Urinary ATP Synthase Subunit β Is a Novel Biomarker of Renal Mitochondrial Dysfunction in Acute Kidney Injury


*Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, South Carolina 29425, †Department of Medicine, Division of Nephrology, Medical University of South Carolina, Charleston, South Carolina 29425 and ‡Ralph H. Johnson Veterans Administration Medical Center, Charleston, South Carolina 29425

1These authors contributed equally to this study.
2To whom correspondence should be addressed at Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, 280 Calhoun St., MSC140, Charleston, SC 29425. Fax: 843-792-2620. E-mail: schnell@musc.edu.

ABSTRACT

Although the importance of mitochondrial dysfunction in acute kidney injury (AKI) has been documented, noninvasive early biomarkers of mitochondrial damage are needed. We examined urinary ATP synthase subunit β (ATPSβ) as a biomarker of renal mitochondrial dysfunction during AKI. Mice underwent sham surgery or varying degrees (5, 10, or 15 min ischemia) of ischemia/reperfusion (I/R)-induced AKI. Serum creatinine, BUN, and neutrophil gelatinase-associated lipocalin were elevated only in the 15 min I/R group at 24 h. Immunoblot analysis of urinary ATPSβ revealed two bands (full length ~52 kDa and cleaved ~25 kDa), both confirmed as ATPSβ by LC-MS/MS, that increased at 24 h in 10- and 15-min I/R groups. These changes were associated with mitochondrial dysfunction evidenced by reduced renal cortical expression of mitochondrial proteins, ATPSβ and COX1, proximal tubular oxygen consumption, and ATP. Furthermore, in the 15-min I/R group, urinary ATPSβ was elevated until 72 h before returning to baseline 144 h after reperfusion with recovery of renal function. Evaluation of urinary ATPSβ in a nonalcoholic steatohepatitis model of liver injury only revealed cleaved ATPSβ, suggesting specificity of full-length ATPSβ for renal injury. Immunoblot analyses of patient urine samples collected 36 h after cardiac surgery revealed increased urinary ATPSβ levels in patients with postcardiac surgery-induced AKI. LC-MS/MS urinalysis in human subjects with AKI confirmed increased ATPSβ. These translational studies provide evidence that ATPSβ may be a novel and sensitive urinary biomarker of renal mitochondrial dysfunction and could serve as valuable tool for the testing of potential therapies for AKI and chemical-induced nephrotoxicity.

Key words: acute kidney injury; ischemia-reperfusion; mitochondria; biomarker; ATP synthase β
Mitochondria play a major role in Ca$^{2+}$ homeostasis, reactive oxygen species generation and redox balance, cellular proliferation, and apoptosis in AKI. Mitochondrial dysfunction is also a component of many chronic diseases including diabetes, neurodegeneration, and aging (Avula et al., 2014; Che et al., 2014).

Ischemia is a common pathophysiologic mechanism in many forms of AKI. Ischemic insult occurs when there is a reduced blood flow to the kidney which may occur after drug or toxicant exposure or as a component of vascular disease, sepsis, or volume depletion and hypotension (Bonventre, 2010; Bonventre and Weinberg, 2003). Mitochondrial damage is a major contributor to the tubular cell injury observed in the initiation and progression of ischemic AKI (Bonventre, 2010; Bonventre and Weinberg, 2003; Feldkamp et al., 2005; Hall and Unwin, 2007; Jassem et al., 2002). Following restoration of blood flow after ischemia, production of reactive oxygen and nitrogen species within the mitochondria is dramatically increased. Oxidative damage to mitochondrial proteins and lipids causes dysfunction of respiratory complexes, membrane depolarization, ATP depletion, and release of apoptotic proteins contributing to cellular injury and death.

Current organ injury biomarkers do not focus on mitochondrial dysfunction, and tests for mitochondrial integrity are limited to invasive procedures including muscle biopsies, organ ATP measurements, or respiratory measurements in isolated mitochondria (Pfeffer et al., 2013). Although the importance of mitochondrial dysfunction during AKI in animals has been well documented (Funk and Schnellmann, 2013; Stallons et al., 2013, 2014; Whitaker et al., 2013), similar data in humans are sparse as the availability of renal tissue for mitochondrial analysis is limited. Consequently, there is a significant need for early, specific, and noninvasive biomarkers of mitochondrial dysfunction in AKI. Identification and validation of these markers could elucidate novel targets and mechanisms of renal mitochondrial dysfunction in AKI and enable the development of new therapies.

We have previously reported that after I/R and glycerol-induced AKI, a time-dependent loss of renal cortical mitochondrial proteins including ATP synthase subunit β (ATPβ), a component of the electron transport chain (ETC), was indicative of renal mitochondrial dysfunction (Funk and Schnellmann, 2012, 2013; Jesinkey et al., 2014; Stallons et al., 2013). Mitochondrial and renal function fail to fully recover after injury suggesting that mitochondrial function is a key component of the renal repair process. Thus, biomarkers of renal mitochondrial dysfunction may be a suitable proxy for monitoring mitochondrial and renal function in patients after AKI. In this study, we validated urinary ATPβ as a potential translational biomarker of renal mitochondrial dysfunction following AKI.

**MATERIALS AND METHODS**

Mouse renal I/R-induced AKI model. Eight-week-old male C57BL/6 mice (25–30 g) were divided into naive, sham, or I/R groups. Mice in the I/R group were subjected to bilateral renal pedicle clamping as described previously (Funk and Schnellmann, 2012; Jesinkey et al., 2014). Briefly, the renal artery and vein were isolated and blood flow was occluded with a vascular clamp for 5, 10, or 15 min. Mice were placed in metabolic cages 6 h after I/R until 24 h for 18 h urine collections. Urine was collected using a chilled collection system, and protease inhibitors were added immediately after collection to prevent protein degradation. Mice were euthanized 24 h after surgery, at which time serum was collected and kidneys harvested for biochemical and histological analyses. For time course studies, urine, serum and kidneys were collected at 72 and 144 h. Renal function was monitored by serum creatinine and BUN using kits (BioAssay Systems, Hayward, CA) per the manufacturer’s instructions. Urinary neutrophil gelatinase-associated lipocalin (NGAL) was measured by immunoblot and normalized to a standard sample. Renal tissues were fixed in 4.5% buffered formalin, dehydrated, and embedded in paraffin. For general histopathology, sections were stained with hematoxylin and eosin (H&E). All procedures involving animals were performed with approval from the IACUC in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Mouse nonalcoholic steatohepatitis model. Male C57BL/6 mice (8–9 weeks) were fed a liquid diet ad libitum containing 35% of calories from fat using corn oil with 0.5% (w/v) cholesterol for 5 months. Control mice were fed normal chow (Teklad Global 18% Protein rodent diet) containing 6.2% fat (18% of calories from fat) ad libitum. The fatty acid compositions in each diet were as follows: 16% saturated, 31% monounsaturated, 53% polyunsaturated in the nonalcoholic steatohepatitis (NASH) diet, and 32% saturated, 41% monounsaturated and 27% polyunsaturated for the control diet. Mice were placed in metabolic cages for 24 h for urine collection. Blood was collected under pentobarbital anesthesia (80 mg/kg, ip). Liver function was assessed by serum ALT determined using kits from Pointe Scientific (Lincoln Park, MI). For general histopathology, sections were stained with H&E.

**Human urine samples.** We used a subset of human urine samples from a recent study with published details of sample collection, processing, informed consent, and inclusion/exclusion criteria (Alge et al., 2013). Urine samples were obtained as part of NIDDK-funded multicenter trial (NIH no. DK080234) to identify prognostic urine markers from cardiac surgery patients who developed AKI. Protease inhibitors were added to each urine sample, and the supernatant was collected after centrifugation at 1000 × g, and aliquots were frozen at −80°C. Samples were shipped to the MUSC site on dry ice and kept frozen until use. Corresponding data including patient demographics, baseline chemistry measurements, urine collection and maximum values for serum creatinine, electrolytes, surgery type, cardiopulmonary bypass time, preexisting diseases, dialysis status, days to discharge, and mortality status were provided (Table 1). Samples were also collected from patients who had cardiac surgery but did not develop AKI. These samples were collected by the MUSC CTSA Biobank and are linked to medical record numbers, which provided subject demographic and clinical information.

**Chemicals.** Unless stated otherwise, all chemicals and biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Rabbit polyclonal anti-NGAL and mouse monoclonal anti-ATPβ were purchased from Abcam Inc. (Cambridge, MA); mouse monoclonal anti-COX1 was purchased from Life Technologies (Carlsbad, CA); and the loading control glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was obtained from Fitzgerald International Inc. (Acton, MA). Anti-rabbit and anti-mouse secondary antibodies conjugated with horseradish peroxidase were obtained from Pierce (Rockford, IL). All LC-MS/MS reagents were LC-grade pure and purchased from Waters (Milford, MA). Protein-A agarose beads used in the immunoprecipitation protocol were purchased from Roche (Indianapolis, IN).
Seahorse XF96 Extracellular Flux Analyzer as described previously (North Bellerica, MA), and basal and FCCP-uncoupled oxygen consumption in isolated tubular segments. Proximal tubules were isolated from fresh kidneys as described previously (Breggia and Himmelfarb, 2008). Briefly, renal cortical tissue was homogenized in Hanks Buffered Salt Solution (HBSS) containing collagenase (Worthington Biochemical Corp., Lakewood, NJ) and Soybean Trypsin Inhibitor (Sigma-Aldrich, St. Louis, MO). Tubules were incubated on a rocker at 37°C for 30 min. Following digestion, horse serum was added and samples were vortexed for 30 s to inactivate collagenase. Samples were centrifuged at 200 × g for 5 min, the pellet was washed with HBSS and centrifuged at 200 × g again. Tubules were resuspended in RPTC culture media (Whitaker, 2010) and further diluted in media for respirometric analysis. Tubules were transferred to an XF-96 polystyrene cell culture plate (Seahorse Biosciences, North Bellerrica, MA), and basal and FCCP-uncoupled oxygen consumption rate (OCR) were assessed in triplicate using the Seahorse XF96 Extracellular Flux Analyzer as described previously (Beeson et al., 2010). OCR was normalized to protein, assessed by BCA (Sigma-Aldrich, St. Louis, MO), using bovine serum albumin as the standard.

Renal ATP levels. ATP was isolated from fresh kidneys as described previously (Chida et al., 2012). Briefly, renal cortical tissue was homogenized in phenol-TE. DEPC water and chloroform were added and the suspension was vortexed. Samples were centrifuged at 10,000 × g for 5 min at 4°C. The upper layer was diluted 1:100 in DEPC water and ATP was measured using the ATP Determination Kit (Invitrogen, Carlsbad, CA). ATP levels were normalized to tissue wet weight.

Renal and urinary immunoblots. Immunoblots using mouse renal cortical tissue were performed as described previously (Korrapati et al., 2012, 2013). Urine samples from mice and humans were thawed and homogenized in 1 volume of protein lysis buffer (1% Triton X-100, 150 mM NaCl, 10 mM Tris-HCl, pH 7.4; 1 mM EDTA; 1 mM EGTA; 2 mM sodium orthovanadate; 0.2 mM phenylmethylsulfonyl fluoride; 1 mM HEPES, pH 7.6; 1 μg/ml leupeptin; and 1 μg/ml aprotinin) using a Polytron homogenizer. The homogenate was stored on ice for 10 min and then centrifuged at 1000 × g for 2 min at 4°C. The supernatant was collected, and total urinary protein was determined as described above. Equal amounts of protein (10 μg) were separated on 4%–20% gradient SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Membranes were blocked either in 5% dried milk (for NGAL) or BSA in TBST (for all other antibodies) and incubated with 1:1000 dilutions of anti-NGAL, anti-ATPSβ, anti-COX1, and anti-GAPDH overnight at 4°C. After incubation for 2 h at room temperature with secondary antibodies (1:2000) conjugated with horseradish peroxidase, membrane proteins were detected via chemiluminescence. Renal proteins were quantified and normalized with GAPDH. Urinary proteins were quantified and normalized to a standard sample and corrected to total urinary protein.

ATPSβ immunoprecipitation. Immunoprecipitation was performed according to the method described by Abcam Inc. (Cambridge, MA). Briefly, mouse urine samples from the 15 min I/R (severe injury) group were homogenized in lysis buffer with protease inhibitor cocktail and then centrifuged to remove cell debris. Lysates were preclarified to reduce nonspecific binding during the immunoprecipitation. Briefly, a non-ATPSβ mouse IgG1 antibody (50 μl) was added to urine lysates and incubated on ice for 1 h. Protein-A agarose bead slurry (100 μl) was added, and samples were incubated for 30 min at 4°C with gentle agitation. Samples were centrifuged and the supernatant saved for immunoprecipitation. Protein concentration of the preclarified supernatant was determined by BCA as described above. A 500-μl aliquot of preclarified protein was incubated with 10 μg mouse monoclonal anti-ATPSβ (Abcam Inc., Cambridge, MA) overnight at 4°C with gentle agitation. Protein complexes were mixed with protein-A agarose bead slurry (100–100 μl) on ice and incubated overnight at 4°C under rotary agitation. After incubation, samples were centrifuged, and the supernatant was collected and washed in lysis buffer three times via centrifugation at 4°C. The final supernatant was removed and 25–50 μl of 2x loading buffer was added. Samples were boiled at 95–100°C for 5 min, centrifuged, and the supernatant was separated by SDS-PAGE. The gel was stained with Coomasie blue, and bands were excised for LC-MS/MS-based peptide identification as described previously (Ball et al., 2006).

Urinary ATPSβ identification with LC-MS/MS. Frozen urine aliquots were thawed at 37°C for 10 min and centrifuged for 10 min at 1000 × g and 4°C. Total urinary protein and creatinine values were measured. Sample volume used for trypsin digestion and subsequent proteomic analysis was calculated by normalizing total urinary protein to both urine volume and urine creatinine to eliminate biological variability. LC-MS/MS analysis including ATPSβ peptide identification and normalization of spectral counts with internal standard HIV gp160 protein for each sample was performed as described previously (Alge et al., 2013; Korrapati et al., 2012).

Data and statistical analysis. Data are expressed as means ± SEM for all experiments. Multiple comparisons of normally distributed data were performed by one-way ANOVA, as appropriate, and group means were compared using a Student-Newman-Keuls post-hoc test. Single comparisons were analyzed using the Student’s t-test where appropriate. The criterion for statistical differences was P < 0.05 for all comparisons.
RESULTS

Urinary ATPSβ correlates with the degree of renal injury following I/R-induced AKI in mice. Mice were subjected to sham or I/R surgery by bilateral renal pedicle ligation for 5, 10, or 15 min corresponding to mild, moderate, and severe injury, respectively. BUN and serum creatinine were increased only in mice with severe injury at 24 h after reperfusion (Figs. 1A and B). Correlated with renal functional loss, mice in the severe injury group exhibited extensive proximal tubular necrosis throughout the corticomedullary region characterized by eosinophilic tubules with karyolytic nuclei (Supplementary fig. S1). No histological renal damage was observed in mice after sham or mild I/R injury, whereas minimal proximal tubular vacuolization was observed in moderately injured mouse kidneys (Supplementary fig. S1). Urinary NGAL, a renal tubular damage biomarker, was detected in severely injured mice only at 24 h (Fig. 1C). Thus, this model demonstrates three distinct groups for evaluation with varying degrees of renal injury and functional loss.

Using these groups, we evaluated urinary ATPSβ as a biomarker of renal mitochondrial dysfunction during I/R-induced AKI and correlated levels to the severity of renal dysfunction. We identified full length (~52 kDa) and cleaved fragments (~25 kDa) of urinary ATPSβ protein via immunoblot (Fig. 1D). Normalized to total urinary protein, full length ATPSβ increased in mice after moderate and severe injury (Fig. 1D). When normalized to total protein, expression of the cleaved fragment increased in mice only after severe injury (Fig. 1E). The proposed full length and cleaved fragment of ATPSβ were immunoprecipitated from urinary protein isolates (Fig. 1G) and fragments were confirmed to be ATPSβ by LC-MS/MS analysis via identification of the peptide sequence VVDLLAPYAK, which is specific to the ATPSβ N-terminal region.

Increases in urinary ATPSβ are associated with renal mitochondrial protein loss and reduced mitochondrial function. We recently reported that renal mitochondrial disruption, including ATPSβ loss, persists for at least 6 days after I/R-induced AKI (Funk and Schnellmann, 2012, 2013; Jesinkey et al., 2014; Stallons et al., 2013). Confirming that renal cortical mitochondrial proteins decreased in conjunction with increased urinary ATPSβ, we observed that renal COX1 decreased following moderate or severe injury, whereas ATPSβ decreased in the severe injury group at 24 h (Figs. 2A–C). Thus, moderate or severe renal damage is associated with the loss of renal cortical COX1 and ATPSβ and increased urinary ATPSβ.

The loss of mitochondrial proteins has been reported to cause a reduction in mitochondrial function (Jesinkey et al., 2014; Nath et al., 1998). Mitochondrial function was evaluated by assessment of basal and FCCP-uncoupled OCR in isolated proximal tubular segments. FCCP-uncoupled OCR, a mitochondrial stress test, was reduced 50% in mild, moderate, and severe injury groups at 24 h compared with sham controls (Fig. 3A). Basal respiration was not reduced in any group. To evaluate whether the observed decreases in OCR manifest as reduced renal energy content, renal cortical ATP levels were measured. ATP levels were reduced 30–40% following mild, moderate, or severe I/R-induced AKI (Fig. 3B). These multiple mitochondrial...
markers demonstrate renal mitochondrial dysfunction in conjunction with increased urinary ATPSβ.

Urinary ATPSβ levels are a marker of renal functional recovery after I/R-induced AKI. We then examined urinary ATPSβ over time after AKI. Urinary full-length and cleaved ATPSβ remained elevated 72 h after severe injury and returned to baseline at 144 h (Figs. 4A–C). Renal function partially recovers 144 h after severe renal injury in this model (Funk and Schnellmann, 2012, 2013; Jesinkey et al., 2014; Stallons et al., 2013) and the return of ATPSβ to baseline is consistent with this partial recovery of renal function.

Urinary full-length ATPSβ is specific for renal mitochondrial dysfunction. ATPSβ is a ubiquitously expressed component of the ETC; thus extra-renal sources of ATPSβ in the urine cannot be excluded. To assess the specificity of urinary ATPSβ for renal mitochondrial dysfunction, we evaluated its presence in the urine of mice with NASH. Mice exhibited liver damage and dysfunction evidenced by histological changes (ie, fat accumulation) and a rise of serum ALT (Figs. 5A and B). No change in serum creatinine was observed suggesting that renal function was not disrupted (Fig. 5C). Urinary immunoblot analysis detected the cleaved ATPSβ fragment; however, no full-length protein was detected (Fig. 5D). These data provide evidence that
the full-length fragment may specifically arise from renal mitochondrial dysfunction, whereas the cleaved fragment may arise from renal or nonrenal tissues (ie, liver).

**Urinary ATPSβ levels are increased in human patients that developed postcardiac surgery AKI.** To assess ATPSβ as a renal mitochondrial dysfunction biomarker in humans, we analyzed urine collected from patients 36 h after cardiac surgery who either developed AKI or did not. Patient demographic and clinical parameters were collected (Table 1). Baseline and postsurgical renal function were evaluated by serum creatinine and patients who developed AKI showed a 2-fold increase in serum creatinine over baseline indicating severe injury (Fig. 6D). We identified full length (~52 kDa) and cleaved fragments (~25 kDa) of urinary ATPSβ protein in patients who developed AKI (Fig. 6A). Normalization of full length and cleaved urinary ATPSβ to total protein revealed increased full-length ATPSβ in AKI patients (Fig. 6B), but no changes were observed in cleaved ATPSβ levels (Fig. 6C). To validate immunoblot results, we conducted LC-MS/MS-analysis which confirmed increases in the same unique peptide (VVDLLAPYAK) identified in mouse urine isolates (Fig. 6E).

**DISCUSSION**

Mitochondria have been characterized as central mediators of the pathophysiology of AKI resulting from a variety of insults including drug/toxicant exposure, ischemia-reperfusion injury, and sepsis (Avula et al., 2014; Brooks et al., 2009; Che et al., 2014; Feldkamp et al., 2005; Hall and Unwin, 2007; Jassem et al., 2002). Persistent disruption of mitochondrial dynamics, function, and biogenesis has been well documented in animal models of renal injury (Funk and Schnellmann, 2012, 2013; Jesinkey et al., 2014; Stallons et al., 2013, 2014; Whitaker et al., 2013). However, because of the limitations in the availability of renal tissue, little is known about the role of mitochondrial dysfunction in human AKI. Therefore, noninvasive biomarkers of renal mitochondrial dysfunction are needed to enhance our understanding of the role of mitochondria in the initiation and recovery from AKI.
this end, we examined urinary levels of ATPSβ as a potential biomarker of renal mitochondrial dysfunction in a mouse model of I/R-induced AKI and in humans who developed AKI following cardiac surgery.

Mice underwent varying degrees of renal ischemia (5, 10, or 15 min) followed by reperfusion. These groups were characterized as representative of mild, moderate, and severe renal injury by renal histology and by markers of renal function and damage including serum creatinine, BUN, and renal NGAL expression (Figs. 1A–C; Supplementary fig. S1). Immunoblot analysis demonstrated a full-length and cleaved form of ATPSβ in the urine of mice following moderate or severe renal injury (Figs. 1D–F). The observed increase in urinary ATPSβ after severe injury may arise from necrosis and epithelial cell sloughing into the tubular lumen due to profound renal injury consistent with observed increases in urinary NGAL (Bonventre, 2010; Bonventre and Weinberg, 2003). Interestingly, full-length urinary ATPSβ was also elevated after moderate injury in the absence of increases in serum creatinine and urinary NGAL, indicating mitochondrial dysfunction prior to renal dysfunction (Figs. 1A–C; Supplementary fig. S1). Thus, full-length urinary ATPSβ increases with moderate and severe renal damage and this increase precedes elevations of other renal injury markers. In contrast, urinary cleaved ATPSβ increased only after severe injury. Although immunoblot analysis is appropriate for biomarker discovery, use of this method for quantification of urinary proteins is neither as sensitive nor quantitative as ELISA, thus we are likely missing changes in urinary ATPSβ.

At 52–56 kDa, ATPSβ falls around the cutoff for glomerular filtration. Thus, ATPSβ may be filtered, and extra-renal sources cannot be excluded. However, the potential that urinary ATPSβ originates from an extra-renal source during AKI is low as levels are similarly elevated after moderate and severe injury despite significant differences in creatinine and NGAL levels between these groups (Fig. 1B). These results provide evidence that sublethal damage to mitochondria in the absence of necrotic cell death may result in the release of ATPSβ; however, the exact mechanism of release is still unclear.

To validate renal specificity, we measured urinary ATPSβ in a mouse model of NASH that exhibited liver injury without an increase in serum creatinine. We detected no full-length ATPSβ in the urine from these mice; however, the cleaved fragment was detected (Fig. 5). These data support the idea that full-length ATPSβ is a specific marker for renal mitochondrial damage, whereas the cleaved fragment may arise from renal and extra-renal sources. We hypothesize that the cleaved fragment is freely filtered due to its small size, whereas full-length ATPSβ is not filtered as it is near the size exclusion limit for filtration, and it carries a significant negative charge at physiological pH. Our data from cardiac surgery patients also support this hypothesis, as we observed both the cleaved fragment in AKI and no AKI samples, whereas only full-length ATPSβ increased in AKI samples (Fig. 6). The cleaved fragment may be of renal or extra-renal origin in these patients (heart, liver, vasculature, etc.).

We have previously demonstrated a time-dependent decrease in renal cortical mitochondrial protein expression following various forms of AKI in rodent models. In this study, we observed a decrease in renal cortical expression of COX1 24 h after reperfusion in mice with moderate or severe renal injury and ATPSβ in mice with severe injury (Fig. 2). Furthermore, mitochondrial function was compromised 24 h after mild, moderate, or severe I/R injury as assessed by isolated proximal tubule OCR and renal cortical ATP (Fig. 3). Reduction of renal mitochondrial function appears to precede the loss of renal mitochondrial proteins and elevations in urinary ATPSβ in this model, although this may be due the sensitivity of immunoblot analysis. Overall, these results confirm renal mitochondrial dysfunction occurs with increased urinary ATPSβ.

Evaluation of urinary ATPSβ levels at 72 and 144 h after I/R in mice revealed a recovery of ATPSβ levels to near control levels at 144 h after injury (Fig. 4). This recovery mirrors the partial recovery of renal function as measured by serum creatinine in this model indicating that urinary ATPSβ may serve as a marker of renal functional recovery (Funk and Schnellmann, 2012; Jesinkey et al., 2014). Interestingly, the recovery of ATPSβ...
precedes the recovery of renal cortical expression of mitochondrial proteins observed in this model. We postulate that this result may be due to either (1) the remaining mitochondria are functionally intact and are not shedding mitochondrial proteins or (2) the severe reduction in mitochondrial number, evidenced by reduced renal cortical expression, creates an artificially low expression of shed mitochondrial proteins in the urine.

To examine the translational potential of urinary ATPSβ, we measured levels in the urine of human patients 36 h after cardiac surgery (Fig. 6). Urinary excretion of ATPSβ increased in patients that developed AKI following surgery when compared with those that did not. These human data suggest that increased urinary ATPSβ in AKI patients predicts renal mitochondrial dysfunction compared with subjects without AKI. Additional analyses are needed to correlate changes in ATPSβ with other renal functional markers and patient outcomes. A large group of well-characterized samples in subjects with mild to moderate AKI, and AKI from other etiologies will enable us to elucidate confounding variables and enhance the predictive power of ATPSβ in human AKI. However, as human renal cortical samples (eg, biopsies) are not readily available, it will be difficult to directly link urinary ATPSβ with renal mitochondrial function.

**FIG. 6.** Urinary ATP synthase subunit β (ATPSβ) is elevated in human patients following cardiac surgery-induced AKI. ATPSβ protein expression was measured via immunoblot in urine collected 36 h after cardiac surgery in patient with either no AKI or AKI (A). The full length and cleaved fragments were quantified via densitometry (B, C) and normalized to total urinary protein. Renal function was assessed by serum creatinine (D). LC-MS/MS analysis from urine of AKI patients after cardiac surgery revealed a fragmentation pattern consistent with the tryptic peptide VVDLLAPYAK, unique for human ATPSβ (E). Data are expressed as mean ± SE (n = 16). *Different from no-AKI controls (P < 0.05).
These studies provide evidence that urinary ATPSβ increases in mice subjected to I/R-induced AKI and that this increase correlates with renal mitochondrial dysfunction. Furthermore, urinary ATPSβ has translational potential for detection of renal mitochondrial dysfunction in postoperative AKI in humans. There are no current reports of noninvasive biomarkers of renal mitochondrial dysfunction after AKI with the exception of urinary cytochrome c in drug-induced AKI (Small and Gobe, 2012). However, use of cytochrome c is limited due to rapid, transient changes in tissue expression and poor renal specificity. Thus, our studies offer evidence that urinary full-length ATPSβ may be the first sensitive and specific translational biomarker of renal mitochondrial dysfunction in AKI. However, additional validation is needed to ascertain its preclinical and clinical applicability. Characterization of ATPSβ as a biomarker of renal mitochondrial disruption and the roles of miRNAs and proteolytic/autophagic pathways in the regulation of ATPSβ and renal mitochondrial function in AKI will assist in the development of new therapeutic targets.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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