

Modulation of ingested water absorption by Palestine sunbirds: evidence for adaptive regulation

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Summary

Nectarivorous birds feed on dilute sugar solutions containing trace amounts of amino acids and electrolytes. To meet their high mass-specific energy demands they must often deal with exceptionally high proportionate water fluxes. Despite nectar intake rates that may reach more than five times body mass per day, hummingbirds appear to absorb all ingested water. Here, we report the results of experiments designed to examine the relationship between nectar intake and water turnover in nectar-feeding Palestine sunbirds (*Nectarinia osea*). Like hummingbirds, sunbirds ingested large amounts of water. At the lowest sucrose concentration (292 mmol l⁻¹), food intake rates reached 2.2 times body mass. Fractional and total water turnover increased linearly with water ingestion, but the fraction of ingested water absorbed by sunbirds decreased from 100% to 36% with increasing water intake rate. Palestine sunbirds may therefore avoid absorbing, and thus having to eliminate, up to 64% of

their ingested water load when feeding on dilute nectars. To our knowledge, this is the first documentation of regulation of water flux across the gastrointestinal tract to the body. Our data suggest that sunbirds regulate transepithelial water flux independently of sugar absorption. These intriguing results open the door to many questions about how water transport is regulated in the vertebrate gastrointestinal tract. We suggest that intestinal water and body water form two separate but interacting pools in nectar-feeding birds. Convergence in diet has led to the evolution of many similar traits in hummingbirds and sunbirds. The physiological traits of these two groups that allow the processing of a water and sugar diet, however, may be very different.

Key words: Palestine sunbird, *Nectarinia osea*, hummingbird, adaptive regulation, water absorption, water intake, water turnover, nectar.

Introduction

To fuel their exceptionally high mass-specific energy demands, nectar-feeding birds often experience water fluxes closer to those experienced by amphibians and freshwater fish than to those of endothermic vertebrates (Beuchat et al., 1990). Extremely high water flux rates have been measured in many species of nectarivorous and frugivorous birds (Rooke et al., 1983; Powers and Nagy, 1988; Weathers and Stiles, 1989; Williams, 1993; Powers and Conley, 1994; Goldstein and Bradshaw, 1998; Lotz and Nicolson, 1999; McWhorter and Martínez del Río, 1999; Nicolson and Fleming, 2003). McWhorter and Martínez del Río (1999) found that, depending on sugar concentration, broad-tailed hummingbirds (*Selasphorus platycercus*) consumed volumes of nectar ranging from 1.6 to 5.4 times their body mass per day. Beuchat et al. (1990) estimated that Anna's hummingbirds (*Calypte anna*) consume a volume of nectar equal to three times their body mass per day under energetically demanding conditions.

Until recently, the physiological challenges associated with the simultaneous regulation of energy intake and water and ion homeostasis by these animals remained relatively unexplored.

Beuchat et al. (1990) hypothesized that when hummingbirds are ingesting large volumes of dilute nectar, perhaps only a small fraction is absorbed in the small intestine, leaving the rest to pass quickly through the intestinal tract. This hypothesis would explain the ability of these birds to process such large volumes of water rapidly but requires the rapid absorption of sugars and electrolytes and strict regulation of transepithelial water flux (Skadhauge, 1981; Beuchat et al., 1990). If ingested water is largely absorbed across the intestine, as appears to be the case in most vertebrates (Powell, 1987), nectar-feeding birds would be faced with significant renal challenges for water elimination and glucose and electrolyte recovery when feeding on dilute nectar (Beuchat et al., 1990). McWhorter and Martínez del Río (1999) developed a model based on

pharmacokinetic techniques to estimate the fractional absorption of ingested water across the gastrointestinal tract of birds. Their model estimates fractional water absorption as the proportion of ingested water that contributes to body water turnover (McWhorter and Martínez del Rio, 1999). McWhorter and Martínez del Rio (1999) tested and rejected the hypothesis of Beuchat et al. (1990) in broad-tailed hummingbirds; they found that approximately 80% of ingested water contributed to the turnover of the body water pool and that fractional water absorption was not correlated with food or water intake rate or diet energy density.

Although nectar-feeding birds are convergent in diet, and indeed often in appearance and behavior, it is unclear if the physiological mechanisms by which they cope with a nectar diet are also convergent. Nectar poses peculiar problems to the animals that feed on it because it is a relatively dilute solution of sugars containing trace amounts of amino acids and electrolytes (Baker, 1975, 1977; Baker and Baker, 1983). Here, we revisit the hypothesis of Beuchat et al. (1990) in another lineage of nectar-feeding birds. We report the results of experiments designed to examine the relationship between nectar intake, water absorption and water turnover in the Palestine sunbird [*Nectarinia osea* (Bonaparte 1856)], an Old World nectarivore in the family Nectariniidae. Based on previous measurements in hummingbirds, we hypothesized that water absorption by sunbirds would be essentially complete at all sucrose concentrations naturally encountered in floral nectars. Alternately, we hypothesized that if water absorption were modulated, fractional absorption would decrease to some obligatory minimum with increasing water intake. This hypothesis was based on the observation that nutrient absorption does not take place without concomitant transport of water, whether *via* hydration spheres of molecules in nutrient transporters (e.g. Loo et al., 1996, 1998) or paracellular solvent drag (e.g. Pappenheimer and Reiss, 1987; Pappenheimer, 1990). As a corollary to our alternate hypothesis, we predicted that absorbed water loads would be greater when sugar assimilation rates are higher. Because researchers generally assume that water turnover in nectar-feeding animals can be used to approximate nectar intake, given that ingested water comes only from food (von Helversen and Reyer, 1984; Kunz and Nagy, 1988; Powers and Nagy, 1988; Weathers and Stiles, 1989; Tiebout and Nagy, 1991), our results also test the primary assumption of a significant body of work on the field energetics and water fluxes of nectarivorous animals.

Materials and methods

Bird capture and maintenance

Male Palestine sunbirds *Nectarinia osea* (Bonaparte 1856) (body mass 5.74 ± 0.07 g, $N=10$) 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 ($30^\circ 51'$ N, $34^\circ 46'$ E; Israel Nature and National Parks Protection Authority permits 5981 and 7686). The birds were

housed individually in outdoor aviaries ($1.5 \text{ m} \times 1.5 \text{ m} \times 2.5 \text{ m}$) 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, Abbott Park, IL, USA) 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* spp.) at least twice a week. During experiments, birds were housed individually in opaque Plexiglas cages ($0.3 \text{ m} \times 0.3 \text{ m} \times 0.3 \text{ m}$) 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 \times 0.01 g; Ohaus Corporation, Florham Park, NJ, USA) so body mass could be monitored continuously. Birds were allowed to acclimate to cages and experimental temperatures 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. The food intake rates of Palestine sunbirds that have been switched among diets of varying energy density stabilize within 4 h (T. J. McWhorter, C. Martínez del Rio and B. Pinshow, unpublished data).

Experimental design

Experiment 1: fractional absorption of ingested water as a function of water intake rate

We relied on the behavioral responses of nectar-feeding birds to food of varying energy density in the design of this experiment. Typically, nectar-feeding birds decrease their food intake rate with increasing sugar concentration (Martínez del Rio et al., 2001). Manipulation of sugar concentration therefore leads to a wide range of variation in the quantity of food ingested. We used a repeated-measures design in which we measured water absorption in four sunbirds fed on four dietary sugar concentrations (292 mmol l^{-1} , 584 mmol l^{-1} , 876 mmol l^{-1} and 1168 mmol l^{-1} sucrose) at one ambient temperature ($30 \pm 2^\circ \text{C}$), randomizing the order in which diets were presented to subjects.

Experiment 2: fractional absorption of ingested water as a function of sucrose assimilation

When ambient temperatures decrease, birds must consume and assimilate more sugar to meet increased energy demands for thermoregulation. We measured water absorption in six sunbirds feeding on 584 mmol l^{-1} sucrose solutions at both $15 \pm 1^\circ \text{C}$ and $30 \pm 2^\circ \text{C}$ in a repeated-measures design to determine the effect of sucrose assimilation rate. We randomized the order in which subjects were exposed to the two temperatures.

Experimental measurements

Water turnover rates were estimated by injecting 1.85×10^5 Bq of $^3\text{H}_2\text{O}$ 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 the nearest 0.0001 g before and after injection. Excreted fluid samples were collected, using glass microcapillary tubes, immediately after excretion and placed in separate scintillation vials. Samples were collected at irregular intervals for approximately 30 h, excluding the dark portion of the photoperiod during which sunbirds do not excrete. Sample collection was not initiated until approximately 40 min after injection, allowing sufficient time for complete equilibration of ^3H with body water (estimates of equilibration time vary from 15 min to 30 min in small birds; Williams and Nagy, 1984; Speakman, 1997). Liquid scintillation cocktail (ACS II; Amersham, Piscataway, NJ, USA) was added to all excreted fluid and injection samples, which were counted, correcting for quench and lumex, in a Packard Tri-Carb 1600TR Liquid Scintillation Analyzer. Fractional water turnover rate ($K^3\text{H}$; measured in h^{-1}) was estimated by fitting negative exponential functions to the relationship between the specific activity of ^3H in excreted fluid and time. In most cases, ^3H specific activity was high enough on the second day to estimate water turnover and absorption. Because birds were not injected on the second day, these measurements provided a test for the effects of handling and injection on water turnover and absorption during the first day. Food intake rate ($\mu\text{l h}^{-1}$) was recorded over the course of each experimental trial by measuring the change in food level to the nearest 0.5 mm in a tube of constant internal diameter, correcting for evaporation and food spillage.

Total body water volume (TBW; measured in μl) was estimated using isotope dilution (Nagy, 1983; Speakman, 1997). Briefly, a small blood sample (approximately 50 μl) was taken approximately 4 h after injection by puncturing the brachial vein. The water microdistilled from this sample (Nagy, 1983) was analyzed for specific activity of ^3H as described above. The slope of the relationship between specific activity of ^3H in excreted fluid and time was extrapolated to the zero time concentration of marker in body water. We used this modification of the isotope dilution technique described by Speakman (1997) because of the sensitivity of small birds to repeated blood sampling. We assumed that the rate of disappearance of marker from the blood was equal to the rate of appearance in the excreted fluid. The specific activity of marker in each fluid would, of course, not be equal because of renal and post-renal modification of urine and the mixing of urine with gut contents. After the final experimental run, one bird was killed with CO_2 and dried to constant mass at 80°C to confirm TBW estimated by isotope dilution. The TBW of this bird measured by dehydration (3591 μl or 63.8% of body mass) was 1.6% higher than the average volume for this individual estimated by isotope dilution.

Estimating water absorption in sunbirds

We used the mass balance approach developed by McWhorter and Martínez del Rio (1999) to estimate the fraction of ingested water that was absorbed by sunbirds (f_w). Simply stated, this method determines the proportion of ingested water that contributes to the turnover of the TBW pool. Assuming that birds were in neutral water balance, f_w was estimated as:

$$f_w = (K^3\text{H} \times \text{TBW} - \dot{V}_M) \dot{V}_I^{-1}, \quad (1)$$

where \dot{V}_I ($\mu\text{l h}^{-1}$) is the rate of water intake, and \dot{V}_M ($\mu\text{l h}^{-1}$) is the rate of metabolic water production. We assumed that metabolic water production was due only to carbohydrate catabolism. Indeed, the respiratory quotient (RQ) of actively feeding sunbirds and hummingbirds indicates carbohydrate catabolism (RQ=1.0; C. Hambly, B. Pinshow, E. J. Harper and J. R. Speakman, unpublished data; Suarez et al., 1990; Powers, 1991). Birds in this study were in mass balance during all experimental trials, so we further assumed that the rate of carbohydrate catabolism was equal to the rate of sucrose assimilation. We calculated the rate of sucrose assimilation as the product of sucrose intake rate and assimilation efficiency. Sucrose assimilation efficiency was estimated as the fraction of ingested sucrose that was assimilated in an independent set of experiments (0.9992 ± 0.0004 , mean \pm s.d., $N=8$). Sucrose assimilation efficiency was independent of sugar concentration.

*Statistical analysis**Experiment 1*

To describe the relationship between fractional water absorption (f_w) and water intake rate (\dot{V}_I) and to assess differences among subjects and treatment days, we constructed a linear model with f_w as a dependent variable, and the reciprocal of water intake (\dot{V}_I^{-1}), individual bird and treatment day as independent variables. We used the reciprocal transformation of \dot{V}_I to obtain a linear relationship (i.e. $f_w = a + b\dot{V}_I^{-1}$, where a and b are constants) because visual inspection of the relationship between f_w and \dot{V}_I resembled a hyperbola that tended asymptotically to a constant value for large values of \dot{V}_I . Because relationships between volumetric food intake and sugar concentration in nectar-feeding birds are power functions (Martínez del Rio et al., 2001), we determined the effects of subject and treatment day on food, water and sucrose intake rates using linear models of \log_e -transformed intake and sucrose concentration data. We similarly used \log_e -transformed data to determine the significance of the relationship between water absorbed per mass sucrose assimilated and sucrose concentration. We used linear models on untransformed data to assess significance and subject and treatment day effects in all other cases. Analysis of covariance (ANCOVA) was used to check for differences in the slope of the relationship between water flux and water intake between sunbirds and hummingbirds. We used the Spearman rank correlation test to check for a correlation between diet sucrose concentration and sucrose intake rate.

Experiment 2

Repeated-measures analysis of variance (RM-ANOVA) was used to test for differences in food and sucrose intake rates, fractional water absorption, water absorbed per mass of sucrose assimilated, and the total absorbed water load between temperatures.

All values are presented as means \pm S.E.M.

Results

Experiment 1: fractional absorption of ingested water as a function of water intake rate

Sunbirds consumed significantly less food as dietary sucrose concentration increased ($F_{1,29}=107.0$, $P<0.0001$; Fig. 1B). There was no significant effect of subject ($F_{3,29}=1.7$, $P>0.1$) or

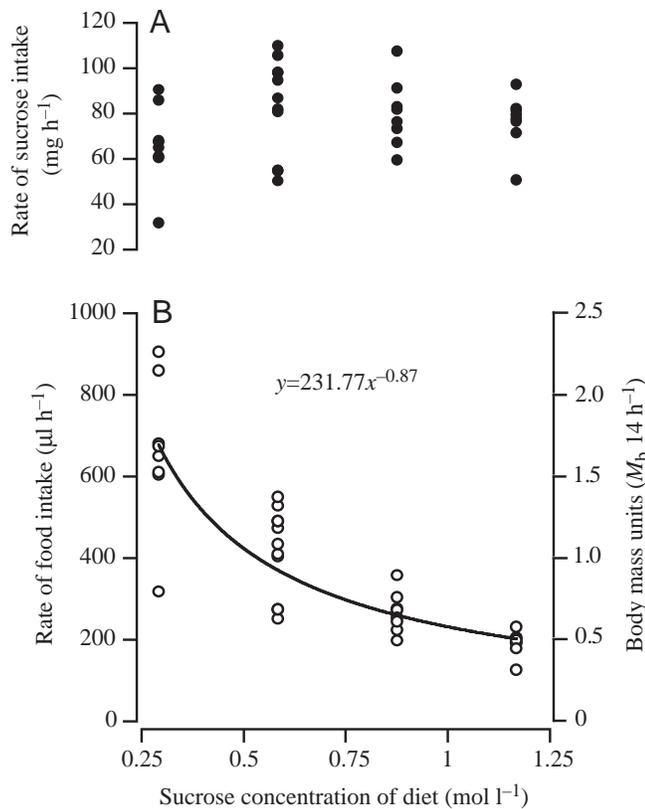


Fig. 1. Behavioral responses of sunbirds to varying sucrose concentration in food. (A) The rate of sugar intake did not increase significantly with sucrose concentration ($r_s=0.12$, $P=0.49$, $N=35$). Mean sucrose intake was 77.17 ± 3 mg h^{-1} (17.94 ± 0.7 kJ day^{-1}). (B) Volumetric food intake rate declined significantly with dietary sucrose concentration ($F_{1,29}=107.0$, $P<0.0001$). The relationship was well described by a power function ($r^2=0.76$) with an exponent that was not significantly different from 1.0 ($t=-1.52$, d.f.=33, $P>0.1$). Changes in food energy density from 0.292 mol l^{-1} to 1.168 mol l^{-1} sucrose led to an approximately 3.5-fold variation in food (and thus water) intake. The right-hand axis shows food intake in multiples of body mass (5.74 ± 0.07 g, $N=4$) per 14 h. At low sucrose concentrations, sunbirds consumed 0.8–2.2 times their body mass in food in 14 h of daylight.

treatment day ($F_{1,29}=1.5$, $P>0.2$) on food intake rate, so we removed these variables from the model. The relationship between food intake and sucrose concentration was adequately described by a power function ($y=231.77x^{-0.87}$, $r^2=0.76$; Fig. 1B). The exponent of this relationship was not significantly different from 1.0 ($t=-1.52$, d.f.=33, $P>0.1$). Hence, although food, and thus water, intake rate varied approximately 3.5-fold between the lowest and the highest sucrose concentration, sunbirds did not increase their sucrose intake significantly with increasing sucrose concentration ($r_s=0.12$, $P=0.49$, $N=35$; Fig. 1A). Sucrose intake averaged 77.17 ± 3 mg h^{-1} (17.94 ± 0.7 kJ day^{-1}). At low sucrose concentrations, sunbirds consumed 0.8–2.2 times their body mass in food in 14 h of daylight (Fig. 1B).

The relationships between the specific activity of 3H in excreted fluid (d.p.m. μl^{-1}) and time were well described by exponential functions (r^2 ranged from 0.57 to 0.96, $N=35$). The decline in the specific activity of 3H in excreted fluid with time therefore seemed to follow one-compartment, first-order kinetics. Fractional water turnover rate ranged from 0.037 h^{-1} to 0.117 h^{-1} and was linearly correlated with water intake rate ($F_{1,29}=169.50$, $P<0.0001$). Because there was no significant effect of subject ($F_{3,29}=1.4$, $P>0.2$) or treatment day ($F_{1,29}=2.0$, $P>0.1$) on K^3H as a function of \dot{V}_I , we removed these variables from the model and estimated a common relationship ($K^3H=1.15 \times 10^{-4} \dot{V}_I + 0.03$; $r^2=0.84$). When birds were feeding on the most dilute nectar (292 mmol l^{-1} sucrose), approximately 10% of their TBW pool was turning over each hour. Average TBW estimated by isotope dilution was 3470 ± 86 μl (or $63.6 \pm 0.7\%$ of body mass, $N=4$).

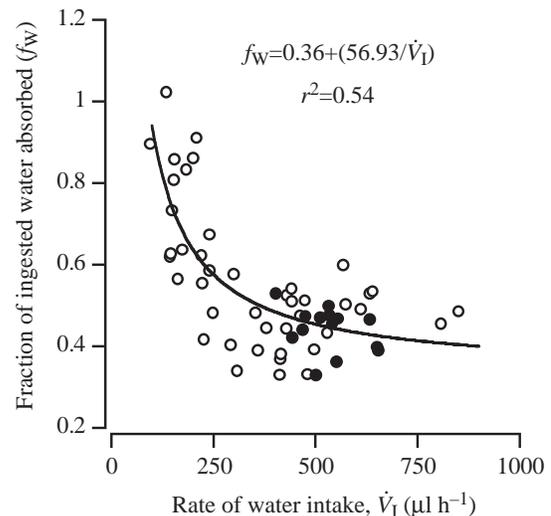


Fig. 2. Fractional absorption of ingested water (f_w) across the gut of Palestine sunbirds ranged from 0.33 to 1.02 (mean \pm S.E.M., 0.59 ± 0.04 , $N=35$) and declined significantly and non-linearly with water intake rate ($F_{1,29}=40.03$, $P<0.0001$, $r^2=0.54$). Palestine sunbirds may therefore avoid absorbing up to 64% of ingested water when feeding on dilute nectars. In sunbirds feeding on 584 mmol l^{-1} sucrose solutions, f_w was not significantly different between $15^\circ C$ (filled circles) and $30^\circ C$ (unfilled circles; 0.44 ± 0.02 vs 0.43 ± 0.02 , respectively; $F_{1,5}=0.22$, $P=0.66$).

Fractional water absorption (f_w) ranged from 0.33 to 1.02 (averaging 0.59 ± 0.04 , $N=35$). Because we found no significant effect of subject ($F_{3,29}=0.29$, $P>0.8$) or treatment day ($F_{1,29}=3.1$, $P>0.08$) on f_w , but a highly significant effect of \dot{V}_I^{-1} ($F_{1,29}=40.03$, $P<0.0001$), we estimated a common relationship between f_w and \dot{V}_I^{-1} . The reciprocal transformation adequately described the relationship between f_w and \dot{V}_I (Fig. 2). These results suggest that Palestine sunbirds may avoid absorbing up to 64% ($1-0.36=0.64$) of ingested water when feeding on dilute nectars. Fractional water absorption was also positively correlated with sugar concentration in food ($y=0.32x+0.37$, $r^2=0.34$, $F_{1,29}=17.13$, $P<0.0003$), which is not surprising given the negative relationship between water intake rate and sucrose concentration. Because we found no significant effects of subject ($F_{3,29}=0.43$, $P>0.7$) or treatment day ($F_{1,29}=0.86$, $P>0.3$) on f_w as a function of sucrose concentration, we removed these variables from the model. Water flux estimated from fractional water turnover rate (K_{3H}) and total body water (TBW) measurements ranged from $112.97 \mu\text{l h}^{-1}$ to $463.83 \mu\text{l h}^{-1}$ and increased linearly with water intake rate ($F_{1,29}=237.29$, $P<0.0001$). Because we found no effects of subject ($F_{3,29}=0.2$, $P>0.8$) or treatment day ($F_{1,29}=1.95$, $P>0.1$), we estimated a common relationship between water flux and water intake rate ($K_{3H} \times \text{TBW} = 0.42\dot{V}_I + 81.64$; $r^2=0.89$; Fig. 3). The slope of this relationship was significantly less than 1.0 (0.42 ± 0.03 , $t=22.8$, $\text{d.f.}=33$, $P<0.001$) and significantly lower than that of the same relationship in broad-tailed hummingbirds (ANCOVA_{slopes} $F_{1,35}=27.8$, $P<0.0001$).

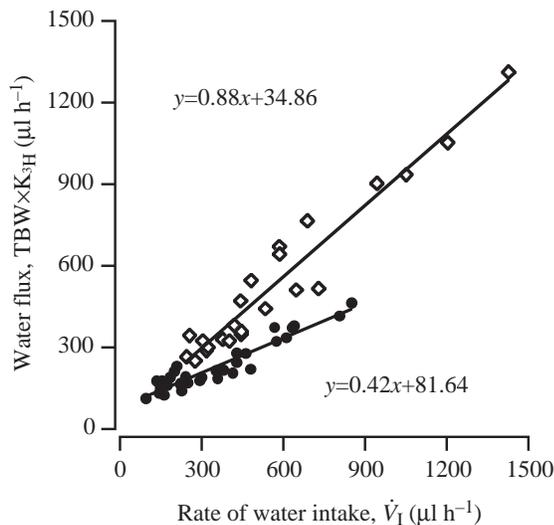


Fig. 3. Water flux in sunbirds (filled circles) estimated from fractional water turnover rate (K_{3H}) and total body water (TBW) measurements ranged from $112.97 \mu\text{l h}^{-1}$ to $463.83 \mu\text{l h}^{-1}$ and increased linearly with water intake rate ($r^2=0.89$, $F_{1,29}=237.29$, $P<0.0001$). The slope of this relationship was significantly less than 1.0 (slope \pm s.e.m. = 0.42 ± 0.03 , $t=22.8$, $\text{d.f.}=33$, $P<0.001$), and significantly lower than that of the same relationship in broad-tailed hummingbirds (unfilled diamonds; slope \pm s.e.m. = 0.88 ± 0.05 , ANCOVA_{slopes} $F_{1,35}=27.8$, $P<0.0001$; data for hummingbirds from McWhorter and Martínez del Río, 1999).

The volume of water absorbed per mass of sucrose assimilated ($\mu\text{l mg}^{-1}$) declined significantly with the sucrose concentration of the diet ($y=1.47x^{-0.9}$, $r^2=0.78$, $F_{1,29}=106.66$, $P<0.0001$; Fig. 4A). There was no significant effect of subject ($F_{3,29}=0.5$, $P>0.6$) or treatment day ($F_{1,29}=0.44$, $P>0.5$), so we removed these variables from the model. Absorbed water load ($f_w \times \dot{V}_I$; measured in $\mu\text{l h}^{-1}$) was positively correlated with food intake rate ($F_{1,29}=152.53$, $P<0.0001$; Fig. 4B). There was no significant effect of subject ($F_{3,29}=0.64$, $P>0.5$) or treatment day ($F_{1,29}=0.83$, $P>0.3$), so we removed these variables from the model and estimated a common relationship ($y=0.40x+25.09$, $r^2=0.84$).

Experiment 2: fractional absorption of ingested water as a function of sucrose assimilation

Sunbirds feeding on 584 mmol l^{-1} sucrose solutions consumed approximately 1.3 times more food and sucrose at

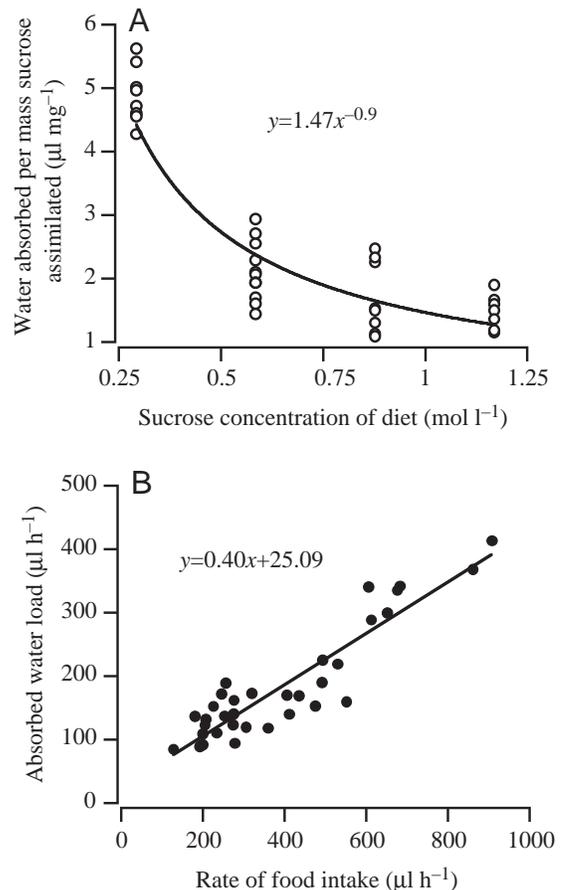


Fig. 4. Water absorption as a function of sucrose assimilation and food intake rate in sunbirds. (A) The volume of water absorbed per mass of sucrose assimilated declined significantly with the sucrose concentration of the diet ($r^2=0.78$; $F_{1,29}=106.66$, $P<0.0001$), despite constant sucrose intake, suggesting that sunbirds regulate transepithelial water flux independently of sugar absorption. (B) The absorbed water load (fractional absorption of ingested water \times water intake rate) was positively correlated with food intake rate ($r^2=0.84$; $F_{1,29}=152.53$, $P<0.0001$).

15°C than at 30°C ($624.52 \pm 29.83 \mu\text{l h}^{-1}$ vs $487.23 \pm 25.47 \mu\text{l h}^{-1}$ and $124.84 \pm 5.96 \text{ mg h}^{-1}$ vs $97.4 \pm 5.09 \text{ mg h}^{-1}$, respectively; $F_{1,5}=6.6$, $P=0.05$ for both variables). These values translate into energy intake rates of $29.01 \pm 1.39 \text{ kJ day}^{-1}$ and $22.64 \pm 1.18 \text{ kJ day}^{-1}$, respectively. Fractional water absorption was not significantly different between temperatures (0.44 ± 0.02 vs 0.43 ± 0.02 at 15°C and 30°C, respectively; $F_{1,5}=0.22$, $P=0.66$; Fig. 2). The volume of water absorbed per mass sucrose assimilated did not differ between temperatures ($1.94 \pm 0.09 \mu\text{l mg}^{-1}$ vs $1.88 \pm 0.08 \mu\text{l mg}^{-1}$ at 15°C and 30°C, respectively; $F_{1,5}=0.21$, $P=0.67$). Although the absorbed water load ($f_w \times \dot{V}_i$) was approximately 1.3 times greater at 15°C than at 30°C, it did not differ significantly between treatments ($237.34 \pm 11.49 \mu\text{l h}^{-1}$ vs $184.72 \pm 14.46 \mu\text{l h}^{-1}$, respectively; $F_{1,5}=5.06$, $P=0.074$). We suspect that lack of statistical significance in this case was the result of low power due to small sample sizes.

Discussion

Nectar-feeding birds vary their food intake rate in response to sugar concentration; sunbirds in this study maintained constant rates of energy intake despite a 3.5-fold variation in food intake rate between the lowest and the highest sucrose concentrations (Fig. 1). This behavioral response allowed us to explore their physiological responses to a wide range of ingested water loads. Contrary to our hypothesis, and in contrast to the results reported by McWhorter and Martínez del Rio (1999) for broad-tailed hummingbirds, the fraction of ingested water absorbed (f_w) by Palestine sunbirds decreased with water intake rate (Fig. 2). The functional relationship between f_w and water intake rate was an asymptotic function that tended towards 0.36 as ingested water load became large. This result implies that sunbirds can avoid absorbing, and thus having to eliminate, up to 64% of their ingested water load when feeding on dilute nectars. To our knowledge, this is the first documentation of apparent regulation of water flux across the gastrointestinal tract to the body in vertebrates.

Although fractional absorption decreased with increasing sucrose concentration, the absorbed water load increased with food intake rate (Fig. 4B). The volume of water absorbed per mass sucrose assimilated decreased with sucrose concentration in food (Fig. 4A), despite constant sucrose intake. This suggests that sunbirds can regulate transepithelial water flux independently of sugar absorption. These intriguing results open the door to many questions about how water transport is regulated in the vertebrate gastrointestinal tract. In this discussion, we explore the differences in water turnover and fractional absorption between sunbirds and hummingbirds, members of separate evolutionary radiations of nectar-feeding birds. We posit that differences in mechanisms of sugar absorption and mass-specific food intake rates between these groups may explain the apparent ability of sunbirds to modulate water absorption. We discuss the implications of our findings for estimating food intake in nectarivorous animals based on water flux rates and suggest that intestinal water and

body water form two separate but interacting pools in nectar-feeding birds.

Our results provide empirical support for the hypothesis posed by Beuchat et al. (1990) for nectar-feeding birds. Sunbirds did not absorb all ingested water, and the fraction of water absorbed in the intestine decreased with the ingested water load. Our results and those of McWhorter and Martínez del Rio (1999) highlight important differences between sunbirds and hummingbirds. Fractional water turnover rates in Palestine sunbirds ranged from 0.037 h^{-1} to 0.117 h^{-1} , while those in broad-tailed hummingbirds ranged from 0.12 h^{-1} to 0.61 h^{-1} (McWhorter and Martínez del Rio, 1999). In other words, sunbirds feeding on the most dilute nectar (292 mmol l^{-1} sucrose) turned over approximately 10% of their TBW pool each hour, compared with over 50% in hummingbirds. Because daily food intake by broad-tailed hummingbirds may reach 5.4 times their body mass while that of Palestine sunbirds only reaches approximately 2.2 times body mass in birds feeding on 292 mmol l^{-1} sucrose solutions, this difference may not be surprising. However, when similar rates of water intake are considered, hummingbirds and sunbirds show large differences in fractional and total water turnover rates. The slope of the linear relationship between water flux and water intake rate (Fig. 3) provides a relative estimate of the fraction of ingested water that contributes to body water turnover. If 100% of ingested water were contributing to the turnover of the TBW pool at all water intake rates, the slope of this relationship would be equal to 1. The slope of this relationship in sunbirds was significantly less than one and shallower than that of the same relationship in hummingbirds. Sunbirds appear to regulate water flux from the gastrointestinal tract to the body, whereas hummingbirds do not. Convergence in diet has led to the evolution of many similar traits in hummingbirds and sunbirds (e.g. elongated bill, small body size and pugnacity). The physiological traits of these two groups that allow the processing of a water and sugar diet, however, may be very different.

The mechanisms of intestinal water absorption in nectar-feeding birds are unknown but are probably facilitated by sugar uptake. Active transport appears to account for essentially all intestinal glucose absorption in hummingbirds (Karasov et al., 1986). Loo et al. (1996) have shown that the translocation of each glucose molecule by the mammalian intestinal Na^+ /glucose cotransporter (SGLT1) is coupled with the transport of up to 260 water molecules (potentially transporting 4.8 liters of water per mol of glucose). Hummingbirds, which appear to absorb all ingested water, also exhibit the highest rate of carrier-mediated glucose uptake measured in a vertebrate (Karasov et al., 1986). McWhorter and Martínez del Rio (1999) estimated that the amount of water potentially accompanying mediated glucose absorption in broad-tailed hummingbirds exceeded the water content in food by 1.7–5.5-fold, depending on sucrose concentration. Sunbirds in this study assimilated on average $3.1 \times 10^{-3} \pm 1.6 \times 10^{-4} \text{ mol}$ of glucose in 14 h. The mediated uptake of this quantity of glucose could be responsible for the transport of 15 ml of

water. Average daily water intake by sunbirds in this study ranged from 2 ml to 8.7 ml. As in hummingbirds, this amount exceeds the water ingested in food by a large margin (approximately 1.7–7.5-fold, depending on sucrose concentration). This comparison is perplexing because sunbirds appear to be able to modulate water absorption whereas hummingbirds do not.

Modulation of intestinal water absorption requires the rapid absorption of dissolved sugars and efficient extraction of electrolytes and amino acids present at low levels in ingested nectar (Beuchat et al., 1990). It also requires that the permeability of the intestine to transepithelial water flux is regulated. How may sunbirds regulate water flux while rapidly absorbing osmotically active sugars and electrolytes? One possibility is that the permeability of the intestine to transepithelial water flux by solvent drag increases with sugar concentration. This would require that sunbirds have a low capacity for mediated glucose uptake relative to hummingbirds and significant passive absorption of nutrients at high sugar concentrations. Although passive absorption appears to be insignificant in hummingbirds (Karasov et al., 1986), it is an important route for nutrient absorption in some passerine and psittacine birds (Karasov and Cork, 1994; Caviedes-Vidal and Karasov, 1996; Afik et al., 1997; Chediack et al., 2001). It would be instructive to measure the capacity for mediated glucose uptake and determine whether the magnitude of passive absorption of carbohydrate probes varies with water intake (given constant energy intake) in sunbirds. Another possibility is that water cotransported into enterocytes during mediated nutrient absorption does not contribute to turnover of the TBW pool but rather is secreted rapidly back into the intestinal lumen (Chang and Rao, 1994). Our estimates of the capacity for water absorption *via* mediated Na⁺/glucose cotransport in birds are based on measurements made by Loo et al. (1996) on the mammalian SGLT1 expressed in the *Xenopus* oocyte. Their measurements, however, sought to isolate water transport by that cotransporter and represent one element in a complex membrane system. The links between nutrient absorption, electrolyte balance and the regulation of transepithelial water flux in birds remain unknown.

Beuchat et al. (1990) raised their hypothesis to explain the ability of hummingbirds to cope with extraordinary water fluxes. Daily food intake by broad-tailed hummingbirds may reach 5.4 times their body mass while that of Palestine sunbirds only reaches approximately 2.2 times body mass in birds feeding on 292 mmol l⁻¹ sucrose solutions. Metabolic mass-specific sucrose intake rate (mg h⁻¹ kg^{-0.75}) is approximately three times higher in hummingbirds than in sunbirds. Why may sunbirds modulate water absorption while hummingbirds do not? Perhaps there are significant physiological differences in nutrient absorption and the regulation of transepithelial water flux between these groups. It is also possible that the extraordinarily high mass-specific energy demands of hummingbirds lead to water intake rates that simply overwhelm their physiological capacities to regulate water absorption. We speculate that water ingestion and subsequent

absorption are unlikely to constrain energy intake by sunbirds. The apparent ability of sunbirds to modulate water absorption may allow them to feed profitably on dilute floral nectars by minimizing the metabolic cost of recovering glucose and electrolytes filtered in the kidney. Indeed, we have preliminary data suggesting that glomerular filtration rates in sunbirds are lower than expected based on allometric estimates.

Implications for doubly labeled water studies

Water turnover in nectar-feeding animals has often been used to approximate nectar intake, assuming that ingested water comes only from food (von Helversen and Reyer, 1984; Kunz and Nagy, 1988; Powers and Nagy, 1988; Weathers and Stiles, 1989; Tiebout and Nagy, 1991). These approximations are based on the assumption that isotope concentrations in water leaving the body are the same as those in the body water at the same time (Lifson and McClintock, 1966). Differences in isotope concentrations between these pools can arise from both physical and biological fractionation (Lifson and McClintock, 1966; Speakman, 1997; Visser et al., 2000). Biological fractionation is due to incomplete mixing of the isotope label between the body and ingested water. Although physical fractionation can be accounted for mathematically, the issue of incomplete mixing has received very little attention (Visser et al., 2000). Nagy and Costa (1980) argued that biological fractionation might occur in birds eating bulky, energy-dilute foods with consequent high gastrointestinal passage rates, but there are no data to support this argument. Visser et al. (2000) recently determined that ingested water reaches isotopic equilibrium with the body water pool regardless of water intake rate in red knots (*Calidris canutus*), which may have water fluxes up to 17 times greater than predicted for free-living birds. By contrast, our results and those of McWhorter and Martínez del Río (1999) suggest that biological fractionation is occurring in nectar-feeding birds, i.e. that intestinal water and body water form two separate but interacting pools. Our model estimates the proportion of ingested water that contributes to the turnover of the TBW pool. We assumed that the rates of appearance of marker in excreted fluid and disappearance from TBW were equal, rather than assuming that the concentrations of markers were equal. If complete equilibration of intestinal water and body water were occurring, our model would estimate f_w as 1.0 regardless of water flux rate, which was not the case for either sunbirds or hummingbirds. Thus, our results tell a cautionary tale for the estimation of food intake based on water flux rates in nectar-feeding animals: nectar intake will be underestimated if water absorption is not complete. Our data also suggest that additional attention needs to be paid to the issue of biological fractionation when using stable and radioactive hydrogen isotopes to measure whole body rates of water turnover in animals.

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