# Renal gene expression in *Squalus acanthias* following hyposmotic stress

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Reabsorption of urea from shark renal filtrate minimizes excretion of this important osmolyte. One important transporter involved in this process is the urea transporter ShUT that is expressed in bundle tubules[1]. Urea reabsorption via ShUT is likely driven by countercurrent transport although the precise mechanisms are unknown. Experimental interventions that alter renal blood and tissue urea content (eg. hyperosmotic and hyposmotic stress) may induce differentially-regulated expression of fluid and solute transport pathways and regulatory factors in the kidney. A case in point: exposure to dilute seawater has previously been shown to increase GFR, urine flow, and urea excretion in the skate kidney[2, 3], and to reduce gene expression of the homologous skate urea transporter, SkUT, in skate kidney homogenates[4].

For much of its length, the nephron of marine elasmobranchs lies in the venous portal system (mesial zone); however, a relatively short length lies within a closed peritubular sheath (bundle zone). Capillary-like vessels and five structurally- and functionally-defined nephron segments forming 2 hairpin loops exist within the bundle zone. This arrangement of nephron segments and vessels resembles the renal countercurrent architecture of worms, leeches, birds, and mammals[3, 5]. Studies of countercurrent transport in all these animals – and particularly from intense investigations in mammalian systems – indicate that multiple membrane transport pathways in contiguous epithelia and endothelia play unified roles to accomplish countercurrent transport.

One of our goals is to establish a method for quantitating gene expression in isolated shark tubules, and then to compare expression levels of genes encoding membrane transporters and regulatory proteins following experimental intervention. This information can then be used to formulate potential models of countercurrent transport. In this study we have used conventional and real-time RT-PCR to assess relative quantities of transport-related genes expressed in kidney homogenates following hyposmotic stress.

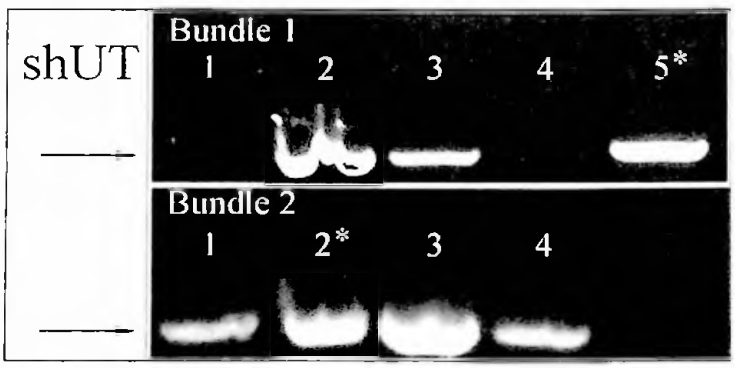


**Figure 1. Expression of shUT (498 bp) in 4 of the 5 different tubule segments present in two separate bundles from a single shark kidney. Early distal tubules are identified with an asterisk. Remaining segments were not identified.**

Effects of hyposmotic stress were tested through gradual dilution of seawater. Freshwater was added to a 4 ft. diameter tank containing continuous-flow, 100% seawater so that sharks experienced 2 days at ~85% seawater, 4 days at ~80% seawater, and 1 day at ~75% seawater. Animals were sacrificed on day 7, kidneys were removed, and tissue was processed or stored at –80° C. Tissue urea was determined by the method of Rahmatullah and Boyde[6]. Reverse transcription (RT) of messenger RNA and amplification of first strand cDNA by conventional polymerase chain reaction (PCR) were carried out as previously reported[1]. Real time RT-PCR was carried out with SYBR-green reporter (Stratagene reagents and MX4000 instrumentation). The reference dye ROX was used to normalize reporter dye signals. Target gene expression levels in each sample were normalized to β-actin[7], whose expression appeared unaffected by seawater dilution. In order to control for genomic contamination, reverse transcriptase was omitted during the cDNA synthesis step in additional reactions and these

showed no reaction product. Following amplification, dissociation plots showed all primer pairs produced single amplification peaks.

Analysis of gene expression in 6 kidneys with conventional RT-PCR showed the early distal tubule (diluting segment) and several additional bundle segments exhibit strong shUT expression. Expression of shUT in multiple tubule segments obtained from each of two separate bundles from a single animal is shown in Fig. 1. Interestingly, the urea transporter from the dogfish *Triakis scyllia* (87.5% protein homology) has been immunolocalized only to a single bundle segment, the bundle collecting tubule[8]. We observed no ShUT expression in mesial segments adjacent to bundles.

**Table 1. Renal tissue urea content and renal expression levels* of ShUT, NKCC1, and PLMS for sharks immersed in 100% or 75% seawater.**

| | Tissue Urea Content (mmoles/kg wet wt) | ShUT | NKCC1 | PLMS |
|---|---|---|---|---|
| **100% seawater** | 303.2 ± 4.8 | 1.00 | 1.00 | 1.00 |
| **75% seawater** | 267.2 ± 8.8† | 1.43 ± 0.67 | 1.41 ± 0.39 | 0.97 ± 0.40 |

*Relative gene expression levels reported as fold-change compared to 100% seawater (mean ± se, N = 3). No significant changes were observed.

† Significantly different from 100% seawater, Student's 2 sample t-test, $P < 0.05$. N = 3 for each treatment.

We investigated tissue urea levels and expression levels of shUT, NKCC1, and shark phospholemman (PLMS, a putative regulator of Na-K-ATPase) in kidneys from sharks immersed in 100% seawater or 75% seawater (Table 1) as described above. A significant decrease in urea content was observed for animals in 75% seawater. A small but statistically insignificant increase was observed in whole-kidney expression levels for shUT and NKCC1 expression, and no change was observed for PLMS expression. This contrasts with a near 3-fold decrease in renal skUT following immersion of skates in 50% seawater for 5 days[4]. An absence of change in shark renal shUT expression with 75% seawater would suggest that non-genomic regulation of urea reabsorption may exist. In order to more fully understand elasmobranch countercurrent transport, it will be informative to assess expression of these and other transporters in individual tubule segments, as well as the nephron permeabilities for urea, NaCl, and water, before and following variable degrees of osmotic stress.

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