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Hummingbirds arrest their kidneys at night: diel variation in glomerular filtration rate in *Selasphorus platycercus*

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Summary

Small nectarivorous vertebrates face a quandary. When feeding, they must eliminate prodigious quantities of water; however, when they are not feeding, they are susceptible to dehydration. We examined the role of the kidney in the resolution of this osmoregulatory dilemma. Broad-tailed hummingbirds (*Selasphorus platycercus*) displayed diurnal variation in glomerular filtration rate (GFR). During the morning, midday and evening, GFRs were 0.9±0.6, 1.8±0.4 and 2.3±0.5 ml h^{-1}, respectively. At midday, GFR increased linearly with increased water intake. During the evening, hummingbirds decreased renal fractional water reabsorption linearly with increased water intake. Broad-tailed hummingbirds appeared to cease GFR at night (–0.1±0.2 ml h^{-1}) and decreased GFR in response to short-term (~1.5 h) water deprivation. GFR seems to be very responsive to water deprivation in hummingbirds. Although hummingbirds and other nectarivorous birds can consume astounding amounts of water, a phylogenetically explicit allometric analysis revealed that their diurnal GFRs are not different from the expectation based on body mass.

Key words: hummingbird, *Selasphorus platycercus*, glomerular filtration rate, renal fractional water reabsorption, diurnal variation, phylogenetically independent contrast, nectarivory, glomerular intermittency.

Introduction

Nectarivorous vertebrates face an osmoregulatory challenge. When feeding, they ingest astounding volumes of water (Martínez del Rio et al., 2001), yet they must prevent dehydration when they are not feeding (Powers, 1992). Therefore, achieving water balance requires the capacity to both eliminate and conserve water. Water conservation, however, requires different morphological characters and physiological processes from those necessary for water elimination (Dantzler, 1989; Goldstein and Skadhauge, 2000). Hummingbirds, because of their small body sizes (Dunning, 1992) and high mass-specific metabolisms (Suarez, 1992), are particularly challenged by this dilemma (Beuchat et al., 1990). How do hummingbirds meet these conflicting demands? In this article, we report the results of several experiments designed to shed light on the kidney's role in resolving this quandary.

As a consequence of ingesting food that is principally water (Baker, 1975), hummingbird water fluxes range from one to seven times their body mass (M_b) per day (Martínez del Rio et al., 2001). Because hummingbirds absorb essentially all ingested water that enters the gastrointestinal tract (McWhorter and Martínez del Rio, 1999), the renal system must play a critical role in maintaining water balance. To avoid overhydration (Faenestil, 1977), hummingbirds must rapidly eliminate a large fraction of ingested water. How do hummingbird kidneys respond to these high water loads? Glomerular filtration rate (GFR) sets the pace of water reabsorption and/or elimination by the kidney. Although GFR appears to be less sensitive to water loading than to water deprivation (Williams et al., 1991), we hypothesized that hummingbirds would increase GFR to eliminate excess ingested water (McWhorter et al., 2004). A second complementary possibility is that renal fractional water reabsorption (FWR) would decrease as water load increases (Goldstein and Bradshaw, 1998). Although the need to process large water loads may be, in part, ameliorated by high evaporative water loss (EWL) rates (Powers, 1992), these water losses can constitute a serious problem for hummingbirds when they are not feeding. Their inability to concentrate urine (Lotz and Martínez del Rio, 2004) in combination with their high EWL rates suggests a potentially acute risk of dehydration for hummingbirds. Water conservation is therefore necessary when they are not feeding, for example at night and during extended periods of flight.

How do hummingbirds reduce urinary water losses during non-feeding periods? GFR decreases in response to water deprivation in several bird species (Yokota et al., 1985;

Williams et al., 1991; Goldstein and Skadhauge, 2000). Because hummingbirds do not feed at night, they are likely to be dehydrated in the early morning and need to conserve water (Fleming et al., 2004). We hypothesized that GFR would be lower during both the night and morning relative to the evening (Goldstein and Rothschild, 1993). We also predicted that hummingbirds would reduce GFR during an episode of water deprivation.

Materials and methods

Hummingbird care

After mist-netting male broad-tailed hummingbirds (*Selasphorus platycercus* Swainson; M_b=3.60±0.40 g, N=10) in Albany County, Wyoming, USA (41°20′ N, 106°15′ W), we housed them in individual cages (0.6×0.6×0.6 m) kept at 24±1°C on a 13 h:11 h photoperiod (photophase: 07:00–20:00 h MST). Hummingbirds fed *ad libitum* on two maintenance diets. Between 08:00 and 18:00 h, they fed on a 13.0% (mass percent) solution of Nektar-Plus (Guenter Enderle, Tarpon Springs, FL, USA) supplemented with vitamins (0.4%; Nekton-S; Guenter Enderle) and sucrose (5.0%). From 18:00 to 08:00 h, they fed on a 25% sucrose solution. Hummingbirds had to hover to feed and were acclimated to captivity for two weeks before experiments began.

Experimental design

We conducted two experiments. The first investigated diel variation in renal function in hummingbirds feeding naturally. The second experiment probed the effect of food (and thus water) deprivation on renal function. In experiment 1, we measured both renal FWR and GFR from roughly 18:00 to 19:59 h (‘evening’). In the same experiment, we measured GFR from 20:00 to 06:59 h (‘night’) and from 07:00 to approximately 08:30 h (‘morning’). Experiment 2 was conducted from approximately 11:00 to 15:00 h. In this experiment, we first measured GFR in hummingbirds feeding voluntarily (‘midday’) and then removed the sucrose solutions from their cages (‘fast’). After this ~1.5 h fast, we returned the sucrose solutions and continued measuring GFR in freely feeding hummingbirds.

During experiments, hummingbirds were housed individually in opaque Plexiglas® cages (0.3×0.3×0.3 m). One cage panel was a Mylar®-coated, one-way glass mirror. Each cage contained one perch that was fitted with an insulated Cu–Cn thermocouple (±0.1°C; ΩOmega Corporation, Stamford, CT, USA) and suspended from an electronic balance (±0.01 g; Scout II; Ohaus Corporation, Florham Park, NJ, USA). Hummingbirds were acclimated to these cages for 2 days before each trial.

Hummingbirds increase their food intake when the sugar concentration of their food decreases (Martínez del Rio et al., 2001). To vary ingested water loads, we fed hummingbirds 292 and 876 mmol l^{-1} sucrose solutions. The fractional water contents of these solutions are 0.94 and 0.81, respectively. In this report, ‘food intake’ is the volume of sucrose solution ingested; ‘water intake’ is the ingested volume of preformed water; and ‘food/water’ refers to the sucrose solutions. Hummingbirds fed *ad libitum* on these sucrose solutions for ~4 h before a trial. We assigned trial order and sucrose concentration randomly for each hummingbird, and hovering was required to feed. All measurements were conducted at 24±1°C and the photoperiod held constant.

GFR and renal FWR estimates in hummingbirds

We estimated GFR using a single injection of [^{14}C]L-glucose (Chang et al., 2004) and a modified version of the slope-intercept method (Hall et al., 1977; Florijn et al., 1994). Our sole modification was that the marker disappearance rate from plasma is matched by its rate of appearance in excreta. In addition to the assumption of constant GFR made by the slope-intercept method (Hall et al., 1977; Florijn et al., 1994), our modification assumes constant renal FWR. Therefore, our method of estimating GFR can only be applied with a single compartment model of marker clearance. This same modification was used by McWhorter et al. (2004). It allows the investigation of renal function in unanesthetized free-flying birds.

Using our modified version of the slope-intercept method, three parameters are needed to estimate GFR: (1) Q_i, the quantity of marker injected (disints min^{-1}, hereafter d.p.m.); (2) $A_{i(0)}$, the zero-time intercept concentration of marker in plasma (d.p.m. ml^{-1}); and (3) $\dot{K}_{14C}$, the fractional turnover rate of marker (h^{-1}). Marker distribution space (S_P; in ml) is then:

$$S_P = Q_i \times A_{i(0)}^{-1} , \quad (1)$$

where $\dot{K}_{14C}$ is used to extrapolate to $A_{i(0)}$ from a single blood sample taken from each bird ~2 h after injection. GFR (ml h^{-1}) is then:

$$GFR = \dot{K}_{14C} \times S_P . \quad (2)$$

We determined $\dot{K}_{14C}$ as the exponent of exponential decay functions fitted to the relationship between the concentration of marker in excreta and time (Hall et al., 1977). Because hummingbirds do not void excreta during fasts, we estimated $\dot{K}_{14C}$ during the night and food/water deprivation periods as:

$$\dot{K}'_{14C} = (E_L - E_F) \times t^{-1} , \quad (3)$$

where E_L and E_F are the natural logarithms of specific activity in the last and first excreta samples (d.p.m. ml^{-1}) predicted by $\dot{K}_{14C}$ before and after the period of non-feeding, respectively, and t is the length (h) of the non-feeding period. Equation 3 can be substituted for $\dot{K}_{14C}$ in equation 2 to estimate mean GFR (GFR′) during the period when no excreta is voided so that:

$$GFR' = \dot{K}'_{14C} \times S_P . \quad (4)$$

We estimated renal FWR as:

$$FWR = 1 - (P_M \times U_M^{-1}) , \quad (5)$$

where P_M and U_M are the concentrations of marker in plasma and ureteral urine (d.p.m. μl^{-1}), respectively (Goldstein, 1993).

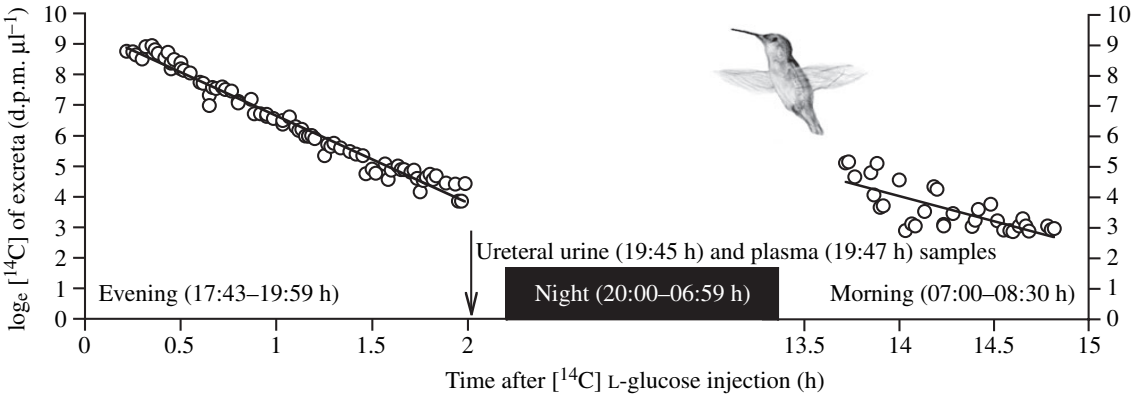

Fig. 1. Semi-logarithmic plot of data from one representative broad-tailed hummingbird illustrating (1) our protocol for estimating evening, night and morning glomerular filtration rates (GFR) and evening renal fractional water reabsorption and (2) that ^{14}C-labeled L-glucose appearance in excreta with time follows single-compartment, first-order kinetics. This particular humming bird had an evening and morning GFR of 1.9 and 1.1 ml h^{-1}, respectively (determined using equation 2); night GFR for this bird was 0.0 ml h^{-1} (determined using equation 4). Although data of [^{14}C] of excreta are log$_e$-transformed here for clarity, our analyses were performed on non-transformed data (Motulsky and Ransnas, 1987). We injected this particular hummingbird at 17:43 h and collected excreta samples until 19:43 h. Ureteral urine and plasma samples were taken from this hummingbird at 19:45 and 19:47 h, respectively. Our morning excreta collections suggested that whole-kidney GFR was interrupted overnight: there were no differences between the [^{14}C] of excreta in the first morning and last evening samples.

Experimental measurements

S_P, *GFR and renal FWR*

We injected each hummingbird in the pectoralis muscle with 9.25×10^4 Bq of [1-^{14}C]-L-glucose (Lot #345-058-050; Moravek Biochemicals, Brea, CA, USA) dissolved in 10 µl of deionized water. Injections were at ~18:00 and ~11:00 h for experiments 1 and 2, respectively. After injections, we collected excreta samples for >1 h. Following the initial excreta collection for experiment 1, we collected both a ureteral urine, using a close-ended polyethylene cannula (Goldstein and Braun, 1989), and blood sample (~10 µl). The blood sample was obtained by clipping a single toenail. We collected these samples between 19:40 and 19:59 h. We resumed collecting excreta the following morning. Fig. 1 illustrates our procedure for experiment 1. In experiment 2, we collected excreta samples before and after an ~1.5 h food/water deprivation period. Excreta samples were collected, using glass capillary tubes, from the wax paper that lined the cage bottom. We counted d.p.m. (LS 6000IC; Beckman Coulter, Fullerton, CA, USA) after dissolving injectate aliquots, excreta, ureteral urine and plasma samples in 7.0 ml liquid scintillation cocktail (EcoLume; ICN Biomedicals, Costa Mesa, CA, USA). All analyses were corrected for ^{14}C background, quench and chemiluminescence.

Body temperature (T_b)

Hummingbirds can enter torpor (Calder and Calder, 1992). To find out if hummingbirds remained normothermic during our measurements, we obtained estimates of T_b using insulated Cu–Cn thermocouples affixed to each perch and digital thermometers (±0.1°C; HH506; ΩOmega Corporation). The length of the perches (20 mm) forced birds to sit atop the thermocouple so that it contacted the abdomen skin surface. We calibrated perching temperatures with cloacal temperatures. Our criterion for hypothermia was any T_b estimate lower than 39.0°C (Calder and Calder, 1992). During the 11 h night phase, we measured T_b every 0.5 h; for all other experiments, we monitored T_b continuously.

Statistical analyses

Because the relationships between food intake and sugar concentration for nectarivorous birds are well described by power functions (Martínez del Rio et al., 2001), we log$_e$-transformed food intake and sucrose concentration data. To determine the effect of food intake rate and subject on GFR, we used repeated-measures analysis of variance (RM-ANOVA). To test for differences among means, we used Tukey's Honest Significant Difference (Tukey's HSD). In all other cases, we used linear models on non-transformed data to assess significance. We report values as means ± 1 S.D.

Results

After injection, the decline in ^{14}C concentration (hereafter [^{14}C]) of excreta with time followed single-compartment, first-order kinetics (Fig. 1). Mean coefficient of determination (r^2) values for log$_e$-transformed data during experiment 1 were 0.83±0.12 (N=10) and 0.43±0.23 (N=10) for the evening and morning, respectively (Fig. 1). During experiment 2, r^2 values were 0.75±0.15 (N=10) and 0.49±0.28 (N=10) before food/water was removed and when it was returned, respectively.

Our estimate of S_P in broad-tailed hummingbirds was 0.74±0.15 ml (N=9), which is approximately 20.6±4.2% of M_b. [^{14}C]L-glucose equilibration time was 19±11 min (N=20). The integrals of the relationship between [^{14}C] of excreta with time indicated that we recovered 97.3±1.1% of Q_i (N=20). Because

subject was a nonsignificant parameter in all our models (P>0.2), we removed this factor from all analyses.

Renal function and time of day

During the evening and morning, food intake rate increased significantly as the sucrose concentration decreased (RM-ANOVA: $F_{1,7}$=10.83, P=0.0133, N=9). During the evening, food intake rates were 1.17±0.37 (N=5) and 0.56±0.14 ml h^{-1} (N=4) on the 292 and 876 mmol l^{-1} solutions, respectively. Food intake rates during the morning were 1.11±0.39 (N=5) and 0.65±0.20 ml h^{-1} (N=4) on the 292 and 876 mmol l^{-1} solutions, respectively. GFR during these same time periods was not influenced by sucrose concentration (RM-ANOVA: $F_{1,7}$=1.54, P=0.25, N=9). We therefore removed sucrose concentration from the analyses described in this section.

There were significant differences among our GFR estimates (RM-ANOVA: $F_{2,7}$=59.9, P<0.0001, N=9), with Tukey's HSD tests revealing that $GFR_{EVENING}$, GFR'_{NIGHT} and $GFR_{MORNING}$ were all different from each other (Fig. 2). $GFR_{EVENING}$ was 2.3±0.5 ml h^{-1} (N=9), ~110% of the allometric prediction (GFR=0.013$M_b^{0.76}$; Bennett and Hughes, 2003; Fig. 2). There were no differences in [^{14}C] of excreta between the last evening and first morning samples (paired t-test: t_8=0.52, P=0.62, N=9; Fig. 1) and GFR'_{NIGHT} was –0.1±0.2 ml h^{-1} (N=9), suggesting an overnight interruption of whole-kidney GFR (Fig. 2). Our GFR'_{NIGHT} estimate was not different from 0 (t-test: t_8=–0.83, P>0.2, N=9). $GFR_{MORNING}$ was 0.9±0.6 ml h^{-1} (N=9) and was lower than $GFR_{EVENING}$ by a factor of 2.6 (Fig. 2).

Contrary to our prediction, water intake rate did not influence GFR during the evening or morning (linear regression: evening, P=0.27, N=9; morning, P=0.34, N=9; Fig. 3A). However, during the evening, renal FWR decreased linearly as water intake rate increased (y=–0.13x+0.89, r^2=0.66, P=0.03, N=7; Fig. 3B).

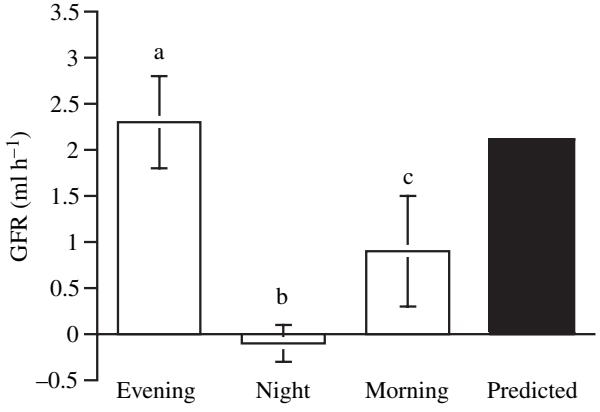

Fig. 2. Diel variation in glomerular filtration rate (GFR) in broad-tailed hummingbirds. Our GFR estimates for the evening, night and morning were 2.3±0.5, –0.1±0.2 and 0.9±0.6 ml h^{-1} (N=9), respectively, and were significantly different from each other. $GFR_{MORNING}$ was lower than $GFR_{EVENING}$ by a factor of 2.6, and GFR'_{NIGHT} was not different from 0. $GFR_{EVENING}$ was approximately 110% of the allometric prediction ($GFR_{PREDICTED}$=2.1 ml h^{-1}; Bennett and Hughes, 2003).

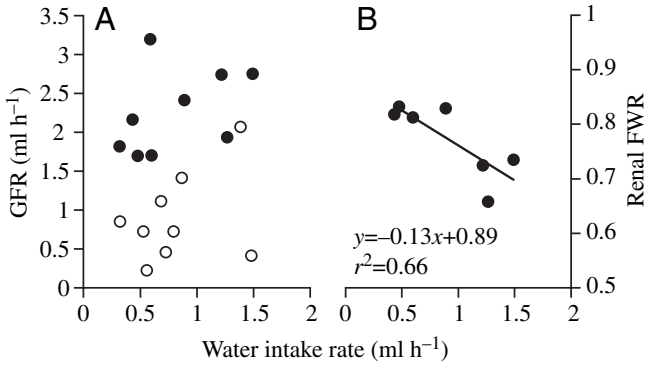

Fig. 3. Renal fractional water reabsorption (FWR) is responsive to water loading in broad-tailed hummingbirds. (A) There was no relationship between water intake rate and glomerular filtration rate (GFR) during the evening (filled circles) or morning (open circles). (B) Hummingbirds decreased renal FWR to dispose of excess ingested water during the evening (y=–0.13x+0.89, r^2=0.66, N=7).

GFR during food/water deprivation

At midday, food intake rate increased significantly as sucrose concentration decreased (RM-ANOVA: $F_{1,7}$=30.44, P=0.0009, N=9). These intake rates were 0.9±0.3 (N=5) and 0.4±0.2 ml h^{-1} (N=4) on the 292 and 876 mmol l^{-1} solutions, respectively. GFR, however, was not affected by sucrose concentration (RM-ANOVA: $F_{1,7}$=0.75, P=0.42, N=9). Following the ~1.5 h food/water deprivation period, sucrose concentration did not affect food intake rate (RM-ANOVA: $F_{1,7}$=0.94, P=0.36, N=9) or GFR (RM-ANOVA: $F_{1,7}$= 0.00, P=0.9930, N=9). We removed sucrose concentration from our analyses presented in this section.

Before food/water removal, GFR_{MIDDAY} was 1.8±0.4 ml h^{-1} (N=9; Fig. 4). During the food/water deprivation period, GFR'_{FAST} (0.9±0.5 ml h^{-1}; N=9) was 50% lower than GFR_{MIDDAY} (Fig. 4). When we returned the food/water,

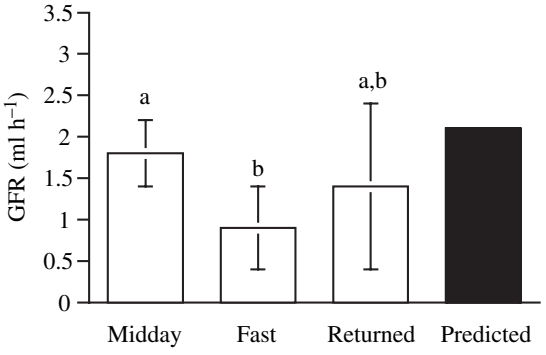

Fig. 4. Glomerular filtration rates (GFR) in broad-tailed hummingbirds before, during and after a ~1.5 h food/water deprivation episode. During food/water removal, mean GFR was significantly reduced relative to the pre-removal period (GFR'_{FAST} and GFR_{MIDDAY} were equal to 0.9±0.5 and 1.8±0.4 ml h^{-1}, respectively; N=9). When we returned the food/water, $GFR_{RETURNED}$ (1.4±1.0 ml h^{-1}; N=9) was not different from either GFR_{MIDDAY} or GFR'_{FAST}. $GFR_{PREDICTED}$ is equal to 2.1 ml h^{-1} (Bennett and Hughes, 2003).

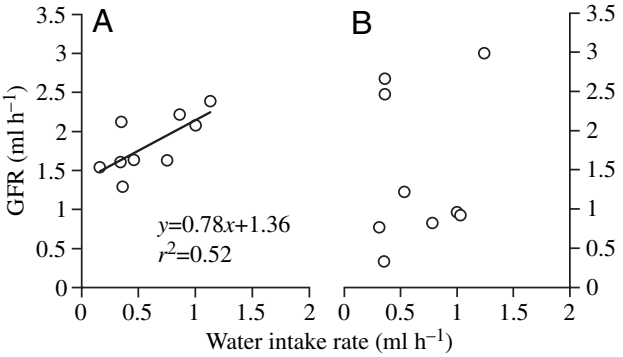

Fig. 5. Glomerular filtration rate (GFR) is responsive to water loading in broad-tailed hummingbirds. (A) During midday, prior to food/water removal, GFR increased linearly with increased water intake rate (y=0.78x+1.36, r^2=0.52, N=9). (B) When we returned the food/water, following the ~1.5 h deprivation period, there was no relationship between water intake rate and GFR.

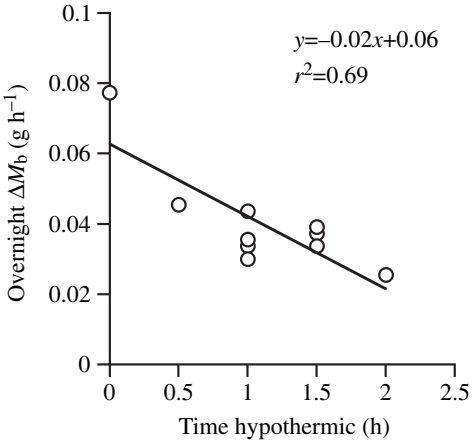

Fig. 6. Overnight body mass (M_b) losses in broad-tailed hummingbirds are influenced by the time spent hypothermic. The rate of change in body mass (ΔM_b; g h^{-1}) during the night decreased as time (h) spent hypothermic increased (y=–0.02x+0.06, r^2=0.69, N=10).

$GFR_{RETURNED}$ was 1.4±1.0 ml h^{-1} (N=9; Fig. 4). Our GFR estimates differed significantly (RM-ANOVA: $F_{2,7}$=9.79, P=0.0094, N=9), but Tukey's HSD tests showed that these differences were only between GFR_{MIDDAY} and GFR'_{FAST}; both GFR_{MIDDAY} and GFR'_{FAST} were not significantly different from $GFR_{RETURNED}$ (Fig. 4). GFR_{MIDDAY} increased significantly as water intake rate increased (y=0.78x+1.36, r^2=0.52, P=0.03, N=9; Fig. 5A). However, $GFR_{RETURNED}$ was not influenced by water intake rate (linear regression: P=0.71, N=9; Fig. 5B).

T_b *and* M_b *estimation*

Hummingbirds were normothermic throughout all experimental trials except at night, where they spent 10.4±5.3% of the 11 h dark phase hypothermic (N=10). The rate of change in M_b (ΔM_b) during the night was –0.04±0.01 g h^{-1} (N=10) and decreased linearly as time spent hypothermic increased (y=–0.02x+0.06, r^2=0.69, P=0.0028, N=10; Fig. 6). During the food/water deprivation period, ΔM_b was –0.25±0.11 g h^{-1} (N=8) and was significantly higher than overnight ΔM_b (paired t-test: t_7=4.94, P=0.0017, N=8).

Discussion

GFR in broad-tailed hummingbirds varied throughout the day and in response to food/water deprivation. Perhaps the most surprising result of this study is the seeming cessation of GFR by hummingbirds during the night. Here, we first consider the diurnal variation in GFR displayed by hummingbirds; then we discuss the renal responses to food/water deprivation, paying particular attention to the observation of an overnight interruption in whole-kidney GFR. We conclude by using a phylogenetic approach to determine whether diurnal GFR in nectarivorous birds conforms to the allometric expectation.

Diurnal variation in renal function

Broad-tailed hummingbirds displayed significant diurnal variation in GFR. They had a low GFR in the morning (0.9±0.6 ml h^{-1}; Fig. 2), an intermediate GFR at midday (1.8±0.4 ml h^{-1}; Fig. 4) and a high GFR in the evening (2.3±0.5 ml h^{-1}; Fig. 2). It is likely that hummingbirds filter slowly in the morning to conserve water and hydrate after a night of water losses (Fleming et al., 2004). Because intake rates during the day are sufficient for birds to hydrate within a few hours (Collins, 1981), the observation of a gradual increase in GFR throughout the day is perplexing but seems to be a pattern shared by other birds. Goldstein and Rothschild (1993) reported a similar pattern in song sparrows (*Melospiza melodia*).

GFR during food/water deprivation

When hummingbirds were deprived of food/water, they reduced mean GFR (Fig. 4). This finding is consistent with the responses to water deprivation observed in other birds (Williams et al., 1991; Goldstein and Skadhauge, 2000). There is, however, one notable difference. In most of the other species examined, the reduction in GFR occurs progressively over a period of several days (Williams et al., 1991). Yet, hummingbirds modulated GFR within 1.5 h of deprivation (Fig. 4). This observation is not surprising, but it illustrates that GFR in hummingbirds is particularly sensitive and responsive to food/water deprivation. Although broad-tailed hummingbirds reduced mean GFR significantly during the deprivation period, they displayed a wide range of responses (Fig. 7). The reduction in mean GFR ranged from moderate (~25%; Fig. 7B) to almost complete (~90%; Fig. 7C). This variation may be explained by differences in water balance status among birds prior to food/water removal.

GFR during the night

Although our observation is not the first evidence of intermittent renal filtration in birds (Braun and Dantzler, 1972;

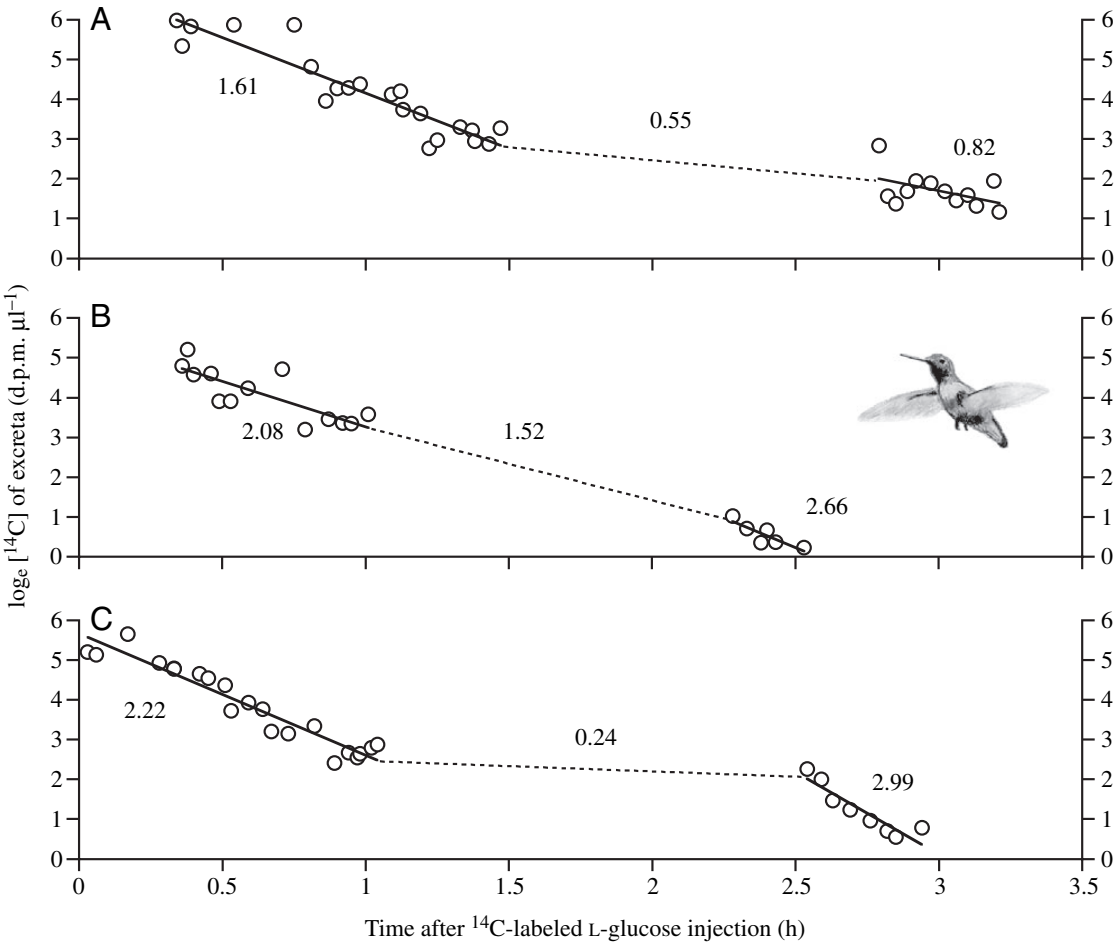

Fig. 7. Despite the overall trend (Fig. 4), broad-tailed hummingbirds showed heterogeneous responses to food/water deprivation. Values associated with each regression line are glomerular filtration rate (GFR; ml h^{-1}) estimates. Solid regression lines denote estimates obtained using equation 2; broken regression lines denote estimates made using equation 4. Although data of [^{14}C] of excreta are log$_e$-transformed here for clarity, our analyses were performed on non-transformed data (Motulsky and Ransnas, 1987). (A) Data from one representative hummingbird. (B) Data from one hummingbird illustrating that GFR′$_{FAST}$ was not always substantially reduced (~25%). (C) Data from one hummingbird showing a GFR′$_{FAST}$ that was considerably reduced (~90%). The hummingbirds in B and C exhibited a GFR$_{RETURNED}$ greater than GFR$_{MIDDAY}$.

Goldstein, 1993), it represents the first account of what appears to be interrupted whole-kidney GFR in a normothermic bird. Our observation of arrested nighttime renal filtration in broad-tailed hummingbirds (Figs 1, 2) is noteworthy for two reasons. First, because hummingbirds were normothermic for ~90% of the night, the cessation of renal filtration was not a result of reduced pressure in the renal arteries due to hypothermia (Glahn et al., 1993). We cannot, however, rule out a nocturnal dip in systemic blood pressure (Miyazaki et al., 2002). Second, a sudden decrease in whole-kidney GFR disrupts homeostatic processes and can have pathological consequences (Anderson and Schier, 2001). How do hummingbirds cope with arresting whole-kidney GFR? This is an intriguing question, but one that is presently open. The ability to interrupt GFR, however, is better understood.

In birds, the reduction in GFR is believed to result from vasoconstriction of the pre-glomerular arterial vessels that supply 'loopless' nephrons (Dantzler, 1989). This vasoconstriction is mediated by arginine vasotocin (Braun, 1976; Giladi et al., 1997; Goecke and Goldstein, 1997). In hummingbirds, more than 99% of all nephrons are loopless (Casotti et al., 1998). Consequently, hummingbirds cannot concentrate urine (Lotz and Martínez del Rio, 2004), but they can reduce urinary water losses by decreasing GFR. This mechanism has a potential drawback. In mammals, the cessation of filtration due to vasoconstriction of afferent arterioles can lead to damage of renal cells from ischemia (Hays, 1992). How do hummingbirds nourish these cells when GFR is suspended?

Birds, like other vertebrates with intermittent glomerular filtration, have a renal portal system (Dantzler, 1989; Smith et al., 2000). Dantzler (1989) hypothesized that this renal portal circulation may perfuse nonfiltering loopless nephrons in the absence of a post-glomerular blood supply. Additionally, other researchers have noted glomerular bypasses in the arterial vasculature of the avian kidney (Siller and Hindle, 1969; Kurihara and Yasuda, 1975). Although these features may allow the perfusion of renal cells when filtration is suspended, their relative importance is unknown.

Table 1. *Glomerular filtration rates (GFR) in birds with differing food habits*

Food habit Species	Body mass (g)*	GFR (ml h^{-1})†	Predicted GFR (ml h^{-1})‡
Nectarivory			
Calypte anna	5.1	2.4	2.7
Selasphorus platycercus§	3.6	2.3	2.1
Anthochaera carunculata	99	21.0	25.6
Nectarinia osea	5.8	1.8	3.0
Omnivory			
Dromaius novaehollandiae	40700	972	2485
Anas platyrhynchos	983	162.0	146.7
Anas platyrhynchos var. *dom.*	2513	446.4	299.4
Aythya valisineria	1052	136.2	154.5
Gallus gallus var. *dom.*	1890	247.8	241.1
Coturnix chinensis	51.4	33.0	15.6
Alectoris chukar	511.7	34.8	89.3
Melopsittacus undulatus	37.5	8.4	12.3
Cacatua roseicapilla	335.9	47.4	64.9
Sturnus vulgaris	77.1	30.0	21.2
Melospiza melodia	18.4	7.8	7.1
Herbivory			
Branta canadensis	3670	374.4	399.3
Callipepla gambelii	158.4	13.8	36.6
Meleagris gallopavo	7400	340.8	680.3
Coturnix pectoralis	107.3	40.8	27.3
Coturnix japonica	122.3	93.0	30.1
Zenaida macroura	119	16.2	29.5
Columba livia	569.3	242.4	96.9
Passer domesticus	22.8	7.8	8.4
Carnivory			
Bucephala islandica	767	243.6	121.5
Falco sparverius	126.0	16.2	30.8
Larus argentatus	1000	264.0	148.6
Larus dominicanus	905	185.4	137.8
Larus glaucescens	900	230.4	137.2

*Source: table 1 in Bennett and Hughes (2003), except where noted otherwise.
†Recalculated from table 1 in Bennett and Hughes (2003).
‡GFR=0.013$M_b^{0.76}$ (Bennett and Hughes, 2003).
§Present study.

GFR and nectarivory

One would expect high GFRs in animals with the astounding water intakes that characterize nectarivorous birds (Yokota et al., 1985; McWhorter et al., 2004). Accordingly, our estimate of GFR in broad-tailed hummingbirds exceeded the allometric prediction (Table 1). The other available data for nectarivorous birds, however, suggest that GFRs are lower than expected (Table 1; Bennett and Hughes, 2003; McWhorter et al., 2004). To find out if diurnal GFR is higher or lower than expected from M_b in nectarivorous birds (Calder and Braun, 1983), we used phylogenetically independent contrasts (PICs) and the method proposed by Garland and Adolph (1994). We used the DNA–DNA hybridization tapestry of Sibley and Ahlquist (1991) as a hypothesis for the phylogenetic relationships and evolutionary distances of birds (Fig. 8A). Briefly, we constructed a regression through the origin with all the standardized phylogenetic contrasts of $\log_{10}(M_b)$ and $\log_{10}$(GFR). This regression excluded the nectarivorous species. We then determined whether the contrasts including nectarivorous birds were within or outside the 95% confidence interval for this relationship.

Before phylogenetic correction, the relationship between M_b (g) and GFR (ml h^{-1}) was described by a power function (y=–0.85$x^{0.74}$, r^2=0.90, N=28; Fig. 8B). The exponent obtained using PICs (0.72±0.10; N=23; Fig. 8C) was similar to that obtained from the phylogenetically uncorrected regression (0.74±0.26; Fig. 8B). The points for the contrasts that included the clades of nectarivorous birds fell within the 95% confidence interval for the regression line. Despite the high water fluxes that characterize nectarivorous birds, these

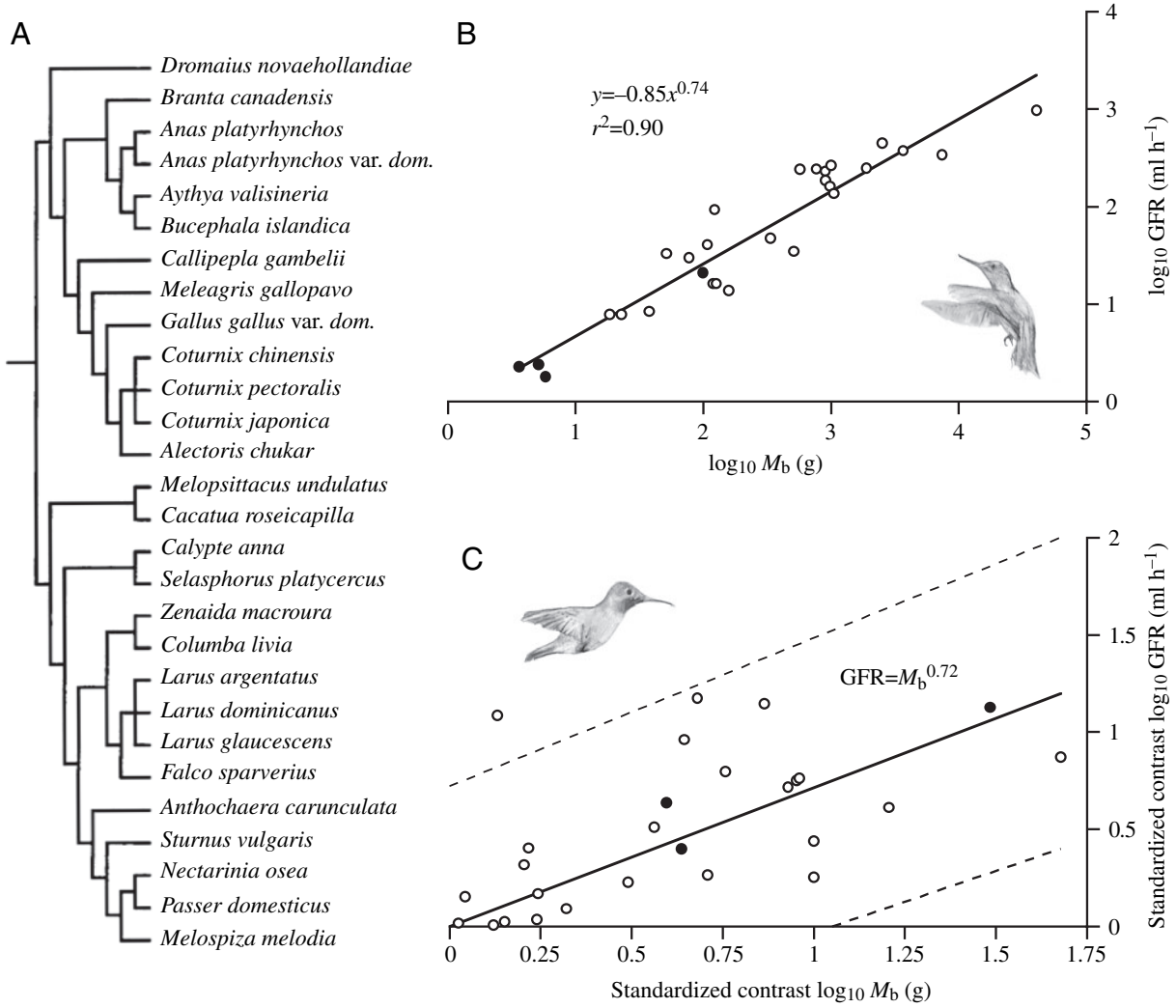

Fig. 8. Do nectarivorous birds have different diurnal glomerular filtration rates (GFRs) compared with equal-sized birds with other dietary habits? (A) Phylogeny of avian species with GFR data (Table 1). (B) The relationship between body mass (M_b; g) and GFR (ml h^{-1}) was described by a power function with an exponent equal to 0.74±0.26 (N=28). (C) The phylogenetically independent contrasts between $\log_{10}(M_b)$ and $\log_{10}$(GFR) for nectarivorous birds (filled circles) are within the 95% confidence interval (represented by the broken lines) of the regression line relating the standardized contrasts of $\log_{10}(M_b)$ and $\log_{10}$(GFR) for species with other dietary habits. The exponent of this phylogenetically corrected relationship equals 0.72±0.10 (N=23).

animals do not seem to have unusual rates of glomerular filtration. This conclusion, however, must be treated with caution. An overnight mean GFR of 0 may qualify broad-tailed hummingbirds as outliers. If labile GFRs (Goldstein and Rothschild, 1993; present study) are common among birds, the time of GFR measurement cannot be ignored in comparative analyses.

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