

Research Article

## Circadian changes in urinary $\text{Na}^+/\text{K}^+$ ratio in humans: is there a role for aldosterone?

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**Keywords:**

$\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Na}^+/\text{K}^+$  ratio, aldosterone, 12h-day, 12h-night, urine

**ABSTRACT**

**Background:** There are indications that the renal excretion of  $\text{Na}^+$  and  $\text{K}^+$  is affected by the body's circadian rhythm. Aldosterone is known to be the major determinant of urinary  $\text{Na}^+/\text{K}^+$  ratio. However, recent reports suggest that the circadian rhythm of  $\text{K}^+$  excretion does not depend on endogenous aldosterone. We therefore aimed to investigate the diurnal and nocturnal changes in urinary  $\text{Na}^+/\text{K}^+$  ratio, and to test if aldosterone plays a key role. **Methods:** We investigated the  $\text{Na}^+/\text{K}^+$  ratios and aldosterone excretion in 12h-day and night urine. Ethical approval was obtained for 24 healthy male subjects, aged 20-30 years, who were included in this study. 12h-day and 12h-night urine samples were collected. Urine concentrations of  $\text{Na}^+$  and  $\text{K}^+$  were analysed using flame photometry and the amount in mmol of these electrolytes was calculated. Urine aldosterone concentrations were analysed using the enzyme immunoassay method. Urinary  $\text{Na}^+/\text{K}^+$  ratios were calculated by dividing the amount of  $\text{Na}^+$  by that of  $\text{K}^+$ , both in mmol/12h. Results are presented as mean  $\pm$  SEM, and analysed using unpaired Student's t-test. **Results:** While a significantly higher  $\text{Na}^+/\text{K}^+$  ratio was observed in the 12h-night urine compared with the 12h-day urine ( $4.22 \pm 0.18$  vs  $2.91 \pm 0.18$ ,  $p < 0.001$ ,  $n=24$ ), aldosterone excretion ( $\mu\text{g}/12\text{h}$ ) was similar in both the day and night urine. The significantly increased  $\text{Na}^+/\text{K}^+$  ratio in the nighttime urine observed in this study was shown to be as a result of a significant decrease ( $p < 0.001$ ) in  $\text{K}^+$  excretion at night. Correlation analysis revealed no significant relationships between aldosterone and the  $\text{Na}^+/\text{K}^+$  ratio in both the day and night urine. **Conclusion:** Our results suggest that an aldosterone independent mechanism may be responsible for the night time dip in the renal excretion of  $\text{K}^+$ . Understanding this mechanism will provide more insights into how this pathway may be targeted in hypertension caused by non-dipping night time renal  $\text{K}^+$  excretion.

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### INTRODUCTION<sup>1</sup>

Urinary  $\text{Na}^+/\text{K}^+$  ratio represent the mechanistic relationship which exist between the renal excretion of  $\text{Na}^+$  and  $\text{K}^+$ .  $\text{Na}^+$  and  $\text{K}^+$  are arguably the two most important electrolytes in the body due to the relative importance of their functions.  $\text{Na}^+$  play key roles in solute transport and in the activities of excitable and contractile tissues, with  $\text{K}^+$  particularly contributing to vaso-relaxation. The overall regulation of arterial blood

pressure depends on how well the kidneys regulate the excretion of  $\text{Na}^+$  and  $\text{K}^+$  in urine.

There are indications that the excretion of  $\text{Na}^+$  and  $\text{K}^+$  into urine is affected by the body's circadian rhythm. Since the control of  $\text{Na}^+$  excretion by the kidneys is critically important for maintaining blood pressure (Firsov et al., 2012), it is plausible that the circadian clock mechanism contributes to the maintenance of blood pressure. Recent evidence suggests that the circadian clock plays significant role in the control of arterial blood pressure (Nikolaeva et al., 2012). Blood pressure has been reported to follow a circadian rhythm with about 10 – 15% lower values observed at night compared with the daytime values (Bankir et al., 2008). This nocturnal dipping in blood pressure may be as a result of the circadian rhythm, which exists in the renal handling of  $\text{Na}^+$  and  $\text{K}^+$ .

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Furthermore, urinary Na<sup>+</sup>/K<sup>+</sup> ratio is known to be the primary indicator of plasma aldosterone activity due to its role in the renal control of Na<sup>+</sup> and K<sup>+</sup> excretion (Wiederholt et al., 1972). Aldosterone is the major regulator of Na<sup>+</sup> reabsorption and K<sup>+</sup> secretion in the distal nephron. In response to changes in extracellular Na<sup>+</sup> and K<sup>+</sup> fluctuations, aldosterone is released from the zona glomerulosa of the adrenal cortex and its actions in the distal nephron result in the increase in the membrane expression and conductance of the epithelial Na<sup>+</sup> channels (Rossier et al., 2002). There is evidence that the circadian clock controls plasma aldosterone levels (Doi et al., 2009), which could be responsible for the diurnal and nocturnal changes in the renal excretion of Na<sup>+</sup> and K<sup>+</sup>. However, previous reports have shown that the circadian rhythm of K<sup>+</sup> excretion does not depend on endogenous aldosterone (Rabinowitz et al., 1986; Rabinowitz et al., 1996; Firsov and Bonny, 2010). The aim of this study was to investigate the diurnal and nocturnal changes in urinary Na<sup>+</sup>/K<sup>+</sup> ratios and to test if any relationships exist between urinary aldosterone and Na<sup>+</sup>/K<sup>+</sup> ratios.

Understanding the nocturnal and diurnal changes in the renal excretion of Na<sup>+</sup> and K<sup>+</sup> will provide more insights into how these excretory patterns may be targeted in the control of hypertension. Our results confirm the well-established dip in urinary K<sup>+</sup> excretion at night, which is shown to be responsible for the increased nighttime urinary Na<sup>+</sup>/K<sup>+</sup> ratio demonstrated in this study. Our findings also suggest that aldosterone may not be responsible for the diurnal and nocturnal fluctuations in urinary Na<sup>+</sup>/K<sup>+</sup> ratios.

## METHODS

### *Subjects*

Subjects were recruited from the University of Benin community. After detailed explanation of the experimental procedure, all subjects gave their consent to participate in this study. Only subjects that were not on any medications, have not consumed alcohol three days prior to the day of experiment and were without any history of renal or cardiovascular diseases were included in this study. All subjects that did not fulfil these criteria were excluded from the study. Preliminary examination of the fractional excretion of Na<sup>+</sup> and K<sup>+</sup> in urine was carried out, and all Twenty-four healthy male subjects, aged 20 – 30 years, included in this study had normal renal function. All subjects reported to the Physiology Laboratory of the University of Benin on the same day, and urine collection was carried out for 24 hours. All protocols employed in this study comply with the guidelines and regulations of the College of Medical Sciences Ethical Committee, University of Benin.

### *Protocol*

All subjects reported to the laboratory by 7am on the day of experiment. After emptying their bladder, urine was collected as follows: 7am – 7pm (12h-day) urine and 7pm – 7am (12h-night) urine. The duration of the experiment was 24 hours, equally divided into day and night periods.

### *Urine biochemistry*

Urine Na<sup>+</sup> and K<sup>+</sup> concentrations were measured using the flame atomic absorption spectrophotometry (Toffaletti and Jones, 1992). Urine aldosterone was analysed using the enzyme immunoassay method (DRG international Inc., USA) according to manufacturer's instructions. The volume of urine collected from each subjects was measured using a measuring cylinder, calibrated in litres.

### *Statistical analysis*

Data are presented as means ± SEM. Graphs and statistical analysis were carried out using GraphPad Prism 5.0 software. Unpaired Student's t-test was used to compare results between the test groups and p-values less than 0.05 (p<0.05) was considered significant. Correlation analysis was used to test if any relationships exist between urinary aldosterone and the Na<sup>+</sup>/K<sup>+</sup> ratios.

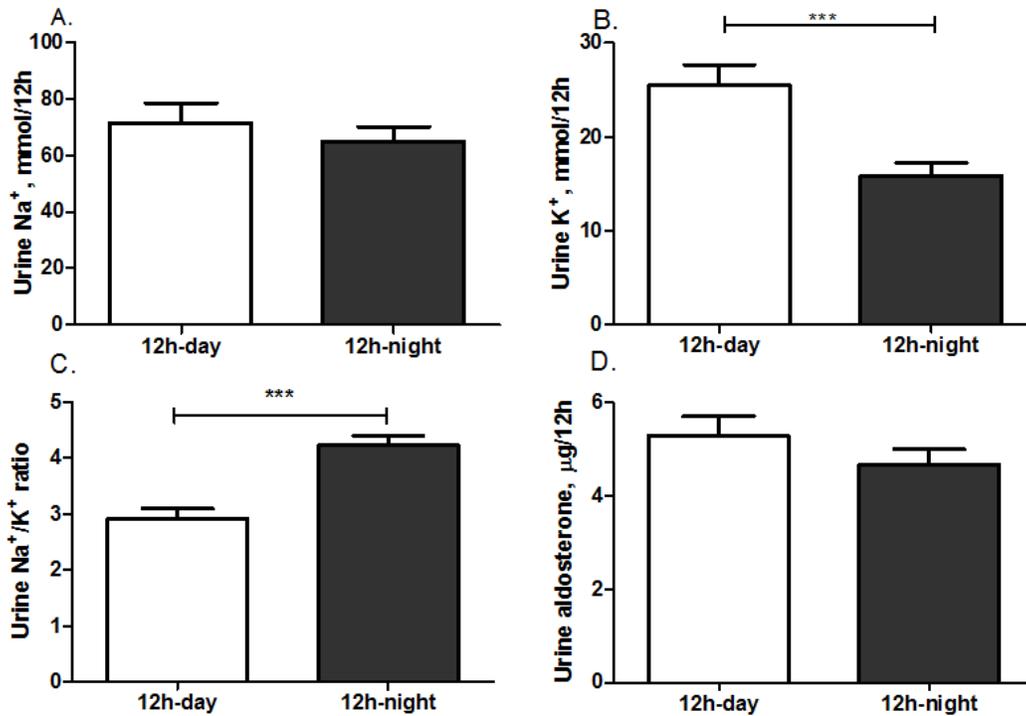
## RESULTS

### *Lowered K<sup>+</sup> excretion is responsible for the increased night-time urinary Na<sup>+</sup>/K<sup>+</sup> ratio*

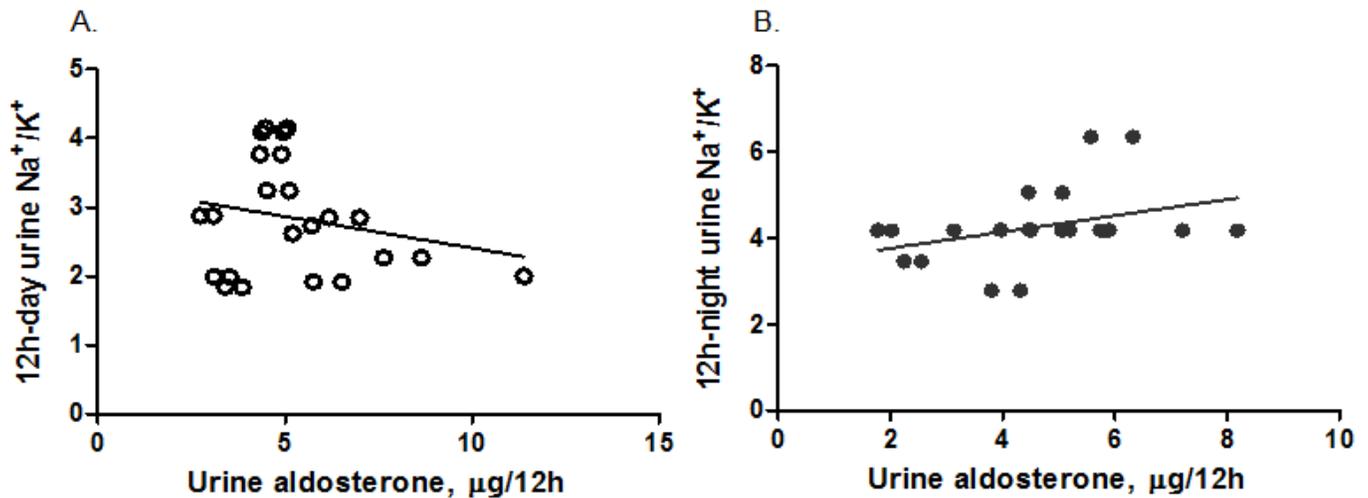
K<sup>+</sup> excretion in 12h- day and night urine samples was calculated (concentration of K<sup>+</sup> x volume of urine) and expressed in mmol/12h. Similarly, Na<sup>+</sup> and aldosterone excretion in urine were also calculated. Our results reveal a significant dip in the excretion of K<sup>+</sup> at night (Fig. 1B), which results in the increase in night-time urinary Na<sup>+</sup>/K<sup>+</sup> ratio (Fig. 1C). However, the 12h-day and 12h-night excretion of Na<sup>+</sup> and aldosterone were found to be similar (Fig. 1A and 1D). These results indicate that the circadian clock mechanism is a key regulator of renal K<sup>+</sup> excretion. Furthermore, the absence of nocturnal and diurnal variations in the renal excretion of Na<sup>+</sup> and aldosterone suggests that the circadian clock mechanism regulating renal K<sup>+</sup> excretion may be independent of Na<sup>+</sup> and aldosterone.

### *No relationship exists between aldosterone and urinary Na<sup>+</sup>/K<sup>+</sup> ratios*

To investigate if any relationships exist between aldosterone and Na<sup>+</sup>/K<sup>+</sup> ratios in the 12h-day and 12h-night urine, we correlated aldosterone excretion with the amount of each electrolyte (Na<sup>+</sup> and K<sup>+</sup>), and the Na<sup>+</sup>/K<sup>+</sup> ratios. Our results indicate that no relationships



**Fig. 1.** Day and night excretion of Na<sup>+</sup> (A) K<sup>+</sup> (B), Na<sup>+</sup>/K<sup>+</sup> (C) and aldosterone (D). Values are means ± SEM (n = 22 – 24), \*\*\*p<0.001.

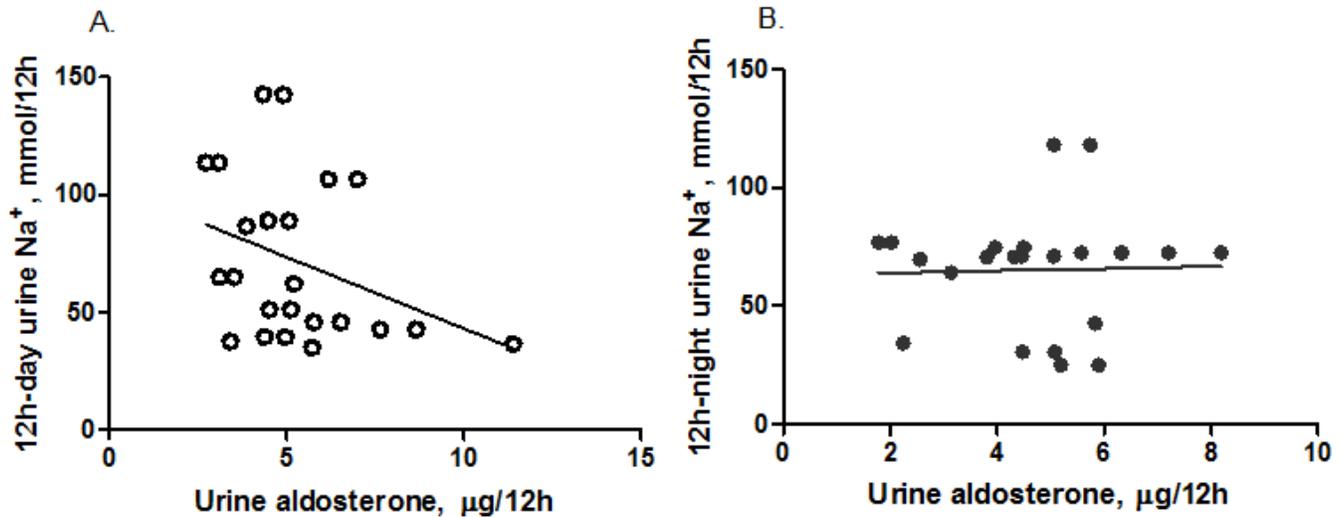


**Fig 2.** Relationship between aldosterone and Na<sup>+</sup>/K<sup>+</sup> ratios in 12h-day (A) and 12h-night (B) urine. No significant relationships exist between aldosterone and Na<sup>+</sup>/K<sup>+</sup> ratios in both the daytime and night-time urine (n = 22-23).

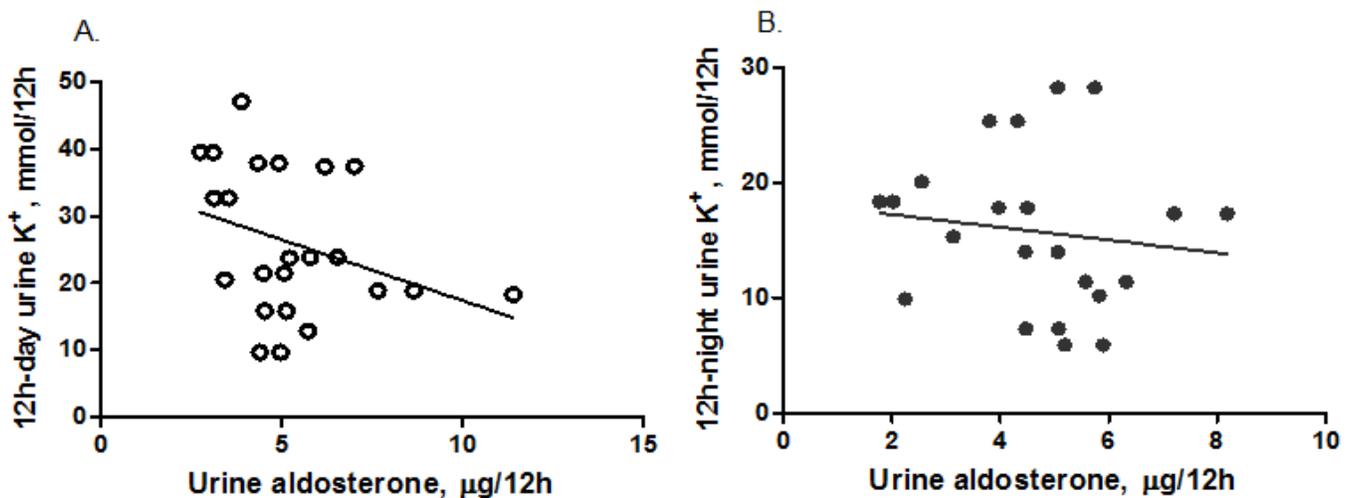
exist between urinary aldosterone and Na<sup>+</sup>/K<sup>+</sup> ratios in both the 12h- day and night urine (Figs. 2A and 2B). To further investigate the underlying cause of the lack of relationship between urinary aldosterone and Na<sup>+</sup>/K<sup>+</sup> ratios in the 12h- day and night urine, we correlated the urinary aldosterone with each electrolytes (Na<sup>+</sup> and K<sup>+</sup>). Again, our results show that no relationships exist between urinary aldosterone and Na<sup>+</sup> (Figs. 3A and 3B), and K<sup>+</sup>(Figs. 4A and 4B) in both the 12h- day and night urine.

**DISCUSSION**

Dysregulation of the circadian clock mechanisms involved in the regulation of major electrolytes (Na<sup>+</sup> and K<sup>+</sup>), which are playing a key role in blood pressure control has been suggested as a possible cause of hypertension (Sachdeva and Weder, 2006). In this study, we investigated the nocturnal and diurnal variations in the renal excretion of Na<sup>+</sup> and K<sup>+</sup>; and their regulatory hormone, aldosterone. While the renal excretion of Na<sup>+</sup> and aldosterone is suggested to be independent of the circadian clock mechanism, renal K<sup>+</sup>



**Fig 3.** Relationship between aldosterone and Na<sup>+</sup> in 12h-day (A) and 12h-night (B) urine. No significant relationships exist between aldosterone and Na<sup>+</sup> in both the daytime and night time urine (n = 22-23).



**Fig 4.** Relationship between aldosterone and K<sup>+</sup> in 12h-day (A) and 12h-night (B) urine. No significant relationships exist between aldosterone and K<sup>+</sup> in both the daytime and night time urine (n = 22-23).

excretion was shown to exhibit significant circadian rhythms. Due to this circadian rhythm of K<sup>+</sup>, the urinary Na<sup>+</sup>/K<sup>+</sup> ratio also exhibit day and night rhythms. Previous evidence suggests that aldosterone is the key regulator of urinary Na<sup>+</sup>/K<sup>+</sup> ratio (Wiederholt et al., 1972); however, our findings suggest that an aldosterone independent mechanism of K<sup>+</sup> excretion, which is strongly affected by the circadian clock may play a key role in the regulation of urinary Na<sup>+</sup>/K<sup>+</sup> ratio. Reports on aldosterone and K<sup>+</sup> balance have shown that aldosterone excess causes hypokalaemia due to excess secretion of K<sup>+</sup> in the distal nephron (Hassan-Smith and Stewart, 2011). Aldosterone levels in humans have been reported to exhibit a circadian rhythm with peak

levels observed at night (Fu and Lee, 2003). Consequently, higher nighttime K<sup>+</sup> excretion in urine is expected compared with the daytime. However, our results suggest otherwise. Studies in adrenalectomised animals have shown that in the absence of endogenous aldosterone, the circadian excretion of K<sup>+</sup> remains unchanged (Rabinowitz, 1996). Taken together, aldosterone may not be responsible for the circadian excretion of K<sup>+</sup> in urine.

There is increasing evidence for the role of the circadian clock in the control of K<sup>+</sup> channels in the heart (Jeyaraj et al., 2012). However, the effect of the circadian clock on the membrane expression and conductance of K<sup>+</sup> channels in the distal nephron is still

under investigation. Emerging evidence suggests that the circadian clock significantly regulates the expression of K<sup>+</sup> channels and their physiological functions (Zuber et al., 2009). It is plausible that the circadian clock in the suprachiasmatic nucleus of the hypothalamus inhibits several kaliuretic mechanisms at night, including the inhibition of K<sup>+</sup> secreting channel activity in the distal nephron. Future studies in rats and mice may be designed to investigate the effects of day and night cycles on the messenger RNA and protein expression levels of K<sup>+</sup> transporters in the kidneys. This will provide more insights into the role of the circadian clock in the control of renal K<sup>+</sup> excretion.

Another key regulator of renal distal tubular K<sup>+</sup> secretion is the rate of Na<sup>+</sup> delivery to the distal nephron (Palmer, 2015). Increased delivery of Na<sup>+</sup> to the distal nephron stimulates the reabsorption of Na<sup>+</sup> in this region, which will make the luminal potential more negative and, thus, increase K<sup>+</sup> secretion (Subramanya and Ellison, 2014). It will be interesting to investigate if increasing the delivery of Na<sup>+</sup> to the distal nephron using loop diuretics will result in a similar dip in nocturnal K<sup>+</sup> excretion demonstrated in this study. The use of loop diuretics will also compensate for the effects of the random salt intake by the subjects, which was not controlled in this study.

In conclusion, our findings support the previously established dip in nocturnal K<sup>+</sup> excretion, which we show here to be responsible for the observed increase in night-time urinary Na<sup>+</sup>/K<sup>+</sup> ratio. Our correlation data suggest that an aldosterone independent mechanism is responsible for the nocturnal dip in K<sup>+</sup> excretion.

#### ACKNOWLEDGEMENT

The authors wish to acknowledge the help offered by Dr. E.S. Idogun of the Chemical Pathology Department, University of Benin. This work was part-funded by E.O. Asowata, B.P. Ilenwabor and a Tetfund/UNIV/BENIN postgraduate research grant. A part of this work has been presented to The Physiological Society (PCB170) at the Physiology 2014 meeting held at the Queen Elizabeth II Conference Centre, London, U.K.

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