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Renal function in Palestine sunbirds: elimination of excess water does not constrain energy intake

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Summary

Although the renal responses of birds to dehydration have received significant attention, the consequences of ingesting and processing large quantities of water have been less studied. Nectar-feeding birds must often deal with exceptionally high water intake rates in order to meet their high mass-specific energy demands. Birds that ingest large volumes of water may either eliminate excess water in the kidney or regulate the volume of water absorbed in the gastrointestinal tract. Because water absorption in the gastrointestinal tract of Palestine sunbirds (*Nectarinia osea*) decreases with increasing water ingestion rate, we predicted that glomerular filtration rate (GFR) in these birds would not be unusually high in spite of large ingested water loads. When feeding on dilute sucrose solutions, sunbirds ingested between 4 and 6 times their body mass in nectar per day, yet they were able to compensate for varying nectar energy density and increased thermoregulatory energy demands with no apparent difficulty. GFR was lower than predicted (1976.22±91.95 μl h^{-1}), and was not exceptionally sensitive to water loading. Plasma glucose concentrations were high, and varied 1.8-fold between fasted (16.08± 0.75 mmol l^{-1}) and fed (28.18±0.68 mmol l^{-1}) sunbirds, but because GFR was low, glucose filtered load also remained relatively low. Essentially the entire glucose filtered load (98%) was recovered by the kidney. Renal fractional water reabsorption (FWR) decreased from 0.98 to 0.64 with increasing water intake. The ability of Palestine sunbirds to reduce the absorption of ingested water in the gastrointestinal tract may resolve the potential conflict between filtering a large excess of absorbed water in the kidney and simultaneously retaining filtered metabolites.

Key words: Palestine sunbird, *Nectarinia osea*, glomerular filtration rate, nectar, glucose, osmoregulation, water balance, kidney, renal function.

Introduction

Studies of renal processes in birds have emphasized dehydration over diuresis (see Braun, 1993). The nature of the relationship between water load and glomerular filtration rate (GFR) has therefore not been described for birds experiencing a large range of water loads (Goldstein and Bradshaw, 1998; Goldstein and Skadhauge, 2000). Nectar-feeding birds are of special interest because they are capable of ingesting astounding volumes of water (reviewed by Martínez del Rio et al., 2001). It is generally believed that GFR is more variable and more responsive to water status in birds than in mammals (Williams et al., 1991; Dantzler, 1992; Osono and Nishimura, 1994; Goldstein, 1995). GFR decreases in response to water deprivation in many avian species (Williams et al., 1991; Goldstein and Skadhauge, 2000) and appears to increase only moderately in response to water loading (Skadhauge and Schmidt-Nielsen, 1967; Braun and Dantzler, 1975; Roberts and Dantzler, 1989). The GFR data available, however, are largely for birds that do not regularly cope with large ingested water loads. The physiological mechanisms that allow nectar-feeding birds to contend with their watery diets, and the consequences of ingesting and processing sizeable quantities of water for energy intake and the maintenance of metabolite and electrolyte homeostasis, are relatively unexplored.

Nectar-feeding birds are faced with the conflicting demands of eliminating excess water and metabolic by-products while retaining electrolytes, metabolites and substrates for energy metabolism (Yokota et al., 1985). Plasma glucose levels in hummingbirds are high and surprisingly variable (ranging from 17 mmol l^{-1} in fasted birds to as much as 40 mmol l^{-1} in feeding individuals; Beuchat and Chong, 1998), resulting in relatively high estimated glucose filtered loads (the product of GFR and the concentration of glucose in plasma). How do

these birds prevent the loss of glucose to urine? In the mammals and birds for which renal glucose recovery has been investigated (summarized in Beyenbach, 1985), the high plasma glucose concentrations found in nectar-feeding birds would lead to severe renal glucose loss and presumably osmotic diuresis. Hummingbirds produce extremely dilute urine (Calder and Hiebert, 1983; Lotz and Martínez del Rio, 2004) and the morphology of their kidneys suggests that they are well suited for water disposal (Johnson and Mugaas, 1970; Casotti et al., 1998; Beuchat et al., 1999). Because hummingbirds also appear to absorb essentially all ingested water (McWhorter and Martínez del Rio, 1999), they probably rely on a large renal capacity for water elimination (and thus energetically expensive renal glucose and electrolyte reabsorption) and on relatively high rates of evaporative water loss (Lasiewski, 1964; Powers, 1992) to maintain water balance. The problem of excess ingested water, however, can be handled both from the supply and disposal sides of the equation. McWhorter et al. (2003) recently found that one species of nectar-feeding sunbird (Nectariniidae) reduces the fractional absorption of ingested water with increasing water intake rate. Sunbirds may therefore avoid a substantial absorbed water load, and thus the associated costs of recovering metabolites in the kidney and potential limitations to energy intake, when feeding on dilute nectars.

Here we report the results of experiments designed to examine the relationship between energy and water intake and kidney function in the Palestine sunbird [*Nectarinia osea* (Bonaparte 1856)], an Old World passerine nectarivore. Despite water intake rates that exceed several times their body mass per day (Lotz and Nicolson, 1999; McWhorter et al., 2003; Nicolson and Fleming, 2003), sunbirds, unlike hummingbirds, may not face exceptional renal water loads. We hypothesized that GFR in the Palestine sunbird would be lower than in hummingbirds and consistent with the allometric prediction of 4.3 ml h^{-1} for a bird of its body mass (Yokota et al., 1985; Williams et al., 1991), and would not be especially sensitive to water loading (Goldstein and Bradshaw, 1998). With this hypothesis in mind, we predicted that sunbirds would have plasma glucose concentrations comparable to those of hummingbirds (Beuchat and Chong, 1998), but relatively lower glucose filtered loads, and would consequently excrete very little glucose (McWhorter and Martínez del Rio, 2000). We further predicted that fractional water reabsorption (FWR) by the kidney would decrease with increasing water load (Goldstein and Bradshaw, 1998).

Materials and methods

Bird capture and maintenance

Male Palestine sunbirds [*Nectarinia osea* (Bonaparte 1856) (body mass 5.81±0.19 g, N=13)] were captured with drop nets on the grounds of Midreshet Ben-Gurion, home of the Sede Boqer Campus of Ben-Gurion University of the Negev, Israel (30°51′N, 34°46′E), under Israel Nature and National Parks Protection Authority permits 5981 and 7686. Birds were housed individually in outdoor aviaries (1.5 m×1.5 m×2.5 m) and fed a maintenance diet of two artificial nectar solutions between experiments. The diets included a 20–25% sucrose equivalent solution and a 15% sucrose solution supplemented with a soy protein infant formula (Isomil™, Abbott Laboratories, Hoofddorp, The Netherlands) diluted to approximately 2.5 g protein per 100 g sucrose. Food and water were available *ad libitum*. Birds were also offered freshly killed fruit flies (*Drosophila* sp.) at least twice a week. During experiments, birds were housed individually in opaque Plexiglas® cages (0.3 m×0.3 m×0.3 m) with individual light sources. The front of these cages was coated with a reflective Mylar™ polyester film to create a one-way mirror effect that permitted observation of birds in a darkened room with minimal disturbance. One of the perches in the center of each cage was fitted to hang from an electronic balance (Scout II 200 g×0.01 g, Ohaus Corporation, Florham Park, NJ, USA) so body mass could be monitored continuously. Birds were allowed to acclimate to cages for 2–3 days before experiments began and were left undisturbed in outdoor aviaries for a minimum of 7 days between trials. The study was conducted using light cycles that matched the natural photoperiod (13.25–14.5 h light). Birds were fed experimental diets, which consisted of sucrose solutions made with distilled water, for a minimum of 24 h before trials began.

Experimental design

We relied on the behavioral responses of birds to nectar of varying energy density in the design of this experiment. Typically, nectar-feeding birds reduce their food (hereafter ‘nectar’, normally the source of both energy and water in these animals) intake rate with increasing sugar concentration (López-Calleja et al., 1997; McWhorter and Martínez del Rio, 1999, 2000; McWhorter and López-Calleja, 2000; Martínez del Rio et al., 2001). Manipulation of sugar concentration therefore leads to a wide range of variation in the quantity of nectar (and thus water) ingested. We used a repeated-measures design in which we measured GFR and renal fractional recovery of filtered water (FWR) in eight sunbirds fed five different sugar solutions (146, 292, 584, 876 and 1168 mmol l^{-1} sucrose) at two ambient temperatures (15±1 and 30±2°C). In a separate repeated-measures experiment, we measured urine and excreted fluid osmotic concentration and glucose concentration in eight sunbirds fed on four sugar solutions (146, 292, 584 and 1168 mmol l^{-1} sucrose) at three ambient temperatures (5±2, 15±1 and 30±2°C). In both experiments, we randomized the order in which diet and temperature treatments were presented to subjects. Ambient temperature was varied within the range that these sunbirds normally experience to elicit a wide range of energy demands and thus nectar intake rates. Finally, we measured the plasma glucose concentration of nine sunbirds, both when feeding on their normal maintenance diet (described above) and after a 12 h overnight fast, in a repeated-measures design. Birds were randomly assigned to the first treatment (i.e., fed *vs* fasted) and all measurements were conducted at 25±2°C.

Estimating GFR and FWR in sunbirds

GFR was estimated with a single injection of ^{14}C-labeled inulin, using a modification of the slope-intercept method (Hall et al., 1977; Florijn et al., 1994). The only assumption we made in modifying this method was that the rate of marker disappearance from plasma was equal to the rate of appearance in excreta. The concentration of marker would of course be different among plasma, urine and excreta because of reabsorption of filtered water in the kidney and mixing of urine with gut contents in the cloaca. Our method allowed us to measure renal function in unanesthetized, actively feeding birds with minimal disturbance. GFR (μl h^{-1}) was estimated as:

$$\mathrm{GFR} = Q_\mathrm{i} \times K_{^{14}\mathrm{C}} \times A_{\mathrm{i}(0)}^{-1}, \quad (1)$$

where Q_i is the quantity of marker injected (disints min^{-1}), $K_{^{14}\mathrm{C}}$ is the fractional inulin turnover rate (h^{-1}), and $A_{\mathrm{i}(0)}$ is the zero-time intercept concentration of marker in plasma (disints min^{-1} μl^{-1}). Fractional inulin turnover rate was estimated by fitting negative exponential functions (Hall et al., 1977) to the relationship between the concentration of ^{14}C in excreta and time. The slope of the fractional inulin turnover curve was then used to extrapolate the plasma marker concentration of a single blood sample, taken 2–3 h after injection, to the zero-time intercept concentration (and thus also estimate the inulin distribution space). This method was used because of the sensitivity of small birds to repeated blood sampling. Fractional recovery of filtered water in the kidney (FWR) was estimated as 1 minus the ratio of marker concentration in plasma (P_M) to that in urine (U_M) (FWR=1–[$P_\mathrm{M}\times U_\mathrm{M}^{-1}$]).

Experimental measurements

GFR and FWR measurements

We injected 4.63×10^4 Bq of inulin-[^{14}C]-carboxylic acid (molecular mass 5175±95; Amersham, Piscataway, NJ, USA) in 15 μl of distilled water into the *pectoralis* of each bird approximately 1.5 h after the lights came on. Injection volumes were verified gravimetrically by weighing syringes (25 μl, Hamilton Company, Reno, NV, USA) to ±0.0001 g before and after injection. Fresh excreta samples were collected for 2–3 h, after which a ureteral urine sample was collected with a closed-ended polyethylene cannula (Goldstein and Braun, 1989) and a blood sample (approximately 50 μl) was collected by puncturing the brachial vein. We separated plasma from blood cells before radioisotope analysis. Liquid scintillation cocktail (ACS II, Amersham) was added to all excreta, plasma, urine and injection samples, which were counted correcting for quench and lumex (chemiluminescence) in a Packard Tri-Carb 1600TR Liquid Scintillation Analyzer (Perkin-Elmer Life and Analytical Sciences, Boston, MA, USA).

Excreted fluid and ureteral urine glucose and osmotic concentration measurements

Fresh excreta samples were collected from actively feeding sunbirds over a 30 min period, pooled for each bird separately, and immediately frozen for later analysis. After excreta collection was completed, we captured birds and collected a ureteral urine sample with a closed-end polyethylene cannula (Goldstein and Braun, 1989). We measured the osmotic concentration of the samples using an Osmette II freezing point depression osmometer (Precision Systems Inc., Natick, MA, USA), and glucose concentration using a clinical diagnostic kit (Procedure No. 315, enzymatic determination by the Trinder reaction; Sigma Chemical, St Louis, MO, USA).

Plasma glucose measurements

We collected blood samples (approximately 30 μl) by puncturing the brachial vein 1 h after the lights came on. Fed birds were allowed to feed normally for 1 h before sampling. Plasma was separated from the blood sample and immediately assayed for glucose concentration as above.

Statistical analysis

Since relationships between nectar intake rate and sugar concentration in nectar-feeding birds are power functions (López-Calleja et al., 1997; McWhorter and Martínez del Rio, 1999, 2000; McWhorter and López-Calleja, 2000; Martínez del Rio et al., 2001; Nicolson and Fleming, 2003), we determined the effects of temperature and individual bird (subject) on nectar intake rate using linear models of $\log_e$-transformed intake and sucrose concentration data. Analysis of covariance (ANCOVA) was used on $\log_e$-transformed data to compare the slope and intercept of this relationship among experimental temperatures. The relationships between the osmotic and glucose concentrations of ureteral urine and excreted fluid and water intake rate were best described by power functions, so we similarly applied linear models to $\log_e$-transformed data. We used linear models on untransformed data to assess significance and subject and temperature effects in all other cases. Repeated-measures analysis of variance (RM-ANOVA) was used to assess differences in plasma glucose concentration between fed and fasted birds. All values are presented as means ± S.E.M.

Results

GFR and FWR measurements

Sunbirds consumed significantly less nectar as sucrose concentration in the diet increased ($F_{1,27}$=382.1, P<0.0001, N=37; Fig. 1B). Nectar intake rate was significantly higher at 15°C than at 30°C (approximately 1.4-fold, averaged for all diet sucrose concentrations; $F_{1,27}$=42.15, P<0.0001). There was no significant effect of subject ($F_{7,27}$=2.19, P=0.07) on nectar intake rate, so we removed this variable from the model. We described the relationship between nectar intake and sucrose concentration using a power function for each temperature separately (Fig. 1B). The exponents of these relationships were not significantly different from –1 (15°C, t=1.87, d.f.=19, P>0.05; 30°C, t=0.72, d.f.=16, P>0.05) or from each other (ANCOVA$_{\mathrm{slopes}}$ $F_{1,33}$=2.76, P=0.11). Sucrose intake rate was 1.6-fold greater at 15°C than at 30°C

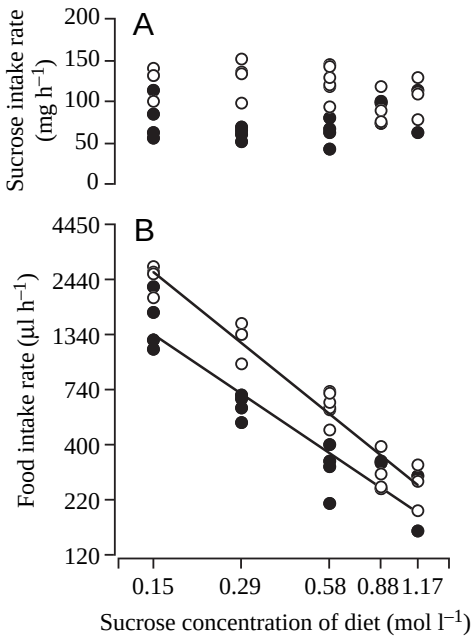

Fig. 1. Palestine sunbirds reduced their nectar intake rates in response to increased sucrose concentration in nectar. Energy intake therefore remained relatively constant at a level that appeared to be dictated by ambient temperature, and hence by thermoregulatory demands. (A) Sucrose intake by sunbirds was not significantly correlated with sucrose concentration in nectar, despite nectar intake rates that varied 7.2-fold (for 30°C; filled circles) to 9.5-fold (for 15°C; open circles) between the lowest and the highest sucrose concentrations. Sucrose intake was 1.6 times greater at 15°C than at 30°C, averaging 119.45±5.08 mg h^{-1} (27.76±1.18 kJ day^{-1}) at 15°C and 75.28±5.17 mg h^{-1} (17.49±1.2 kJ day^{-1}) at 30°C. (B) Sunbirds consumed significantly less nectar as dietary sucrose concentration increased. Nectar intake rate was significantly higher at 15°C than at 30°C. We described the relationship between nectar intake and sucrose concentration using a power function for each temperature separately (15°C, $y=313.24x^{-1.11}$, $r^2=0.95$; 30°C, $y=224.63x^{-0.93}$, $r^2=0.87$). The exponents of these relationships were not significantly different from –1. When feeding on the most dilute sucrose solution (0.146 mol l^{-1}), sunbirds consumed between 4 and 6 times their body mass in nectar in 14 h of daylight, depending on ambient temperature. Note that both axes in B and the *x*-axis of A are logarithmic scales.

($F_{1,27}$=42.59, P<0.0001), but was not correlated with dietary sucrose concentration ($F_{1,27}$=2.13, P=0.16). Hence, although nectar, and thus water, intake rate varied from 7.2- to 9.5-fold (for 30°C and 15°C, respectively) between the lowest and the highest sucrose concentrations, sunbirds did not increase their sucrose intake significantly with increasing sucrose concentration (Fig. 1A). Sucrose intake averaged 119.45±5.08 mg h^{-1} (27.76±1.18 kJ day^{-1}) at 15°C and 75.28±5.17 mg h^{-1} (17.49±1.2 kJ day^{-1}) at 30°C. When feeding on 0.146 mol l^{-1} sucrose solutions, sunbirds consumed between 4 and 6 times their body mass in nectar in 14 h of daylight, depending on temperature.

The relationships between the concentration of ^{14}C-labeled inulin in excreta (disints min^{-1} μl^{-1}) and time were well described by negative exponential functions (r^2=0.61–0.99, N=37). The decline in the concentration of ^{14}C-labeled inulin in excreta with time therefore followed one-compartment, first-order kinetics (Fig. 2). Fractional inulin turnover rate ($K_{^{14}C}$) was significantly higher at 30°C (1.816±0.098 h^{-1}) than at 15°C (1.513±0.085 h^{-1}, ANOVA $F_{1,35}$=5.45, P=0.025). Inulin distribution space estimated by the intercept method ranged from 19.14 to 23.49% of body mass (21.11±0.57%, N=8; multiple estimates for individual subjects averaged).

Glomerular filtration rate (GFR) in Palestine sunbirds ranged from 820.7 to 3597.31 µl h^{-1} (1976.22±91.95 µl h^{-1}, N=37; Fig. 3). There was a significant effect of temperature ($F_{1,34}$=9.7, P=0.004) and water intake rate ($F_{1,34}$=8.47, P=0.006) on GFR, but no significant effect of subject ($F_{7,27}$=1.99, P=0.11), so we removed the latter variable from the model. To examine the effects of water intake independently of temperature, we constructed separate linear models for measurements at each temperature. GFR was correlated with water intake rate at 15°C ($y=0.37x+1435.8$, r^2=0.3, $F_{1,18}$=7.56, P=0.013), but not at 30°C ($F_{1,15}$=0.91, P=0.36). Mean GFR was significantly higher at the higher temperature (1792.4±129.78 *vs* 2192.48±111.65 µl h^{-1} for 15 and 30°C, respectively; $ANCOVA_{temperature}$ $F_{1,34}$=9.7, P=0.004).

Fractional water reabsorption (FWR) in the kidney ranged from 0.64 to 0.98 (0.82±0.02, N=29) and decreased significantly with water intake rate as predicted ($F_{1,19}$=6.65, P=0.018; Fig. 4). Because there were no significant effects of subject ($F_{7,19}$=1.21, P=0.34) or temperature ($F_{1,19}$=0.08, P=0.77), we removed these variables from the model and

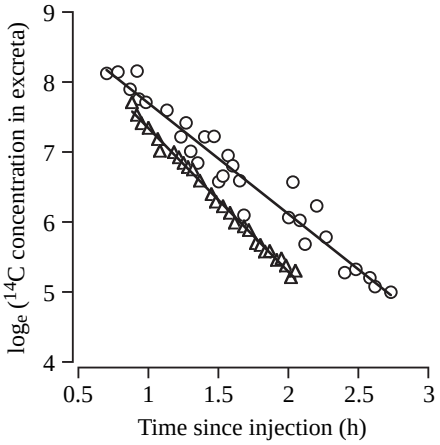

Fig. 2. The relationships between the concentration of ^{14}C-labeled inulin in excreta (disints min^{-1} μl^{-1}) and time were well described by exponential functions (r^2=0.61–0.99, N=37). The decline in the concentration of ^{14}C-labeled inulin in excreta with time therefore followed one-compartment, first-order kinetics. Data are shown here for two individuals and were semi-log_e transformed for clarity. Analysis was performed on untransformed data (Motulsky and Ransnas, 1987).

estimated a common relationship between FWR and water intake rate (y=–1.6×10^{-4}x+0.91, r^2=0.34).

Excreted fluid and ureteral urine glucose and osmotic concentration measurements

Osmotic concentration declined significantly with increasing water intake rate ($F_{1,40}$=48.36, P<0.0001), and was significantly greater in ureteral urine than in excreted fluid ($F_{1,31}$=57.91, P<0.0001; Fig. 5B). Since there were no effects of subject ($F_{9,31}$=0.91, P=0.53) or temperature ($F_{2,31}$=1.72, P=0.2), we removed these variables from the model. We described the relationship between osmotic concentration and water intake rate using separate power functions for ureteral urine and excreted fluid (y=18045.61$x^{-0.82}$, r^2=0.49, $F_{1,11}$=10.47, P=0.008, N=13, and y=1101.14$x^{-0.57}$, r^2=0.65, $F_{1,28}$=51.1, P<0.0001, N=32, respectively; Fig. 5B). Ureteral urine osmotic concentration ranged from 14.96 to

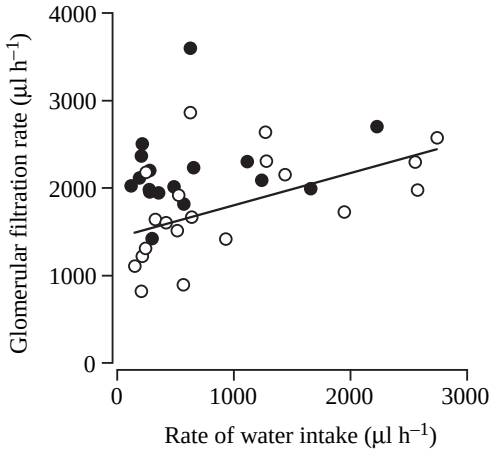

Fig. 3. Glomerular filtration rate (GFR) as a function of rate of water intake and ambient temperature in Palestine sunbirds. GFR ranged from 820.7 to 3597.31 µl h^{-1} and was correlated with water intake rate at 15°C (open circles; y=0.37x+1435.8, r^2=0.3), but not at 30°C (filled circles). Mean GFR was significantly higher at the higher temperature (1792.4±129.78 *vs* 2192.48±111.65 µl h^{-1} for 15 and 30°C, respectively).

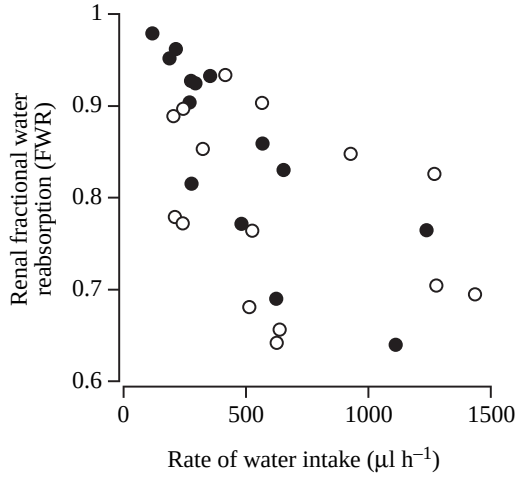

Fig. 4. Fractional water reabsorption (FWR) in the kidney ranged from 0.64 to 0.98 (0.82±0.02, N=29) and decreased significantly with water intake rate as predicted (y=–1.6×10^{-4}x+0.91, r^2=0.34). There was no significant effect of ambient temperature (15°C, open circles; 30°C, filled circles) on FWR as a function of water intake rate.

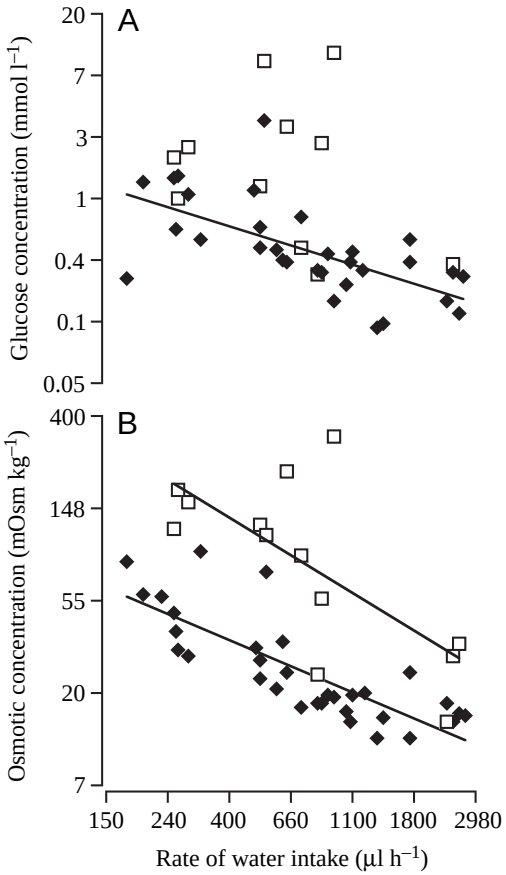

Fig. 5. Glucose and osmotic concentrations in excreted fluid and ureteral urine of Palestine sunbirds varied with rate of water intake. (A) Glucose concentration declined significantly with increasing water intake rate, and was significantly higher in ureteral urine (open squares) than in excreted fluid (filled diamonds). Glucose concentration was not significantly correlated with water intake rate when ureteral urine data were considered separately, probably because of small sample size, particularly at higher rates of water intake. The relationship between glucose concentration in excreted fluid and rate of water intake was adequately described by a power function (y=26.18$x^{-0.62}$, r^2=0.4, N=31). Glucose concentration in ureteral urine ranged from 0.28 to 10.39 mmol l^{-1} (2.97±1.05, N=11), and that in excreted fluid ranged from 0.12 to 3.52 mmol l^{-1} (0.6±0.12, N=31). (B) Osmotic concentration declined significantly with increasing water intake rate, and was significantly greater in ureteral urine than in excreted fluid. We described the relationship between osmotic concentration and water intake rate using separate power functions for ureteral urine and excreted fluid (y=18045.61$x^{-0.82}$, r^2=0.49, N=13, and y=1101.14$x^{-0.57}$, r^2=0.65, N=32, respectively). Osmotic concentration of ureteral urine ranged from 14.96 to 329 mOsm kg^{-1} (115.5±25.28, N=13), and that of excreted fluid ranged from 12.33 to 95 mOsm kg^{-1} (30.82±3.82, N=32). Note that the scales of all axes are logarithmic.

329 mOsm kg^{-1} (115.5±25.28, N=13), and that of excreted fluid ranged from 12.33 to 95 mOsm kg^{-1} (30.82±3.82, N=32).

Glucose concentration declined significantly with increasing water intake rate ($F_{1,37}$=13.47, P=0.0008), and was significantly higher in ureteral urine than in excreted fluid ($F_{1,28}$=17.1, P<0.0003; Fig. 5A). There were no effects of subject ($F_{9,28}$=0.9, P=0.54) or temperature ($F_{2,28}$=2.21, P=0.13), so we removed these variables from the model. Glucose concentration was not significantly correlated with water intake rate when ureteral urine data were considered separately ($F_{1,9}$=0.67, P=0.43, N=11), probably because of small sample size, particularly at higher rates of water intake. The relationship between glucose concentration and water intake rate in excreted fluid was adequately described by a power function (y=26.18$x^{-0.62}$, r^2=0.4, $F_{1,27}$=8.21, P=0.008, N=31; Fig. 5A). Glucose concentration in ureteral urine ranged from 0.28 to 10.39 mmol l^{-1} (2.97±1.05, N=11), and that in excreted fluid ranged from 0.12 to 3.52 mmol l^{-1} (0.6±0.12, N=31).

Plasma glucose measurements

Plasma glucose concentration was significantly greater in fed (28.18±0.68 mmol l^{-1}) than in fasted sunbirds (16.08±0.75 mmol l^{-1}; $F_{1,7}$=335.44, P<0.0001, N=8).

Discussion

The behavioral response of sunbirds to changes in nectar energy density allowed us to explore their physiological responses to a wide range of ingested water loads. Sunbirds maintained constant rates of energy intake despite water intake rates that varied as much as 9.5-fold between the lowest and highest sucrose concentrations (Fig. 1). They consumed between 4 and 6 times their body mass in nectar per day when feeding on dilute sucrose solutions, depending on ambient temperature. Such phenomenal water ingestion rates would lead to pathological consequences in many terrestrial vertebrates (Lumeij and Westerhof, 1988; Gebel et al., 1989; Gevaert et al., 1991; de Leon et al., 1994), yet sunbirds were able to compensate for varying nectar energy density and increased thermoregulatory energy demands with no apparent difficulty. Our results suggest that water processing does not limit energy intake in Palestine sunbirds within the range we tested.

In general, the data support our predictions. Glomerular filtration rate was lower than expected (46% of the value predicted based on body mass; Yokota et al., 1985; Williams et al., 1991; however, these allometric predictions are based on larger, usually anesthetized birds and therefore may well not extrapolate to small unanesthetized birds), and was not exceptionally sensitive to water loading (Fig. 3). When standardized to metabolic body mass (kg$^{0.75}$), mean GFR in Palestine sunbirds (93.91±4.37 ml kg$^{-0.75}$ h^{-1}) was approximately 60% and 75% of that in two species of hummingbirds (see below). Plasma glucose concentrations were high and varied 1.8-fold between fasted and fed sunbirds, but because GFR was low, glucose filtered load also remained relatively low (0.056 mmol h^{-1} in fed birds). Essentially the entire glucose filtered load (98%) was recovered by the kidneys. Renal fractional water reabsorption decreased from 0.98 to 0.64 with increasing water load (Fig. 4), comparable to observations in nectar-feeding red wattlebirds (*Anthochaera carunculata*; Goldstein and Bradshaw, 1998). The fraction of ingested water absorbed by Palestine sunbirds decreases with water intake rate (McWhorter et al., 2003), however, so their low GFR and high proportional renal recovery of glucose is not surprising. They deal with the problem of water over-ingestion by not absorbing all the water that they consume, rather than by absorbing it and then filtering it in the kidney. In this discussion, we explore the consequences of these adaptations to high water loads for the simultaneous maintenance of water and energy balance. We posit that the energetic cost of recovering filtered metabolites, and the potential for these processes to limit energy intake, are much lower in sunbirds than in hummingbirds (Nicolson and Fleming, 2003).

Water ingestion and subsequent absorption in intestine has the potential to constrain an animal's energy intake rate by exceeding its capacity for water disposal (McWhorter and Martínez del Rio, 1999; Martínez del Rio et al., 2001). Water loads (preformed water in nectar plus metabolic water) greater than the sum of evaporative water loss and maximum renal water elimination (GFR minus a minimum fractional water reabsorption necessary to retain filtered metabolites) will overwhelm osmoregulatory processes and lead to water intoxication unless the animal decreases nectar intake. Nectar intake by sunbirds in this study increased with no detectable plateau as diet sucrose concentration and ambient temperature decreased (Fig. 1). Indeed, the slopes of the relationships between nectar intake and diet sugar concentration at both 15°C and 30°C were not significantly different from –1, indicating that birds were compensating completely for changes in nectar energy density (Martínez del Rio et al., 2001). In addition, the 1.6-fold higher average sucrose intake rate observed at 15°C corresponds almost exactly to the 1.5-fold increase in metabolic rate observed in Palestine sunbirds between ambient temperatures of 15°C and 30°C in the laboratory (C. Hambly, B. Pinshow, E. J. Harper and J. R. Speakman, unpublished data). The sugar concentrations in the diets used in this study span the range of sugar concentrations found in the nectar of bird-pollinated plants (Pyke and Waser, 1981; Gryj et al., 1990; Stiles and Freeman, 1993). Our results suggest, therefore, that water processing does not limit energy intake in Palestine sunbirds over the range of sugar concentrations that they encounter naturally.

McWhorter et al. (2003) found that the fraction of ingested water absorbed (f_W) by Palestine sunbirds decreased from 100% to 36% with increasing water intake rate ($\dot{V}_I$). In addition, Goldstein and Bradshaw (1998) found evidence suggesting that dietary water was not completely absorbed from the gut of nectar-feeding red wattlebirds under conditions of high water intake. Therefore, in spite of water intake rates

that exceed several times their body mass per day (Lotz and Nicolson, 1999; McWhorter et al., 2003; Nicolson and Fleming, 2003), Palestine sunbirds may not face exceptional renal water loads when feeding on dilute nectars. In Fig. 6 we compare water intake rate, estimated water load and urine flow rate [GFR–(GFR×FWR)] as a function of diet sucrose concentration for birds in this study (data for both temperatures combined). Water load was estimated as water absorption rate [$f_W \times \dot{V}_I$, where $f_W=0.36+(56.93\times \dot{V}_I^{-1})$; McWhorter et al., 2003] plus metabolic water production (estimated based on sucrose assimilation rate, assuming carbohydrate catabolism). Estimated water load increases much more slowly with decreasing sucrose concentration in nectar than does water intake rate, and roughly parallels urine flow rate. The difference between water load and urine flow rate represents water lost by evaporation (approximately 30% of water load). The ability of Palestine sunbirds to modulate the absorption of preformed water in nectar substantially reduces the water load that must subsequently be eliminated by the kidney.

Excreted fluid glucose concentrations are comparably low in Palestine sunbirds (0.6±0.12 mmol l^{-1}) and broad-tailed hummingbirds (*Selasphorus platycercus*; 1.3±0.6 mmol l^{-1}; McWhorter and Martínez del Rio, 2000). Does renal glucose processing and conservation differ between sunbirds and hummingbirds? Glucose filtered loads in Palestine sunbirds were relatively low (0.056 mmol h^{-1} in fed birds) in spite of plasma glucose concentrations similar to those of hummingbirds (Beuchat and Chong, 1998). GFR data are available for two species of hummingbirds: *Calypte anna*, body mass 5.1 g, GFR 2.4 ml h^{-1} (125.76 ml $kg^{-0.75}$ h^{-1}; S. Medler, unpublished data), and *Selasphorus platycercus*, body mass 3.6 g, GFR 2.3 ml h^{-1} (156.5 ml $kg^{-0.75}$ h^{-1}; B. Hartman-Bakken, T. J. McWhorter, E. Tsahar and C. Martínez del Rio, unpublished data). Assuming an average plasma glucose concentration of 35 mmol l^{-1} in fed hummingbirds (based on measurements in three species; Beuchat and Chong, 1998), the predicted glucose filtered load would be 0.084 and 0.081 mmol h^{-1} for *C. anna* and *S. platycercus*, respectively, or about 1.5-fold that of the larger sunbird. The glucose filtered load that must be recovered by the kidneys of Palestine sunbirds is 1.9- to 2.4-fold lower than that estimated for hummingbirds when standardized to metabolic body mass (2.26 *vs* 4.4 and 5.47 mmol h^{-1} $kg^{-0.75}$ for *C. anna* and *S. platycercus*, respectively). Although excreta and urine concentrations of other metabolites (e.g. amino acids) and electrolytes were not measured in this study, the above argument may be applied to them as well. The ability of sunbirds to modulate their absorbed water load may therefore resolve the potential conflicts between eliminating excess water and metabolic by-products while retaining electrolytes, metabolites and energy (Yokota et al., 1985).

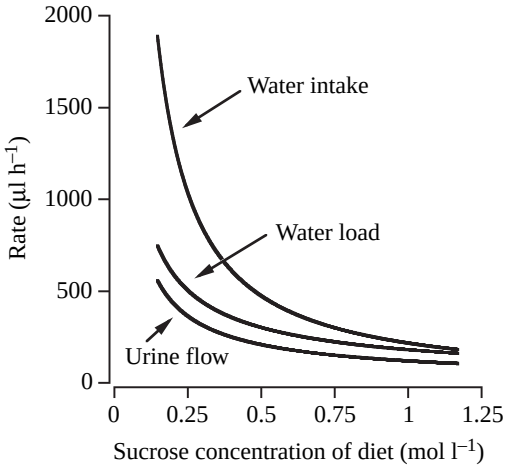

Fig. 6. Water intake rate, estimated water load (ingested water that is absorbed in the gastrointestinal tract plus metabolic water) and urine flow rate as functions of diet sucrose concentration in Palestine sunbirds. Estimated water load increases much more slowly with decreasing diet sucrose concentration than does water intake rate, and roughly parallels urine flow rate. The ability of sunbirds to modulate the absorption of preformed water in nectar substantially reduces the water load that must subsequently be eliminated by the kidney. Data for both temperatures were combined; see text for an explanation of how variables were estimated. No inferential statistics were performed on estimated water loads.

Palestine sunbirds rely on the integrated functioning of two organ systems to maintain water balance in spite of highly variable and often extremely high water intake rates: (1) fractional absorption of dietary water is modulated in the gastrointestinal tract (McWhorter et al., 2003) and (2) FWR is modulated by the kidney. GFR in sunbirds appears to be relatively insensitive to water loading. Similarly, Goldstein and Bradshaw (1998) concluded that changes in urine flow rate in nectar-feeding red wattlebirds were more closely related to modulation of renal FWR than to changes in GFR. The correlation between GFR and water intake rate at 15°C but not at 30°C suggests that GFR in sunbirds is more sensitive to water loading at low ambient temperatures (Fig. 3). Estimated water load (absorbed plus metabolic water) was higher at 15°C, so this is not surprising. However the significantly higher mean GFR at 30°C (at least at low rates of water intake) is perplexing. It is possible that evaporative water loss was higher at 15°C because of increased metabolic demands (Powers, 1992; Williams, 1996) and thus that GFR was modulated in response to water deficit when birds were feeding on concentrated sucrose solutions (Williams et al., 1991). The observed decrease in ureteral urine osmotic concentration with increasing water intake (Fig. 5B) supports our contention that modulation of renal FWR, rather than of GFR, determines renal water elimination in sunbirds. The low osmotic and glucose concentrations of excreted fluid relative to ureteral urine (Fig. 5) support the idea that sunbirds are relying on modulation of ingested water absorption in their gastrointestinal tract to reduce renal water loads, although this could also result from post-renal modification of urine (Braun, 1999). Sunbirds and hummingbirds lose exceptionally small amounts of glucose and electrolytes in excreted fluid (McWhorter and Martínez del Rio, 2000; Lotz and Martínez del Rio, 2004). We posit that the energetic cost of recovering

filtered metabolites, and the potential for these processes to limit energy intake, are much lower in sunbirds than in hummingbirds (see also Nicolson and Fleming, 2003).

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