1 hour of IAP release. The reversibility of the adverse hemodynamics of elevated IAP suggest that no permanent damage to kidney function has taken place. These findings emphasize the potential reversibility of IAH-induced renal dysfunction. However, they also indicate that as long as IAP remains elevated, renal compromise is likely to persist, and only when a reduction in IAP can be achieved is renal function expected to improve. This tenet may be clinically relevant, because mechanical fluid removal is usually applied to provide symptomatic relief and alleviate discomfort or respiratory compromise mainly in patients with tense ascites, rather than to directly alter the underlying pathophysiology of the CRS. Alternatively, PDE-I may be clinically useful to alleviate the renal hemodynamic consequences of IAH. Support for this notion is derived from Mullens et al.[3] In the setting of decompensated heart failure, Mullens et al reported that among 40 patients, 24 (60%) had elevated IAP and

4 (10%) demonstrated IAH despite the absence of overt abdominal symptoms. A strong correlation ($r = 0.77$; $P < .001$) was observed between reduction in IAP and improved renal function in patients with baseline elevated IAP. In a small prospective study of diuretic-resistant ADHF patients with mild IAH, Mullens et al showed that ultrafiltration or paracentesis (if ascites was present) resulted in a significant reduction in IAP and serum creatinine, with an increase in urine output.[32] In those studies, IAH was also associated with worse baseline renal function. Of note, they found that baseline creatinine was significantly higher in patients with elevated IAP. Therefore, increased IAP can slowly and progressively impair renal function. This was also demonstrated in the present study, which also showed that the kidney in CHF is more susceptible to the adverse effects of elevated IAP. A vicious cycle may occur, in which fluid retention increases IAP, and elevated IAP in turn worsens renal function and leads to further fluid retention.

*IAP-Induced Pathologic Changes.* The sensitivity and specificity for (histologic) tubulointerstitial damage of NGAL and other renal biomarkers have shown strong sensitivity and reasonable specificity for the presence and extent of tubular damage.[23–35] The prevalence and extent of structural tubular damage due to IAH is unknown. We found that $U_{NGAL}V$ excretion increased in a pressure-dependent manner in sham-operated rats subjected to elevated IAP. These changes in $U_{NGAL}V$, together with the increase in necrosis and cell shedding into the tubule lumens in response to increased IAP, indicate an adverse impact on renal tissue integrity beyond its deleterious hemodynamic effects. Most likely, a rise in parenchymal pressure[11] may cause direct compression of tubules, as evidenced by the increased cell shedding to the tubule lumens, thus exacerbating hypoperfusion and the ischemic insults.

The present study has several limitations (see below), including the lack of molecular mechanisms for how IAP induces acute tubular injury. In addition, urine $NO_2$ and $NO_3$ levels are missing, as well as KIM-1, a marker of proximal tubule injury in the postischemic rat kidney. Furthermore, tadalafil used in this study acts by inhibiting the PDE5 enzyme but also inhibits kidney PDE11, which may affect kidney function and thus interfere with the suggested mechanism.

*Potential Mechanisms Underlying the Nephroprotective Effect of PDE-5 Inhibition.* PDE-5 inhibition may exert nephroprotection against IAP-induced kidney dysfunction and AKI via several potential mechanisms. PDE-Is have broad systemic effects due to their ability to inhibit cyclic guanosine monophosphate (GMP) breakdown, the second messenger for NO and natriuretic peptides (NPs), resulting in vasodilation.[36] We and others have shown that PDE-Is exert beneficial renal effects in an ischemia-reperfusion rat model[37,38] and for AKI after cardiopulmonary bypass in swine.[39] These beneficial effects included improvement of renal histology and function and attenuation of oxidative stress. In addition, we have demonstrated that tadalafil exerted nephroprotective effects in patients who underwent nephron-sparing surgery, as evidenced by reduced urinary excretion of sensitive biomarkers of AKI,[22,40,41] including NGAL and kidney injury molecule 1.[33] Together, the present findings are in line with the vasodilatory, antiischemic, and antioxidative effect of PDE-Is. However, the impact of tadalafil on $U_{NGAL}V$ was mixed, because the latter depends not only on the extent of tubular injury but also on urine flow.

In conclusion, our data suggest that increased IAP induces renal hypoperfusion and triggers acute tubular injury, which may contribute to the kidney dysfunction in decompensated heart failure accompanied by increased IAP. The beneficial effect of tadalafil on IAP-induced renal impairment suggests a potential therapeutic role for PDE-5 inhibition in decompensated CHF complicated by increased intra-abdominal pressure.

## Study Limitations

Our results must be regarded as hypothesis generating and exploratory; they require validation in other studies. The histologic analysis was not quantitative and should be validated by a larger study aimed at the impact of elevated IAP on histologic renal alterations.

## Disclosures

None.

## References

1. Ronco C, Maisel A. Volume overload and cardiorenal syndromes. Congest Heart Fail 2010;16(Suppl 1)):Si–iv.
2. Verbrugge FH, Dupont M, Steels P, Grieten L, Malbrain M, Tang WH, et al. Abdominal contributions to cardiorenal dysfunction in congestive heart failure. J Am Coll Cardiol 2013;62:485–95.
3. Mullens W, Abrahams Z, Skouri HN, Francis GS, Taylor DO, Starling RC, et al. Elevated intra-abdominal pressure in acute decompensated heart failure: a potential contributor to worsening renal function? J Am Coll Cardiol 2008;51:300–6.
4. de Waele JJ, de Laet I, Kirkpatrick AW, Hoste E. Intra-abdominal Hypertension and abdominal compartment syndrome. Am J Kidney Dis 2011;57:159–69.
5. Aronson D. Cardiorenal syndrome in acute decompensated heart failure. Expert Rev Cardiovasc Ther 2012;10:177–89.
6. Bishara B, Abu-Saleh N, Awad H, Ghrayeb N, Goltsman I, Aronson D, et al. Phosphodiesterase 5 inhibition protects against increased intra-abdominal pressure–induced renal dysfunction in experimental congestive heart failure. Eur J Heart Fail 2012;14:1104–11.
7. Abassi Z, Goltsman I, Karram T, Winaver J, Hoffman A. Aortocaval fistula in rat: a unique model of volume-overload congestive heart failure and cardiac hypertrophy. J Biomed Biotechnol 2011;2011:729497.
8. Abassi ZA, Gurbanov K, Mulroney SE, Potlog C, Opgenorth TJ, Hoffman A, et al. Impaired nitric oxide-mediated renal vasodilation in rats with experimental heart failure: role of angiotensin II. Circulation 1997;96:3655–64.
9. Abassi ZA, Brodsky S, Karram T, Dobkin I, Winaver J, Hoffman A. Temporal changes in natriuretic and antinatriuretic systems after closure of a large arteriovenous fistula. Cardiovasc Res 2001;51:567–76.

10. Bhindi R, Witting PK, McMahon AC, Khachigian LM, Lowe HC. Rat models of myocardial infarction. Pathogenetic insights and clinical relevance. Thromb Haemost 2006;96:602–10.
11. Mohmand H, Goldfarb S. Renal dysfunction associated with intra-abdominal hypertension and the abdominal compartment syndrome. J Am Soc Nephrol 2011;22:615–21.
12. Barnes GE, Laine GA, Giam PY, Smith EE, Granger HJ. Cardiovascular responses to elevation of intra-abdominal hydrostatic pressure. Am J Physiol 1985;248:R208–13.
13. Harman PK, Kron IL, McLachlan HD, Freedlender AE, Nolan SP. Elevated intra-abdominal pressure and renal function. Ann Surg 1982;196:594–7.
14. Kirsch AJ, Hensle TW, Chang DT, Kayton ML, Olsson CA, Sawczuk IS. Renal effects of $CO_2$ insufflation: oliguria and acute renal dysfunction in a rat pneumoperitoneum model. Urology 1994;4:453–9.
15. Numabe A, Nishikimi T, Komatsu K, Frohlich ED. Intrarenal hemodynamics in low- and high-output cardiac failure in rats. Am J Med Sci 1994;308:331–7.
16. Cody RJ, Ljungman S, Covit AB, Kubo SH, Sealey JE, Pondolfino K, et al. Regulation of glomerular filtration rate in chronic congestive heart failure patients. Kidney Int 1988;34:361–7.
17. Coca SG, Parikh CR. Urinary biomarkers for acute kidney injury: perspectives on translation. Clin J Am Soc Nephrol 2008;3:481–90.
18. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, et al. Dual action of neutrophil gelatinase-associated lipocalin. J Am Soc Nephrol 2007;18:407–13.
19. Schmidt-Ott KM, Mori K, Kalandadze A, Li JY, Paragas N, Nicholas T, et al. Neutrophil gelatinase-associated lipocalin–mediated iron traffic in kidney epithelia. Curr Opin Nephrol Hypertens 2006;15:442–9.
20. Mori K, Lee HT, Rapoport D, Drexler IR, Foster K, Yang J, et al. Endocytic delivery of lipocalin-siderophore-iron complex rescues the kidney from ischemia-reperfusion injury. J Clin Invest 2005;115:610–21.
21. Paragas N, Qiu A, Zhang Q, Samstein B, Deng SX, Schmidt-Ott KM, et al. The Ngal reporter mouse detects the response of the kidney to injury in real time. Nat Med 2011;17:216–22.
22. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, et al. Identification of neutrophil gelatinase–associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 2003;14:2534–43.
23. Damman K, van Veldhuisen DJ, Navis G, Voors AA, Hillege HL. Urinary neutrophil gelatinase associated lipocalin (NGAL), a marker of tubular damage, is increased in patients with chronic heart failure. Eur J Heart Fail 2008;10:997–1000.
24. Aghel A, Shrestha K, Mullens W, Borowski A, Tang WH. Serum neutrophil gelatinase–associated lipocalin (NGAL) in predicting worsening renal function in acute decompensated heart failure. J Card Fail 2010;16:49–54.
25. Shrestha K, Shao Z, Singh D, Dupont M, Tang WH. Relation of systemic and urinary neutrophil gelatinase-associated lipocalin levels to different aspects of impaired renal function in patients with acute decompensated heart failure. Am J Cardiol 2012;110:1329–35. https://www.ncbi.nlm.nih.gov/pubmed/22835414 .
26. Damman K, Masson S, Hillege HL, Maggioni AP, Voors AA, Opasich C, et al. Clinical outcome of renal tubular damage in chronic heart failure. Eur Heart J 2011;32:2705–12.
27. Damman K, van Veldhuisen DJ, Navis G, Vaidya VS, Smilde TD, Westenbrink BD, et al. Tubular damage in chronic systolic heart failure is associated with reduced survival independent of glomerular filtration rate. Heart 2010;96:1297–302.
28. Shyam R, Patel ML, Sachan R, Kumar S, Pushkar DK. Role of urinary neutrophil gelatinase–associated lipocalin as a biomarker of acute kidney injury in patients with circulatory shock. Indian J Crit Care Med 2017;21:740–5.
29. Lisowska-Myjak B. Serum and urinary biomarkers of acute kidney injury. Blood Purif 2010;29:357–65.
30. Kozan R, Şare M, Yılmaz TU, Yüksel S, Şeneş M, Çaycı AB, Bozkurt Ş. Effectiveness of new parameters in the evaluation of pneumoperitoneum-related acute kidney injury in rats. Turk J Med Sci 2018;48:1278–84.
31. Gan J, Zhou X. Comparison of urine neutrophil gelatinase-associated lipocalin and interleukin-18 in prediction of acute kidney injury in adults. Medicine (Baltimore) 2018;97:e12570.
32. Mullens W, Abrahams Z, Francis GS, Taylor DO, Starling RC, Tang WH. Prompt reduction in intra-abdominal pressure following large-volume mechanical fluid removal improves renal insufficiency in refractory decompensated heart failure. J Card Fail 2008;14:508–14.
33. van Timmeren MM, van den Heuvel MC, Bailly V, Bakker SJ, van Goor H, Stegeman CA. Tubular kidney injury molecule-1 (KIM-1) in human renal disease. J Pathol 2007;212:209–17.
34. Liangos O, Perianayagam MC, Vaidya VS, Han WK, Wald R, Tighiouart H, et al. Urinary N-acetyl-beta-(D)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. J Am Soc Nephrol 2007;18:904–12.
35. Ding H, He Y, Li K, Yang J, Li X, Lu R, et al. Urinary neutrophil gelatinase–associated lipocalin (NGAL) is an early biomarker for renal tubulointerstitial injury in IgA nephropathy. Clin Immunol 2007;123:227–34.
36. Reffelmann T, Kloner RA. Phosphodiesterase 5 inhibitors: are they cardioprotective? Cardiovasc Res 2009;83:204–12.
37. Sohotnik R, Nativ O, Abbasi A, Awad H, Frajewicki V, Bishara B, et al. Phosphodiesterase-5 inhibition attenuates early renal ischemia-reperfusion-induced acute kidney injury: assessment by quantitative measurement of urinary NGAL and KIM-1. Am J Physiol Renal Physiol 2013;304:F1099–104.
38. Guzeloglu M, Yalcinkaya F, Atmaca S, Bagriyanik A, Oktar S, Yuksel O, et al. The beneficial effects of tadalafil on renal ischemia-reperfusion injury in rats. Urol Int 2011;86:197–203.
39. Patel NN, Lin H, Toth T, Jones C, Ray P, Welsh GI, et al. Phosphodiesterase-5 inhibition prevents postcardiopulmonary bypass acute kidney injury in swine. Ann Thorac Surg 2011;92:2168–76.
40. Devarajan P. Review: neutrophil gelatinase-associated lipocalin: a troponin-like biomarker for human acute kidney injury. Nephrology (Carlton) 2010;15:419–28.
41. Vaidya VS, Ferguson MA, Bonventre JV. Biomarkers of acute kidney injury. Annu Rev Pharmacol Toxicol 2008;48:463–93.