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Circadian Potassium Excretion is Unaffected Following Furosemide Induced Increase in Sodium Delivery to the Distal Nephron

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Summary: The mineralocorticoid aldosterone is widely accepted as a key regulator of K^+ balance as well as urinary K^+ excretion. However, recent evidence suggests that the circadian control of K^+ excretion is independent of aldosterone. The delivery of Na^+ to the distal nephron is known to be an important determinant of aldosterone mediated secretion of K^+ in this segment of the nephron. Examining the link between distal Na^+ delivery and K^+ excretion; and how this link affect circadian K^+ excretion will advance what is currently known about the maintenance of K^+ homeostasis. In the current study, we investigated the effect of furosemide-induced increase in distal tubular Na^+ on K^+ excretion. Na^+, K^+ and aldosterone levels were measured in 12-hour day time and 12-hour night time urine samples following furosemide administration, and compared with controls in 10 apparently healthy male subjects. To confirm the increased delivery of Na^+ to the distal nephron by furosemide, increased Na^+ excretion and aldosterone activity was observed in subjects administered furosemide. Consistent with previous reports, night time K^+ excretion was significantly lower than day time, and this observation was unchanged even with increased Na^+ delivery to the distal tubules. In healthy individuals, aldosterone increases K^+ secretion and this is known to further increase with increased Na^+ delivery to the potassium secreting segment of the nephron. Even though the administration of furosemide increased aldosterone activity and the delivery of Na^+ to the distal tubules, the dip in night time K^+ excretion was unchanged. Our findings suggest that the circadian control of K^+ excretion is not linked to Na^+ levels and thus independent of aldosterone.

Keywords*:* Na^+/K^+ ratio, circadian rhythm, aldosterone, furosemide, distal nephron, kidney

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INTRODUCTION

Potassium ion (K^+) is the most abundant cation in the intracellular fluid and is an important requirement for many physiological functions. It plays a role in the maintenance of resting membrane cell potential necessary for the function of excitable tissues as well as in cardiac function (Kara et al., 2013). It is imperative that the concentration of extracellular K^+ is maintained within narrow physiological limits. The kidneys are primarily responsible for this maintenance by adjusting K^+ excretion to match K^+ intake. The mineralocorticoid aldosterone is widely accepted as a key regulator of urinary K^+ excretion and K^+ balance. Numerous studies have reported circadian rhythms in urinary excretion of K^+ with higher values during the day than at night (Mills, 1966; Moore Ede *et al*., 1975; Doi *et al*., 2009; Gumz and Rabinowitz, 2013). This rhythm is found to be independent of activity, posture and diet (Firsov et al., 2012).

Blood pressure is known to follow a circadian pattern in normal individuals exhibiting a 10-20% decrease at night. This dip in blood pressure has been positively associated with urinary K^+ excretion (Routledge *et al*., 2007; Hermida *et al*., 2007; Bankir *et al*., 2008). Absence of this dip in nighttime blood pressure is associated with hypertension and increased risk of cardiac death and renal damage (Portaluppi *et al*., 1991; Stow and Gumz, 2011). Since renal sodium (Na^+) handling has been linked to the regulation of this nighttime dipping blood pressure (Stow and Gumz, 2011); the renal K^+ handling may be critical as well. Several studies have shown that K^+ supplementation leads to lowering of nocturnal blood pressure (Vij and Peixoto 2009). In this regard, Wilson et al. (1996) demonstrated the beneficial effect of raised extracellular K^+ levels where oral intake of K^+ restored the dipping profile in normal subjects with a non-dipper blood pressure status. Understanding the mechanisms underlying the physiological dip in K^+ excretion will be essential in targeting the renal handling of this electrolyte in the treatment of hypertension.

We have recently reported circadian variations in urinary K^+ with a nighttime dip in K^+ excretion, which was independent of aldosterone activity (Asowata *et al*., 2016). Since renal distal tubular Na^+ concentration

has been shown to affect K^+ excretion (Subramanya and Ellison, 2014), we hypothesise that increasing distal tubular Na^+ levels will abolish the dip in night-time K^+ excretion. The aim of this study was therefore to investigate if increasing the delivery of Na^+ to the distal tubule using furosemide will alter the dip in nighttime urinary K^+ excretion.

MATERIALS AND METHODS

Study design and Subjects

Subjects were recruited from the University of Benin community, Edo State, Nigeria. Only subjects that were not on any medications and were without any history of renal or cardiovascular diseases and risk factors 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^+ and K^+ in urine was carried out, and all ten (10) healthy male subjects (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 days, and urine collection was carried out for 24 hours. Participants were instructed to avoid strenuous physical activity and alcohol consumption for 48 hours preceding sample collection. All protocols employed in this study comply with the guidelines and regulations of the College of Medical Sciences Ethical Committee, University of Benin and all subjects gave their informed consent to participate in the study.

Sample collection

Instructions were given to participants regarding urine collection and 2 sterile plastic containers (2L) were given to each participant. The 24-h urine collection was divided into two time periods: the day period (from 7:00 am to 7:00 pm) and the night period (from 7:00 pm to 7:00 am). Urine samples were collected for two days with control samples collected on the first day. On the second day, 20mg of furosemide was given orally to the subjects twice (at 7am and 7pm) and urine was collected same way as the first day for 12 hours after furosemide ingestion. Subjects reported to the laboratory both days after sample collection.

Sample Analysis

Urine volume was measured using a measuring cylinder, Na^+ and K^+ concentrations were measured using the flame atomic absorption spectrophotometry (Toffaletti and Jones, 1992). Aldosterone concentration in urine was measured using the enzyme immunoassay method (DRG International, Inc., USA).

Statistical Analysis

Statistical analysis was conducted using the GraphPad Prism 5.0 statistical software. Data are reported as means ± SEM. To test for differences in the means across groups, the unpaired t-test was used. The association between urinary parameters in 12- and 24-h urine samples was determined using the Pearson (r) correlation coefficient. Statistical significance was set at $P < 0.05$.

RESULTS

We observed the urinary Na^+/K^+ ratio to be increased in the test group when compared with the control group in both day and night urine samples. As a diuretic, furosemide increased Na^+ excretion while K^+ excretion remained unchanged in this study (Figs. I & II) In addition, aldosterone activity was also increased following furosemide administration (test group) (Fig. I).

Consistent with our previous findings (Asowata *et al*., 2016), the Na^+/K^+ ratio in control subjects is higher at night than during the day (Fig. IIIa). The nighttime fall in urinary K^+ excretion (Fig. IIIc) is found responsible for this higher Na^+/K^+ ratio. This fall in K^+ excretion at night is not correlated with aldosterone levels which remained unchanged (Fig. IIId).

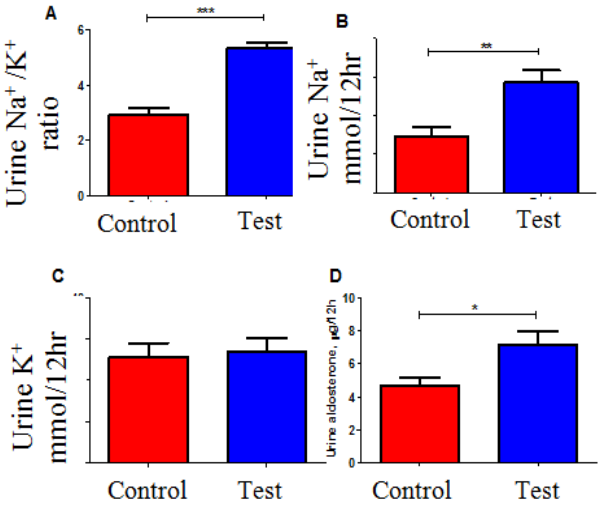

Fig I: Graph showing 12 hour day control and test values for Na^+/K^+ ratio (a), Na^+ excretion (b), K^+ excretion (c) & aldosterone excretion (d). *p<0.05, **p<0.01, ***p<0.001 (n= 10)

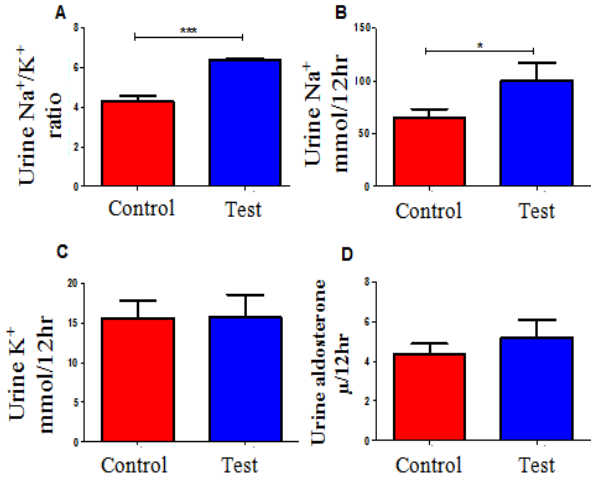

Fig II: Graph showing 12 hour night control and test values for Na^+/K^+ ratio (a), Na^+ excretion (b), K^+ excretion (c) & aldosterone excretion (d). *p<0.05, ***p<0.001 (n=10)

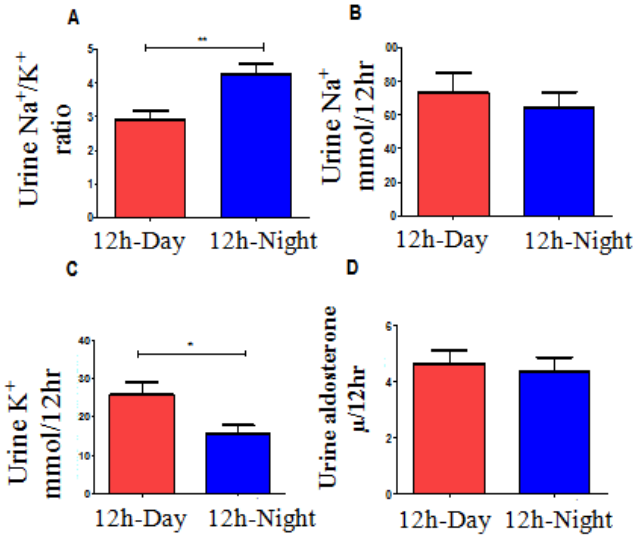

Fig III: Graph showing control values for 12 hour day and 12 hour night Na^+/K^+ ratio (a), K^+ excretion (b), Na^+ excretion (c) & aldosterone excretion (d). *p<0.05, **p<0.01

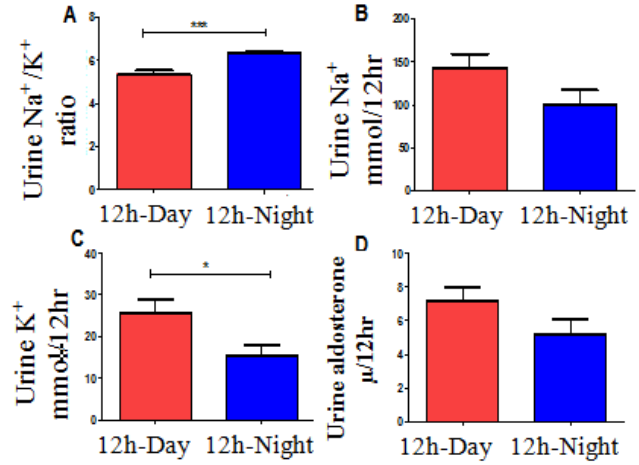

Fig IV: Graph showing test values for 12-hour day and 12-hour night Na^+/K^+ ratio (a), K^+ excretion (b), Na^+ excretion (c) and and alsosterone excretion (d). *p<0.05, ***p<0.001

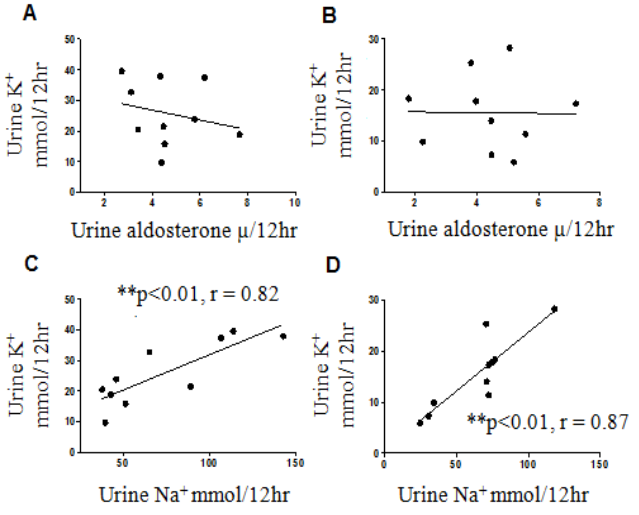

Fig V: Correlation graphs between urine aldosterone & K^+ in 12h-D (a) and 12h-N (b) and urine Na^+ and K^+ in 12h-D (c) and 12h-N (d) in the control group. **p<0.01

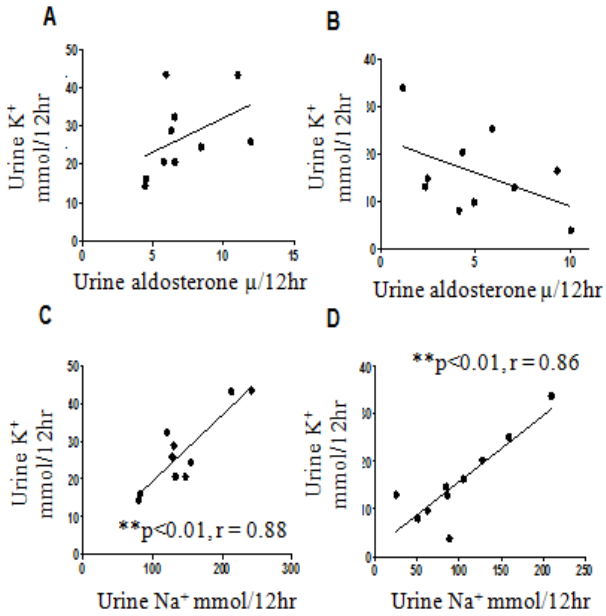

Fig VI: Correlation graphs between urine aldosterone & K^+ in 12h-D (a) and 12h-N (b) and urine Na^+ and K^+ in 12h-D (c) and 12h-N (d) in the test group. **p<0.01.

Following increased the delivery of Na^+ to the distal tubule with the administration of furosemide (test group), Na^+/K^+ ratio remained higher at night than during the day (Fig. IVa). This higher nighttime Na^+/K^+ ratio was due to the K^+ excretion, which remained lower at night (Fig. IVc). Na^+ excretion was unchanged in both day and night urine samples (Fig. IVb). Aldosterone excretion increased compared with control values as expected (Fig. Id), but there was no difference between daytime and nighttime aldosterone excretion in the test group (Fig. IVd). This buttresses the fact that the dip in K^+ excretion at night is independent of aldosterone.

Correlation analysis was used to test for the existence of any relationships between urinary K^+ excretion and aldosterone (Fig. V a & b), and with Na^+ (Fig V c & d) in day and night urine samples of controls. No significant relationship was observed between aldosterone and K^+. However, a strong positive relationship was observed between urinary Na^+ and K^+ showing that K^+ and Na^+ excretions are directly linked. Following furosemide administration, the established relationship between urinary K^+ excretion and aldosterone, and between K^+ and Na^+ in day and night urine samples of controls were unchanged (Fig. VI).

DISCUSSION

A critical determinant of blood pressure is how the kidneys handle salt excretion and understanding how this is carried out is important as hypertension is rarely observed in the absence of renal dysfunction (Stow and Gumz, 2011). The results in this study further highlights a circadian pattern in the excretion of K^+ in urine which is responsible for the higher Na^+/K^+ ratio

in nighttime urine. The two principal determinants of K^+ excretion by the kidneys are; the steroid hormone aldosterone and the distal delivery of K^+ (Palmer, 2015). Plasma aldosterone levels have been reported to follow a circadian pattern with peaks levels occurring during sleep (Doi *et al*., 2009; Fu and Lee, 2003). It was thought that the circadian rhythm in aldosterone secretion played a role in driving the circadian urinary K^+ excretion (Hilfenhaus, 1976; Palmer, 2015). However, results from this study and our previous report show that the dip in nighttime K^+ excretion does not correlate with aldosterone levels. It has also been reported that aldosterone at physiologic levels have little influence on urinary K^+ excretion (Rabinowitz *et al*., 1985) and studies have also reported that circadian rhythm in K^+ excretion remains intact in adrenalectomized animals and adrenal insufficient humans (Rabinowitz, 1996). These reports show that aldosterone might not be responsible for the nighttime dip in K^+ excretion by the kidneys.

The second determinant of K^+ excretion as mentioned above is the amount of Na^+ delivered to the distal tubule. Urinary K^+ excretion is controlled by transport processes occurring at the distal tubular segment which receives about 10% of filtered K^+ (Muto, 2001). Our findings of a significant positive relationship between urinary Na^+ and K^+ provide additional evidence for the role of distal tubular Na^+ levels in modulating K^+ secretion. Steele *et al*. (1994) reported that the circadian pattern in urinary K^+ excretion is determined mostly by circadian changes in the intratubular K^+ concentration, Na^+ levels and urine flow rate in the distal tubule. Our study was aimed to observe the effect of this increased flow rate and Na^+ on the circadian rhythm of K^+ excretion since they influence K^+ excretion independent of aldosterone. It has been suggested as well that the circadian pattern in K^+ excretion is not determined by aldosterone but by the circadian rhythm of Na^+ excretion (Dalton and Rabinowitz, 1989; Ogiyama *et al*., 2013). We expected circadian differences in K^+ excretion to be blunted by the effect of furosemide since Na^+ delivery and concurrently, flow rate will be maintained at a similar level. To test our hypothesis, we used furosemide to increase the delivery of Na^+ to the distal tubules as well as the flow rate of tubular fluid, and then re-examined the relationship between urinary Na^+ and K^+ excretion. Interestingly and contrary to what we expected, the direct relationship between urinary Na^+ and K^+ excretion observed in control subjects were unaffected following furosemide administration.

Furosemide used in this study increases the rate at which Na^+ and fluid reaches the distal tubule by inhibiting the sodium-potassium-chloride 2 (NKCC 2) transporter in the ascending limb of the loop of Henle. This is long known to result in increased K^+ secretion as there is increased Na^+ reabsorption in the distal tubule and this Na^+ reabsorption is coupled to K^+ secretion in this segment of the nephron (Kaissling and Stanton, 1988; Khuri *et al*., 1975). Increased flow rate due to furosemide also reduces luminal K^+ concentration causing a steeper K^+ gradient across the apical membrane. This increases K^+ secretion into the tubular lumen resulting in an increase in its excretion in urine (Kunau et al., 1974; Good and Wright, 1979). However, contrary to our expectations, results from this study show that the night-time dip in urinary K^+ excretion were unaffected despite the influence by furosemide. Our study thus shows that the circadian excretion of potassium is not dependent on the circadian rhythm of sodium excretion. This confirms previous postulations suggesting independent regulations for circadian excretion of Na^+ and K^+ (Poulis *et al*., 1985). Also, there is evidence that different oscillators may drive the Na^+ and K^+ excretion cycles in the kidney (Rabinowitz, 1996).

Increasing evidence suggests that the expression of Na^+ and K^+ channels and transporters in the highly regulated distal nephron is also controlled by the circadian clock (Stow *et al*., 2012; Richards *et al*., 2014; Solocinski and Gumz, 2015; Zuber *et al*., 2009). It is therefore plausible that the suprachiasmatic nucleus (SCN) of the hypothalamus (the central clock) entrains pacemakers in the kidneys (peripheral clock) to bring about circadian rhythm in renal function (Gumz and Rabinowitz, 2013). This could bring about varying K^+ excretion by regulating the expression of potassium channels in the distal tubule such as ROMK, Na/K-ATPase and H/K-ATPase (Sahli *et al*., 2016; Segura *et al*., 2004; Firsov *et al*., 2012).

In conclusion, this study shows that the established dip in nighttime urinary excretion of K^+ is not dependent on the amount of Na^+ reaching the distal tubule or/and aldosterone. It is interesting to note that while our findings indicate the existence of a relationship between urinary K^+ excretion and the amount of Na^+ reaching the distal tubule, this relationship does not contribute to the circadian rhythm in urinary K^+ excretion. We agree with suggestions that mechanisms involving the SCN reduce the expression of K^+ channels at night and thus play a key role in the dip in nocturnal K^+ excretion.

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