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Gastrointestinal and renal responses to water intake in the green-backed firecrown (*Sephanoides sephanoides*), a South American hummingbird

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Hartman Bakken, Bradley, and Pablo Sabat. Gastrointestinal and renal responses to water intake in the green-backed firecrown (*Sephanoides sephanoides*), a South American hummingbird. *Am J Physiol Regul Integr Comp Physiol* 291: R830–R836, 2006. First published April 13, 2006; doi:10.1152/ajpregu.00137.2006.—To maintain water balance, nectar-feeding vertebrates oscillate between meeting the challenges of avoiding overhydration and preventing dehydration. To understand how green-backed firecrowns (*Sephanoides sephanoides*) accomplish this, we examined the response of water-handling processes in the gastrointestinal tract (GIT) and kidney to different rates of water intake during the evening, night, and morning. Fractional water absorption in the GIT was independent of water intake rate (evening: 0.91 ± 0.08; morning: 0.88 ± 0.04). Consistent with this nonregulated water absorption, we found linear increases in water flux, fractional turnover of body water, and the rate of renal water loading as water intake rate increased during both the evening and morning. Despite these relationships, glomerular filtration rate (GFR) was insensitive to water loading (evening: 2.08 ± 0.56 ml/h; morning: 1.84 ± 0.68 ml/h) and less than the allometric expectation (2.92 ml/h). During the evening, fractional renal water reabsorption decreased linearly as the rate of water intake increased. At night, a period of natural fasting for hummingbirds, mean GFR was not different from zero (0.00 ± 0.05 ml/h). These findings indicate that green-backed firecrowns eliminate excess ingested water by decreasing water reabsorption in the kidney; to conserve water, it appears that hummingbirds arrest whole kidney GFR, effectively preventing urinary water losses. After discounting evaporative water losses, our results show that hummingbirds rely principally on their renal system to resolve the osmoregulatory quandary posed by nectarivory.

glomerular filtration rate; glomerular intermittency; osmoregulation; renal water reabsorption; water turnover

NECTAR-FEEDING VERTEBRATES have a curious relationship with water: when feeding, they ingest it in excess (13, 29, 40); yet, during fasts, they are prone to losing it (23, 27, 41). Consequently, water balance in nectarivores demands they meet the disparate challenges of avoiding both overhydration (1, 11) and dehydration (23). In this article, we measure water-handling processes in the gastrointestinal tract (GIT) and kidney of a nectarivorous bird, the green-backed firecrown (*Sephanoides sephanoides*), to determine how this osmoregulatory quandary is resolved.

In general, floral nectar is energetically dilute (2, 35). To satisfy daily caloric requirements, nectarivores must therefore ingest large quantities of water (29). Such water intake rates have the potential to severely disrupt water balance (1, 11). For hummingbirds (Trochilidae), a group of highly specialized nectar feeders (39), daily water intakes are commonly multiples of body mass (M_B; see Refs. 3 and 29). This observation led Beuchat and colleagues (3) to hypothesize that nectar-feeding birds may avoid overhydration by modulating water absorption in the GIT. Such a response would effectively reduce renal water load and explain, in part, how nectar-feeding birds cope with excessive water intake (3). McWhorter et al. (31) found support for this hypothesis in a passerine nectarivore, the Palestine sunbird (*Nectarinia osea*): as water intake rate increased, sunbirds reduced fractional water absorption to as little as 0.36. There is, however, no evidence to support Beuchat et al.'s (3) hypothesized mechanism in hummingbirds: regardless of water intake, fractional water absorption in broad-tailed hummingbirds (*Selasphorus platycercus*) was 0.78 ± 0.03 (mean ± SE; see Ref. 30). We hypothesized that green-backed firecrowns, like confamilial broad-tailed hummingbirds, would be incapable of modulating water absorption. This hypothesis suggests that the renal system, after discounting total evaporative water loss (TEWL), is chiefly responsible for eliminating excess ingested water.

What renal processes do nectarivorous birds use to expel excess water? The available data indicate that they rely more heavily on reducing the reabsorption of filtered water rather than increasing the volume of water filtered (18, 23, 32). Accordingly, we did not expect the glomerular filtration rate (GFR) in green-backed firecrowns to exceed the allometric prediction, but we anticipated that renal water reabsorption would decrease with increasing water intake (18, 23, 32). Renal functions, however, appear to vary with time of day (12, 21, 23). We therefore expected GFR to be lower in the morning relative to the evening (21, 23).

The capacity to eliminate excess ingested water efficiently poses a dilemma when water needs to be conserved. A common water-conserving strategy among terrestrial vertebrates is to produce hyperosmotic urine (9). Hummingbirds, however, have a nearly homogenous cortical-type nephron population (4, 5) and are incapable of producing concentrated urine (27). For animals with a small body size, this dilemma is exacerbated by high mass-specific rates of TEWL (20, 38, 48). Therefore, to conserve water, hummingbirds appear to reduce, even cease, GFR during fasting periods (23). Here we measured GFR during a natural, overnight fast. We hypothesized that green-

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backed firecrowns would reduce GFR at night; however, because green-backed firecrowns are larger than broad-tailed hummingbirds (10), we predicted that they would not arrest GFR.

METHODS

The protocols we followed for this work conformed to the bioethical guidelines established by the University of Chile for animal care and experimentation.

Hummingbird capture and care. Male green-backed firecrowns (M_B = 5.31 ± 0.47 g, n = 6) were captured with mist nets in San Carlos de Apoquindo, Chile (33°23'S, 70°31'W). We housed hummingbirds individually in cages (0.3 × 0.5 × 0.5 m) inside a temperature-controlled room with a natural photoperiod. During captivity, the photophase ranged from 10.97 to 12.63 h/24 h. Average ambient temperature inside this facility, as determined from daily minimum and maximum temperatures recorded each day at ~1200 Chile Time, was 24.6 ± 2.8°C (n = 51). We provided birds with two maintenance foods. The first was a 10.0% (mass%) solution of Nektar-Plus (Guenter Enderle, Tarpon Springs, FL) supplemented with sucrose (10.0%) and vitamins (0.4%, Nekton-S; Guenter Enderle). Hummingbirds fed ad libitum on this solution from ~0900–1800. The second maintenance food was a 25.0% sucrose solution. Because hummingbirds do not eat at night, they fed ad libitum on the second food from ~1800 to lights off and from lights on to ~0900. While captive and during the experiment described below, feeding necessitated hovering.

Water flux, body water turnover, water absorption, and renal water load measurements. We used the mass-balance model developed by McWhorter and Martínez del Rio (30) to measure GIT responses to water intake. We applied this model as previously described (31). Briefly, this approach requires: *1*) $Q_{^3H}$, the quantity of ^3H_2O injected [disintegrations/min (dpm)]; *2*) $I_{^3H}$, the *time 0* intercept concentration of ^3H_2O in body water (dpm/ml); and *3*) $K_{^3H}$ the hourly fractional rate of ^3H elimination. With the use of these parameters, total body water (TBW; ml) is estimated as

$$\mathrm{TBW} = \frac{Q_{^3H}}{I_{^3H}}$$

where $K_{^3H}$ is used to extrapolate to $I_{^3H}$ from a blood sample taken ~1.5 h after injection. To check that this isotope dilution method yielded reasonable estimates, we culled a subset of birds (n = 3) and determined TBW by dehydration at 80°C to constant M_B. Water flux ($\dot{W}$; ml/h), the rate at which water is incorporated into body water, is then

$$\dot{W} = K_{^3H} \times \mathrm{TBW}$$

We estimated the hourly fractional turnover rate of body water (f_T) as

$$f_T = \frac{\dot{W}}{\mathrm{TBW}}$$

To estimate fractional water absorption in the GIT (f_A, previously denoted as f_W; see Refs. 30 and 31), we made several assumptions concerning the rate of metabolic water production ($\dot{V}_M$). Because sucrose assimilation efficiency in hummingbirds is high and independent of the sucrose intake rate ($\dot{S}_I$; see Refs. 28–30), we assumed the fractional assimilation of ingested sucrose was 0.95. We also assumed that hummingbirds were relying solely on carbohydrates to fuel metabolism (43). We measured food intake gravimetrically (±0.0001 g) and calculated rates of sucrose intake (g/h) and water intake (ml/h) after correcting for evaporation. Additionally, we assumed 1 g of sucrose liberates 0.57 ml of water, and 1 ml of water has a mass of 1 g. With these assumptions, $\dot{V}_M$ (ml/h) is

$$\dot{V}_M = \dot{S}_I \times 0.95 \times 0.57$$

and f_A is

$$f_A = \frac{\dot{W} - \dot{V}_M}{\dot{V}_I}$$

where $\dot{V}_I$ is the rate of dietary water intake (ml/h). The rate of renal water loading ($\dot{V}_R$; ml/h) can then be estimated as

$$\dot{V}_R = \dot{V}_I \times f_A + \dot{V}_M$$

GFR and water reabsorption measurements. To estimate GFR, we used versions of the slope-intercept method (15, 22) that accommodate animals sensitive to repeated blood sampling. This allowed us to make measurements in nonrestrained hummingbirds feeding freely.

After McWhorter et al. (32), we estimated GFR (ml/h) during feeding periods as

$$\mathrm{GFR} = K_{^{14}C} \times \frac{Q_{^{14}C}}{I_{^{14}C}}$$

where $K_{^{14}C}$ is the hourly fractional elimination rate of ^{14}C, $Q_{^{14}C}$ is the quantity of L-[^{14}C]-labeled glucose injected (dpm), and $I_{^{14}C}$ is the *time 0* intercept concentration of ^{14}C in plasma (dpm/ml) as predicted by $K_{^{14}C}$ from a blood sample taken ~1.5 h after injection. The quotient of $Q_{^{14}C}$ over $I_{^{14}C}$ gives the L-glucose distribution space (P_B; ml). We estimated mean GFR (GFR′; ml/h) during fasting periods after Hartman Bakken et al. (23) such that

$$\mathrm{GFR}' = K'_{^{14}C} \times P_B$$

where $K'_{^{14}C}$ is the difference between the ^{14}C concentration in the last excreta sample before the fast and the first excreta after the fast over time. After Goldstein (17), we estimated fractional renal water reabsorption (f_R) as

$$f_R = 1 - \frac{P_{^{14}C}}{U_{^{14}C}}$$

where $P_{^{14}C}$ and $U_{^{14}C}$ are the ^{14}C concentrations in plasma and ureteral urine (dpm/ml), respectively.

Assumptions of the mass-balance and single-injection, slope-intercept models. The approach we adopt in this article can only be used with confidence if the clearance of both ^3H and ^{14}C follows single-compartment, first-order kinetics (15, 22, 23, 30–32). We used M_B to gauge if the neutral water balance assumption (30) was met.

Experimental protocol. Nectar-feeding birds consume increasing volumes of food as the sugar concentration decreases (26, 29). To vary rates of water intake naturally, we gave green-backed firecrowns either a 292 or 876 mM sucrose solution. These solutions are ~10 and 30% (mass%), respectively. Each bird fed ad libitum on a single, randomly assigned sucrose solution throughout the experiment, starting 3 h before injections.

Roughly 2 h before lights off, we injected ~9 × 10^4 Bq of ^3H_2O (lot no. 3559–732; Perkin-Elmer, Boston, MA) and ~8 × 10^4 Bq L-[^{14}C]-labeled glucose (lot no. 3406–255; Perkin-Elmer; see Ref. 7) in the pectoralis muscle of green-backed firecrowns. We dissolved both markers in distilled water, and the total volume injected did not exceed 15 μl. Promptly after injecting the birds, we returned them to their individual experimental cages (0.2 × 0.3 × 0.5 m) and began collecting freshly voided excreta. Experimental cages and excreta collection were as previously described (23), except that we lined cage bottoms with aluminum foil rather than wax paper. Approximately 15 min before lights off, we removed each bird from its cage to collect ureteral urine and blood. We acquired the ureteral urine with a closed-ended polyethylene cannula (19); the blood was gathered after clipping a single toenail. We halved this blood sample (17 ± 5 μl) and obtained water by distillation (34) and plasma by centrifugation. We then returned birds to their cages. The following morning, at lights on, we continued to collect freshly voided excreta for ~1.5 h. We made all measurements at 25 ± 1°C. Figure 1 illustrates this protocol.

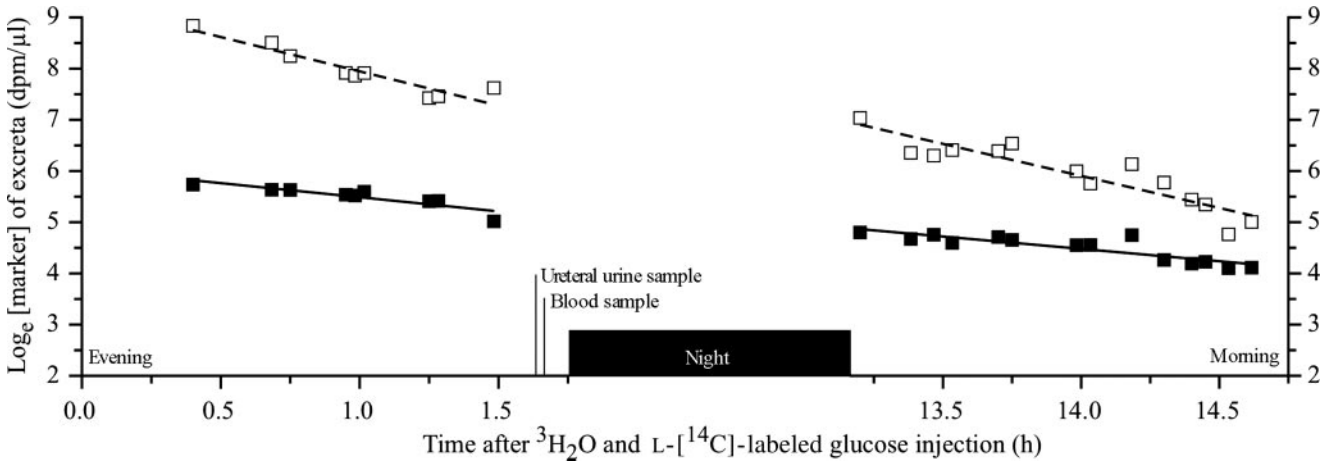

Fig. 1. Data from a representative green-backed firecrown (*Sephanoides sephanoides*) illustrating: *1*) our protocol for measuring gastrointestinal and renal functions during the evening, night, and morning and *2*) that ^3H_2O (■) and L-[^{14}C]-labeled glucose (□) appear in excreta with time according to single-compartment, first-order kinetics. ^{3}H and ^{14}C concentrations of excreta are log$_e$ transformed here; however, our analyses were performed on nontransformed data (33). We injected this particular hummingbird with both ^3H_2O and L-[^{14}C]-labeled glucose at 1703 Chile Time and collected freshly voided excreta samples until 1832. At 1843 and 1845, we collected a ureteral urine and blood sample, respectively. Lights off was at 1849 and the night phase concluded at 0617. At lights on the next morning (0618), we resumed collecting freshly voided excreta samples.

We monitored M_B by hanging the only available perch from an electronic balance (±0.01 g). During this experiment, the photophase ranged from 12.27 to 12.52 h/24 h. We placed all injection aliquots, ^{3}H and ^{14}C background, excreta, ureteral urine, water, and plasma samples in individual polyethylene scintillation vials immediately after collection. We added Ecoscint (National Diagnostics, Atlanta, GA) scintillation cocktail to all samples before measuring counts with a Packard Tri-carb 1600-TR liquid scintillation analyzer (Packard Instruments, Downers Grove, IL). All counts were corrected for ^{3}H and ^{14}C background, quench, chemiluminescence, and ^{14}C spillover. We tested the ^{14}C spillover correction empirically and found that ^{3}H counts were not different in the presence of ^{14}C (paired *t*-test: $t_{11} = -0.81$, $P = 0.4333$, $n = 12$).

Nighttime estimates of hypothermia. To determine what fraction of the night phase green-backed firecrowns spent hypothermic, we made estimates of body temperature by attaching a Cu-Cn thermocouple (±0.1°C) to the perch, as previously described (23). We recorded a body temperature estimate every 0.5 h throughout the night.

Statistical analyses. To determine the effect of sucrose concentration and subject on the rate of water intake and GFR, we used repeated-measures ANOVA (RM-ANOVA). Following these analyses, we used Tukey's Honest Significant Difference to test for differences among means. We analyzed paired data using paired *t*-tests and evaluated means against a hypothesized value with one-sample *t*-tests. In all other cases, we analyzed data using standard least-squares linear regression (LR). Unless stated to the contrary, $n = 6$ for the analyses we conducted. Findings are reported as means ± SD.

RESULTS

Marker equilibration and elimination. Equilibration times for ^{3}H and ^{14}C were 32 ± 16 and 22 ± 8 min, respectively. The relationships of ^{3}H and ^{14}C concentration in excreta with time were well described by negative exponential functions: coefficient of determination (r^2) values during the evening were 0.78 ± 0.19 for ^{3}H and 0.74 ± 0.24 for ^{14}C; during the morning, r^2 values were 0.68 ± 0.15 for ^{3}H and 0.71 ± 0.19 for ^{14}C. The elimination of ^{3}H and ^{14}C concentration in excreta with time, therefore, appeared to follow single-compartment, first-order kinetics (Fig. 1).

Were hummingbirds in neutral water balance? We used M_B to assess the neutral water balance assumption of the mass-balance model. During the evening, M_B after injection did not differ from M_B at lights off (paired *t*-test: $t_5 = -0.67$, $P = 0.5340$). This suggests the assumption of neutral water balance may have been reasonable for the evening trial. However, during the morning, M_B at lights on was significantly less than M_B at the end of the trial (paired *t*-test: $t_5 = 9.31$, $P = 0.0002$). Presumably, this reflects the rehydrating behavior of hummingbirds after the dehydrating night phase (8, 26); yet, it suggests the assumption of neutral water balance may have been violated. Even though ^{3}H and ^{14}C clearances were well described by negative exponential functions during the morning (Fig. 1), we are careful not to make inferences from the morning data.

Body fluid spaces. Our estimate of TBW in green-backed firecrowns was 3.01 ± 0.23 ml, which constitutes 56.6 ± 2.0% of M_B. This estimate, obtained by isotope dilution, did not differ from our estimate obtained by dehydration (57.2 ± 1.0% of M_B, $n = 3$; paired *t*-test: $t_2 = 0.58$, $P = 0.6231$, $n = 3$). We use the isotope dilution estimate for the analyses in this article. L-[^{14}C]-labeled glucose distribution space in green-backed firecrowns was 1.04 ± 0.08 ml, which is 19.6 ± 1.9% of M_B.

Water intake. Water intake rate was not influenced by subject [RM-ANOVA: $F(1,3) = 0.38$, $P = 0.5825$]. We therefore removed this parameter from the analyses in this section. During both the evening and morning, the rate of water intake increased as the sucrose concentration of food decreased [RM-ANOVA: $F(1,4) = 14.36$, $P = 0.0016$]. Water intake rates of birds feeding on 292 mM sucrose were 1.25 ± 0.07 ($n = 3$) and 2.25 ± 0.59 ($n = 3$) ml/h during the evening and morning, respectively. On 876 mM sucrose, rates of water intake during the evening and morning were 0.30 ± 0.06 ($n = 3$) and 0.82 ± 0.19 ($n = 3$) ml/h, respectively. Water intake rates were significantly higher in the morning compared with the evening (paired *t*-test: $t_5 = 3.61$, $P = 0.0153$).

Water flux. Water flux increased linearly as the rate of water intake increased (LR: evening, $y = -0.04 + 0.84x$, $r^2 = 0.97$, $P = 0.0003$; morning, $y = -0.12 + 0.85x$, $r^2 = 0.996$, $P < 0.0001$; Fig. 2*A*). As would be expected with a significantly higher water intake rate during the morning, water flux was

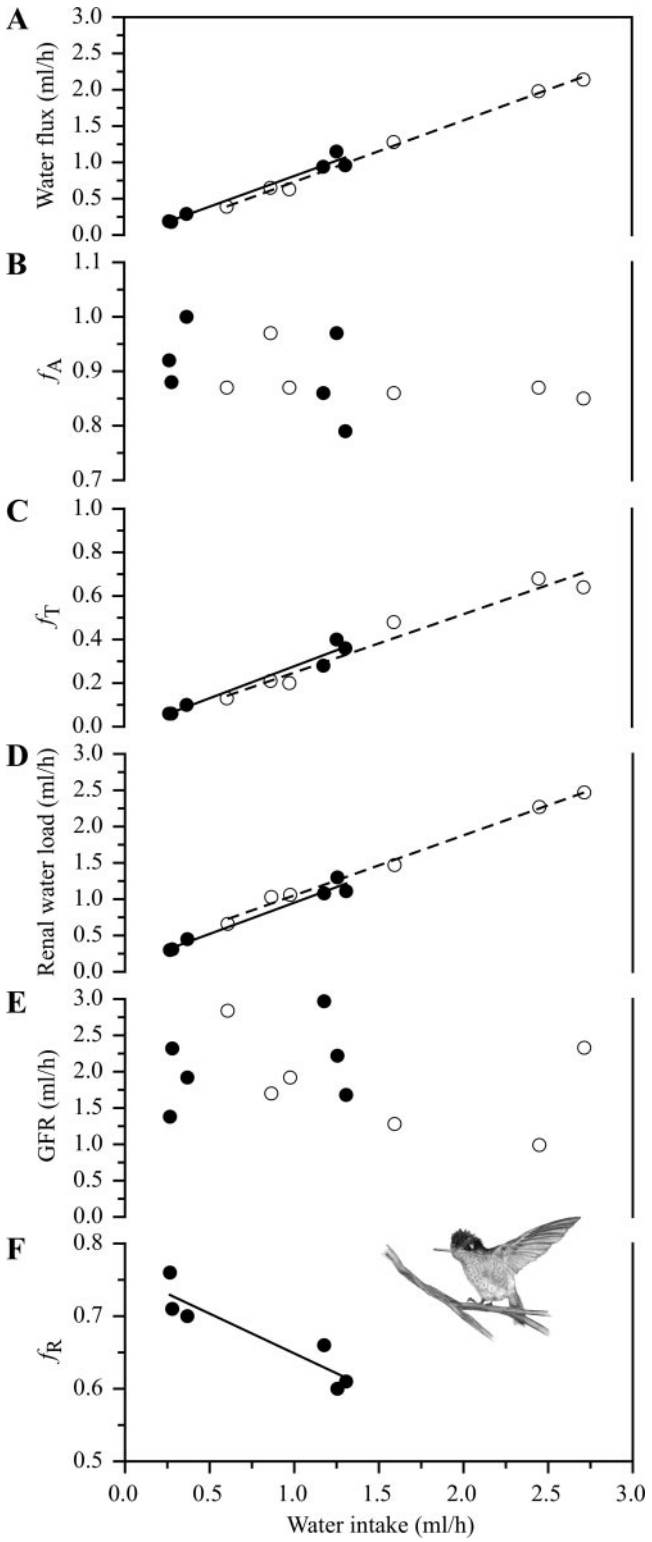

Fig. 2. The influence of water intake rate on water-handling processes during the evening (●) and morning (○) in green-backed firecrowns (*S. sephanoides*). Water flux increased linearly with water intake (*A*). We found no evidence to support the idea that hummingbirds modulate fractional water absorption in the gastrointestinal tract (f_A; *B*). Hourly rates of both fractional body water turnover (f_T; *C*) and renal water load (*D*) increased linearly with water intake. Glomerular filtration rate (GFR) in green-backed firecrowns, however, was not influenced by water intake (*E*). Fractional renal water reabsorption (f_R) decreased linearly with water intake (*F*).

also significantly higher in the morning [RM-ANOVA: $F(1,4) = 2.58$, $P = 0.0326$].

Water absorption in the GIT. We did not find any evidence that green-backed firecrowns modulate water absorption in response to water intake. In the evening, fractional water absorption equaled 0.91 ± 0.08 and was not affected by the rate of water intake (LR: $P = 0.4164$; Fig. 2*B*). Despite the significantly greater rate of water intake observed during the morning, fractional water absorption in the morning was 0.88 ± 0.04 and similarly independent of the rate of water intake (LR: $P = 0.3398$; Fig. 2*B*). Fractional water absorption during the morning and evening was similar [RM-ANOVA: $F(1,4) = 0.00$, $P = 0.9373$].

Turnover of body water. As would be expected with the high and nonregulated water absorption observed in green-backed firecrowns, we found a positive linear relationship between fractional body water turnover and the rate of water intake (LR: evening, $y = -0.02 + 0.29x$, $r^2 = 0.97$, $P = 0.0005$; morning, $y = -0.02 + 0.27x$, $r^2 = 0.95$, $P = 0.0008$; Fig. 2*C*). The fractional turnover rate of body water was greater during the morning compared with the evening [RM-ANOVA: $F(1,4) = 3.37$, $P = 0.0214$].

Renal water load. Because green-backed firecrowns absorbed the majority of ingested water (Fig. 2*B*), our observation of a positive linear relationship between the rate of renal water loading and water intake rate is not unexpected (LR: evening, $y = 0.09 + 0.86x$, $r^2 = 0.97$, $P = 0.0003$; morning, $y = 0.23 + 0.83x$, $r^2 = 0.993$, $P < 0.0001$; Fig. 2*D*). Rates of renal water loading were marginally, albeit not significantly, greater during the morning compared with the evening [RM-ANOVA: $F(1,4) = 1.91$, $P = 0.0508$].

GFR. GFR was not influenced by subject [RM-ANOVA: $F(1,3) = 1.74$, $P = 0.2784$] or the sucrose concentration of food [RM-ANOVA: $F(1,3) = 0.21$, $P = 0.6785$], and we removed these parameters from the analyses in this section. There were significant differences among our GFR estimates [RM-ANOVA: $F(2,4) = 20.98$, $P = 0.0021$]. However, a Tukey's Honest Significant Difference test showed that these differences were only between nighttime GFR′ and the GFRs estimated during the evening and morning (Fig. 3). During the evening, GFR was 2.08 ± 0.56 ml/h (or 0.39 ± 0.08 $ml \cdot h^{-1} \cdot g^{-1}$), ~71% of the allometric expectation ($y = 0.85x^{0.74}$; see Ref. 23 and Fig. 3), and independent of water intake (LR: $P = 0.4858$; Fig. 2*E*). During the morning, GFR equaled 1.84 ± 0.68 ml/h (or 0.37 ± 0.12 $ml \cdot h^{-1} \cdot g^{-1}$; Fig. 3) and was not affected by water intake (LR: $P = 0.4651$; Fig. 2*E*). Morning GFR was ~63% of the allometric prediction (23; Fig. 3). Evening and morning GFRs were similarly independent of renal water load rate (LR: evening, $P = 0.4530$; morning, $P = 0.4392$).

We hypothesized that green-backed firecrowns would not arrest whole kidney GFR during the night; however, our estimate of nighttime GFR′ was 0.00 ± 0.05 ml/h (Fig. 3) and not different from zero (one-sample *t*-test: $t_5 = -0.05$, $P = 0.9619$).

Water reabsorption in the kidney. Although GFR is insensitive to water loading in green-backed firecrowns, water reabsorption appears to be responsive. During the evening, fractional renal water reabsorption decreased linearly with increased water intake (LR: $y = 0.75 - 0.11x$, $r^2 = 0.82$, $P = 0.0133$; Fig. 2*F*). We found a similar relationship between

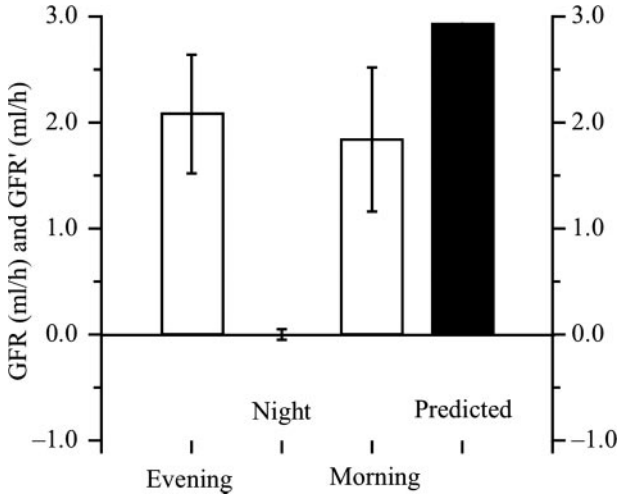

Fig. 3. GFR in green-backed firecrowns (*S. sephanoides*) during different times of day. Our GFR and mean GFR (GFR′) estimates (empty bars) were all lower than the allometric prediction of 2.92 ml/h (filled bar; see Ref. 23). We found that GFR during the evening (2.08 ± 0.56 ml/h) and morning (1.84 ± 0.68 ml/h) were similar. Nighttime GFR′ was 0.00 ± 0.05 ml/h and not different from 0. Data are means ± SD.

fractional renal water reabsorption and the rate of renal water loading (LR: $y = 0.77 - 0.12x$, $r^2 = 0.85$, $P = 0.0090$).

Nighttime hypothermia. Our nighttime body temperature estimates indicate that birds spent 15.1 ± 10.3% (1.75 ± 1.22 h) of the night phase hypothermic. Birds spent an increasing amount of time hypothermic as the length of night increased (LR: $y = -1{,}125.50 + 98.80x$, $r^2 = 0.79$, $P = 0.0180$). Nighttime GFR′ was, however, independent of both time hypothermic (LR: $P = 0.3387$) and percent of the night hypothermic (LR: $P = 0.3402$).

DISCUSSION

Our findings indicate that hummingbirds do not maintain water balance by regulating water-handling processes in the GIT. Rather, to meet the disparate challenges of eliminating and conserving water, hummingbirds appear to rely on their renal system. In our discussion, we examine this conclusion with comparisons to passerine nectarivores. We also consider the influence of TEWL, a substantial route of water loss in small vertebrates (20, 38, 48), on mechanisms of water balance in hummingbirds.

Avoiding overhydration. The water intake rates we measured in green-backed firecrowns, which ranged from 5.1 to 51.0% of body mass each hour (Fig. 2), although remarkable for a terrestrial homeotherm, are not extraordinary among nectar-feeding birds (3, 26, 29). Despite this range of water intake, which spans one order of magnitude (0.27–2.71 ml/h; Fig. 2), we found no evidence to suggest that hummingbirds modulate water absorption to avoid large renal water loads (Fig. 2*B*). From an energy acquisition perspective, where water transport accompanies nutrient absorption (25, 36, 37), this is expected. However, it indicates that passerine nectarivores, shown to modulate water absorption with no apparent effect on nutrient absorption (31), may avoid overhydration differently than hummingbirds. This hypothesis is drawn on only a few studies, however. With eight independent evolutions of nectarivory among birds (28) and opportunistic nectar feeding common (16), our understanding of the determinants of water-handling processes in the GIT is limited.

Despite absorbing ~90% of dietary water and confronting renal water loads that ranged from 0.31 to 2.47 ml/h, GFR was insensitive to water loading (Fig. 2*E*). It appears that eliminating this excess ingested water is accomplished by reducing water reabsorption (Fig. 2*F*) as opposed to increasing the rate at which body water is filtered. Similar findings and conclusions were drawn for two passerine nectarivores, the red wattlebird (*Anthochaera carunculata*) and the Palestine sunbird, where water reabsorption was more responsive to water loading than GFR (18, 32). In the broad-tailed hummingbird, however, both GFR and water reabsorption appear sensitive to water loading, but the relative importance of each mechanism for water elimination may vary with time of day (23).

Avoiding dehydration. Although GFR was not influenced by water loading (Fig. 2*E*), GFR does appear sensitive to water deprivation. During the night, a period of natural fasting for hummingbirds, we found that green-backed firecrowns arrested whole kidney GFR (Fig. 3). With no capacity to concentrate urine (27), the factor driving this response is the likely need to prevent urinary water losses when the only water input is from metabolism. The mechanism that is responsible for glomerular intermittency in birds has been described (9, 42); however, nothing is known with respect to how hummingbirds tolerate this renal "failure." Factors influencing this tolerance, among a potential suite of others, may be their low protein intake and total endogenous nitrogen losses, traits hummingbirds share with other nectar- and fruit-feeding birds (46).

What role does evaporation play in hummingbird water balance? Because our experimental design allows us to calculate the rate of water excretion ($\dot{V}_E$; ml/h), such that

$$\dot{V}_E = \dot{V}_I(1 - f_A) + GFR(1 - f_R)$$

we can address this question by estimating TEWL (ml/h) as

$$TEWL = \dot{V}_I + \dot{V}_M - \dot{V}_E$$

With the use of this approach, TEWL for green-backed firecrowns during the evening was 0.07 ± 0.32 ml/h. Although this estimate closely agrees with the allometric expectation of 0.04 ml/h ($y = 0.300x^{0.678}$; see Ref. 48), it is statistically indistinguishable from zero (one-sample *t*-test; $t_5 = 0.53$, $P = 0.6176$). Despite this, discerning the role of evaporation remains critical to understanding what mechanisms are responsible for maintaining water balance in nectarivores. With the use of our estimate, the green-backed firecrowns in this study lost ~2% of their body water to evaporation each hour. Because the water intake rates reported here were ~4–39 times greater than rates of TEWL, replacing water lost to evaporation appears trivial when hummingbirds are feeding. During fasts, however, insensible water loss is not inconsequential. In a 12-h night with TEWL equal to 0.07 ml/h, green-backed firecrowns would lose ~0.84 ml of body water lost to evaporation, which is roughly 28% of total body water. In terms of osmoregulation, oscillating between feeding and fasting for hummingbirds may be a more extreme scenario than that represented by organisms adapted to mesic and xeric environments. To meet these conflicting water balance demands, we have shown that hummingbirds rely on their renal system (Figs. 2 and 3). However, hummingbirds may be capable of modifying the

lipid composition of the stratum corneum to reduce cutaneous water loss (24, 44, 45) during extended fasts.

"Hummingbirds are the most amphibious of all birds." When William A. Calder III advised us of this, we could not have envisioned how well recent findings would support his insight. When active and feeding, water fluxes among nectar-feeding birds are closer to those of amphibians and freshwater fishes (3, 29). The water fluxes we measured in green-backed firecrowns, which ranged from 0.19 to 2.14 ml/h (Fig. 2*A*), do not contradict this idea and equate to fractional body water turnover rates of 0.06 to 0.69/h (Fig. 2*C*). Amphibians and hummingbirds also share renal morphological traits (4, 5), which may explain why both toads and hummingbirds dramatically reduce GFR during water stress (23, 47, and this study).

Among nectar-feeding vertebrates, small hummingbirds may be the most susceptible to dehydration during fasts (27, 38). However, they are not coping with a unique dilemma. Passerine nectarivores, although typically larger than hummingbirds (10, 13) and possessing a greater urine-concentrating capacity (14), are also susceptible to dehydration during extended periods of fasting (12). The same is true for nectar-feeding bats (41), yet mammals do not appear to modulate GFR to the same extent as birds (9). In general, our understanding of osmoregulation in nectarivorous vertebrates is limited to a small number of hummingbird species and a few passerine nectarivores. However, the gradients of nectarivory (39), mass (10), and renal morphology (4–6, 41) among nectar-feeding vertebrates presents an opportunity to resolve how diet, body size, and phylogeny affect the mechanisms vertebrates use to achieve water balance.

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