

FOOD INGESTION AND WATER TURNOVER IN HUMMINGBIRDS: HOW MUCH DIETARY WATER IS ABSORBED?

TODD J. MCWHORTER^{*,‡} AND CARLOS MARTÍNEZ DEL RIO^{*}

Department of Zoology and Physiology, University of Wyoming, Laramie, WY 82071, USA

^{*}Present address: Department of Ecology and Evolutionary Biology, Biological Sciences West, Room 310, The University of Arizona, Tucson, AZ 85721-0088, USA

[‡]Author for correspondence (e-mail: mcwhorte@u.arizona.edu)

Accepted 27 July; published on WWW 30 September 1999

Summary

Hummingbirds are specialized nectarivores that feed on dilute solutions of sugars with trace amounts of amino acids and electrolytes. Their diets contain excess water that, if absorbed, must be eliminated. It has been hypothesized that in hummingbirds only a small fraction of this dietary water may be absorbed in the intestine. Here, we report the results of experiments designed to examine the relationship between nectar intake and water turnover in hummingbirds. Our results also allow the estimation of water absorption across the intestine and therefore test the hypothesis that ingested water in hummingbirds passes largely unabsorbed through the gastrointestinal tract. We found that fractional and total water turnover increased linearly with water ingestion. At low sucrose concentrations, food intake rates between four and five times body mass per 12 h were not unusual. A simple mass-

balance model suggested that 78 % of ingested water was absorbed in the gastrointestinal tract and hence must be processed by the kidneys. However, fractional water absorption was variable and did not appear to be correlated with food or water intake parameters. Our results do not lend support to the hypothesis that the bulk of dietary water passes through the intestine unabsorbed. Although hummingbird kidneys appear well suited to excrete large volumes of dilute urine, rates of energy assimilation in hummingbirds may be constrained by excess water elimination when these birds are feeding on nectars with a low sugar concentration.

Key words: hummingbird, *Selasphorus platycercus*, food ingestion, water turnover, diet, osmoregulation, nectar.

Introduction

A perpetual challenge facing terrestrial animals is the maintenance of fluid and electrolyte balance under the desiccating conditions that characterize their environments. Osmoregulatory research over the last four decades has largely focused on the physiological challenges to water balance faced by animals living under conditions of limited water availability (Schmidt-Nielsen, 1964). This emphasis on desert animals may have led to an unbalanced view of the water relationships of terrestrial animals in general. Nectar-eating animals exemplify the other end of the osmoregulatory spectrum. Floral nectars are dilute aqueous solutions of sugars containing trace amounts of amino acids and electrolytes (Baker, 1975, 1977; Baker and Baker, 1983). Because they contain large amounts of water, little protein and have relatively high osmotic concentrations, nectar diets can pose special osmoregulatory challenges for animals (Beuchat et al., 1990; Nicholson, 1998).

These challenges may be especially acute for small nectar-eating birds such as hummingbirds, which are perhaps the most specialized nectar-eating birds and are among the most specialized nectarivorous vertebrates (Grant and Grant, 1968). They have exceptionally high mass-specific metabolic

demands and exhibit the highest water flux rates measured in endothermic vertebrates (Weathers and Stiles, 1989). Previous research on hummingbirds has emphasized the physiological and ecological problems of a life with high energetic demands (Calder, 1975, 1994; Hainsworth, 1978, 1981; Hainsworth and Wolf, 1983) and, to a lesser extent, their ability to maintain the high levels of sugar digestion needed to meet those demands (Karasov et al., 1986; Martínez del Rio, 1990). Here, we focus on the maintenance of water and ion homeostasis and on the potential problems associated with simultaneous regulation of energy intake and osmoregulation.

Beuchat et al. (1990) estimate that, to satisfy daily energy requirements, Anna's hummingbirds (*Calypte anna*) routinely consume an amount of nectar equal to approximately 1.6 times their body mass, a value five times higher than that expected for a bird of their size. When energetic demands are greater (e.g. increased thermoregulatory demands because of low ambient temperatures), these birds can consume a volume of nectar equal to approximately three times their body mass per day (Beuchat et al., 1990, and references therein). If this ingested water is completely absorbed, the proportionate water

flux experienced by hummingbirds would exceed even that of freshwater amphibians (Beuchat et al., 1990). Indeed, extremely high water flux rates have frequently been measured in other 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; Nicholson, 1998).

To explain the ability of hummingbirds to process such large volumes of water rapidly, Beuchat et al. (1990) hypothesized that perhaps only a small fraction of the ingested water is absorbed from the small intestine, leaving the rest to pass quickly through the intestinal tract to the cloaca. This scenario requires the rapid absorption of sugars and electrolytes across the intestine and regulation of the transepithelial water flux that would inevitably accompany the absorption of osmotically active substances (Skadhauge, 1981; Beuchat et al., 1990). If the ingested water is largely absorbed across the intestine, as it appears to be in most vertebrates (Powell, 1987), hummingbirds would be faced with significant challenges to renal function when feeding on energy-dilute nectars (Beuchat et al., 1990). Here, we report the results of experiments designed to examine the relationship between nectar intake and water turnover in hummingbirds. Our results also allow the estimation of water absorption across the intestine of hummingbirds and therefore provide a test of the hypothesis of Beuchat et al. (1990).

Researchers have traditionally assumed that water turnover in nectarivorous 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). This hypothesis is based on the assumption that 100 % of ingested water is absorbed in the intestine. Thus, in evaluating the hypothesis of Beuchat et al. (1990), we also tested the validity of the main assumption of a significant body of work on the field energetics of nectar-feeding birds.

Materials and methods

Bird capture and maintenance

Broad-tailed hummingbirds [*Selasphorus platycercus* (Swainson)], body mass 3.34 ± 0.08 g, mean \pm S.E.M.] were captured with mist nets in Albany County, Wyoming, USA ($41^{\circ}20'N$, $106^{\circ}15'W$), and housed individually in wire mesh cages ($0.75 \text{ m} \times 0.75 \text{ m} \times 0.75 \text{ m}$). Birds were captured approximately 2 months before the project began and maintained or increased their body mass while in captivity. During experiments, birds were housed individually in opaque Plexiglas cages ($0.5 \text{ m} \times 0.5 \text{ m} \times 0.5 \text{ m}$) with individual light sources. The front of these cages was a one-way mirror that permitted observation of birds in a darkened room with minimal disturbance. Birds were allowed to acclimate to the cages for 2–3 days before the experiments began. The study was conducted at room temperature ($22 \pm 2^{\circ}\text{C}$) using a natural photoperiod from the time of bird capture (16 h:8 h light:dark). Birds were fed Roudybush Nectar 3 for adult hummingbirds

between experiments (Roudybush, Templeton, CA, USA). During experiments, birds were fed synthetic diets modified from Brice and Grau (1989). They were fed the experimental diet for a minimum of 24 h before trials began.

Experimental design

The experiment consisted of a series of trials in which each bird was randomly assigned to one of four dietary energy densities (292, 584, 876 or 1168 mmol l^{-1} sucrose). We relied on the behavioral response of hummingbirds to varying food energy density (López-Calleja et al., 1997). Typically, nectar-feeding birds show a steep negative relationship between total volumetric food intake and sugar concentration. Manipulation of sugar concentration therefore leads to a wide range of variation in food intake by hummingbirds. 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. Water turnover rates were estimated by injecting approximately $1.85 \times 10^4 \text{ Bq}$ of $^3\text{H}_2\text{O}$ in $15 \mu\text{l}$ of distilled water into the pectoralis muscle of each bird approximately 1.5 h after the lights had been turned on. Injection volumes were verified gravimetrically by weighing syringes ($25 \mu\text{l}$; Hamilton Company, Reno, NV, USA) to the nearest 0.0001 g before and after injection. Plastic-coated paper was drawn through slots in the bottom of the cages to facilitate collection of excreta while minimizing disturbance. Microcapillary tubes ($50 \mu\text{l}$) were used to collect excreta and quantify volume. Fresh excreta samples were collected at irregular intervals for approximately 30 h, excluding the dark portion of the photoperiod during which hummingbirds do not defecate. Samples were not collected until approximately 40 min after injection, allowing sufficient time for complete ^3H equilibration with body water (equilibration time estimates vary from 15 to 30 min in small birds; Williams and Nagy, 1984; Speakman, 1997). Excreta were immediately placed in separate scintillation vials to prevent evaporation. Liquid scintillation cocktail (Ecolume, ICN Research Products, Costa Mesa, CA, USA) was added to all excreta and injection samples, which were counted, correcting for quench and lumex (model LS 6000IC liquid scintillation counter, Beckman Instruments, Fullerton, CA, USA). Fractional water turnover rate ($K_{^3\text{H}}$) was estimated by fitting negative exponential functions to the relationship between ^3H specific activity in excreta 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. Birds were killed by Halothane (Halocarbon Laboratories, Augusta, SC, USA) inhalation overdose after the final experimental run and dried to constant mass at 80°C to measure total body water (TBW). Dehydration was used to measure TBW because isotope dilution methods proved technically difficult

because of the sensitivity of hummingbirds to repeated blood sampling.

Estimating water absorption in hummingbirds

We used a simple mass-balance approach to estimate the fraction of ingested water that was absorbed by hummingbirds. The method was inspired by the model developed by Karasov and Cork (1994) to determine the contribution of a passive pathway to total glucose absorption in birds. This approach used the data on water intake rate (\dot{V}_I in $\mu\text{l h}^{-1}$), total body water (TBW in μl), fractional water turnover rate (K_{3H} in h^{-1}) and rates of sugar catabolism (\dot{M}_S in mg h^{-1}) derived from the experiments described in the previous section. Assuming steady state (i.e. that the birds are in neutral water balance), the input rate (R_I) of water into the total body water is equal to total water turnover:

$$R_I = K_{3H} \times \text{TBW}. \quad (1)$$

The rate of input of water ($\mu\text{l h}^{-1}$) into TBW is equal to the sum of the fraction of ingested water that is absorbed and the rate of metabolic water production:

$$R_I = f_W \times \dot{V}_I + V_M \times \dot{M}_S, \quad (2)$$

where f_W is the fraction of ingested water that is absorbed and V_M is the amount of metabolic water resulting from the catabolism of carbohydrates ($0.56 \mu\text{l mg}^{-1}$). In subsequent calculations, we assume that \dot{M}_S is equal to the rate of sugar assimilation. Thus:

$$K_{3H} \times \text{TBW} = f_W \times \dot{V}_I + 0.56 \dot{M}_S. \quad (3)$$

We estimated f_W for each bird/trial as:

$$f_W = (K_{3H} \times \text{TBW} - 0.56 \dot{M}_S) / \dot{V}_I. \quad (4)$$

Sugar assimilation efficiency was estimated as the fraction of ingested sugar that was assimilated in an independent set of experiments (0.95 ± 0.02 , mean \pm S.D., $N=12$). Sugar assimilation efficiency was independent of sugar concentration. We calculated the rate of sugar assimilation as the product of sugar intake rate and sugar assimilation efficiency. Because TBW was measured by dehydration, we made the additional assumption that TBW did not change significantly between experimental trials and when it was measured. Our model will provide overestimates of fractional water absorption if birds were dehydrated during experimental trials relative to when TBW was measured.

Results

Volumetric food intake declined with sugar concentration (Fig. 1B). The relationship between food intake and sugar concentration was well described by a power function with an exponent that was significantly less than 1 (0.80 ± 0.08 , exponent \pm S.E.M., $t=-2.55$, $P<0.02$). Food intake varied approximately fivefold between the lowest and the highest sugar concentration. Sugar intake increased significantly with sugar concentration ($r_s=0.5$, $P<0.02$, $N=23$) (Fig. 1A).

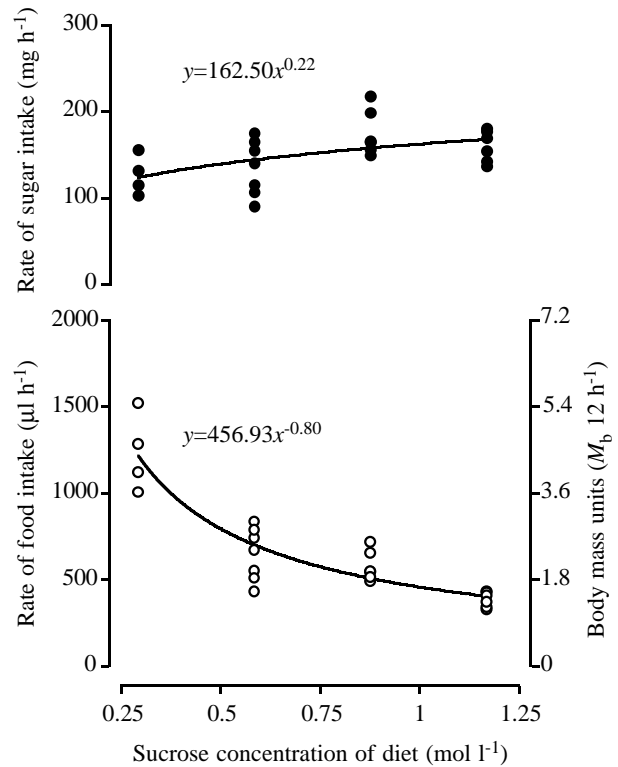


Fig. 1. Behavioral responses of hummingbirds to varying sugar concentration in their food. (A) The rate of sugar intake increased significantly with sugar concentration ($r_s=0.5$, $N=23$, $P<0.02$). The relationship was adequately described by a power function ($y=162.50x^{0.22}$, $r^2=0.25$). (B) Volumetric food intake rate declined with sugar concentration. The relationship was well described by a power function ($y=456.93x^{-0.80}$, $r^2=0.82$) with an exponent that was significantly less than 1 ($t=-2.55$, $P<0.02$). Changes in food energy density from 292 to 1168 mmol l^{-1} sucrose led to an approximately fivefold behavioral variation in nectar (and thus water) intake. The right-hand y-axis shows food intake in multiples of body mass (M_b) ($3.34 \pm 0.08 \text{ g}$, mean \pm S.E.M., $N=4$) per 12 h. At low sucrose concentrations, food intake rates between four and five times body mass per 12 h were not unusual.

The relationships between ^3H specific activity in excreta ($\text{disintegrations min}^{-1} \mu\text{l}^{-1}$) and time were well described by exponential functions (r^2 ranged from 0.56 to 0.98, $N=23$, Fig. 2). Thus, the decline in ^3H specific activity in excreta with time seemed to follow one-compartment, first-order kinetics (Fig. 2). Fractional water turnover rate ranged from 0.12 to 0.61 h^{-1} and was tightly and linearly correlated with water intake ($K_{3H}=4.14 \times 10^{-4}x+0.02$, $F_{1,21}=303.32$, $P=0.0001$). When birds were feeding on the most dilute nectar (292 mmol l^{-1} sucrose), approximately half of their TBW was turning over each hour. Average TBW measured by dehydration was $2.07 \pm 0.03 \text{ ml}$ (mean \pm S.E.M., $N=4$).

Fractional water absorption (f_W) ranged from 0.58 to 1.05 (0.78 ± 0.03 , mean \pm S.E.M., $N=23$) and was not significantly correlated with sugar concentration in food ($F_{3,20}=0.43$, $P>0.5$). Although the majority of ingested water appeared

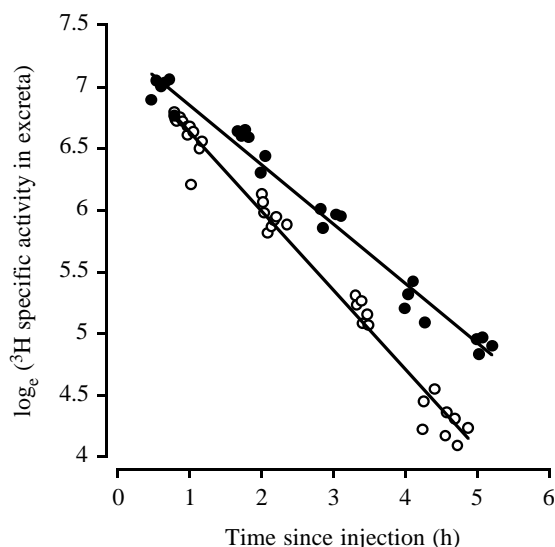


Fig. 2. Kinetics of ^3H excretion. The relationships between ^3H activity in excreta ($\text{disintegrations min}^{-1} \mu\text{l}^{-1}$) and time were well described by exponential functions (r^2 ranged from 0.56 to 0.98, $N=23$). The fractional water turnover rate ($K_{3\text{H}}$) was estimated as the exponent of the exponential relationship between ^3H activity in the excreta and time since injection. Fractional water turnover appeared to follow one-compartment, first-order kinetics. Data for two birds are represented here and were \log_e -transformed for clarity. Analysis was performed on non-transformed data (see Motulsky and Ransnas, 1987).

to be absorbed across the intestine of broad-tailed hummingbirds, absorption was variable and was not correlated with food and water ingestion rates. Water flux estimated from fractional water turnover rate ($K_{3\text{H}}$) and total body water (TBW) measurements ranged from 250.82 to $1311.93 \mu\text{l h}^{-1}$ and increased linearly with water intake ($F_{1,21}=300.52$, $P=0.0001$, Fig. 3). The slope of this relationship was significantly less than 1 (0.88 ± 0.05 , slope \pm S.E.M., $t=-2.5$, $P<0.05$). At low sucrose concentrations, food intake rates between four and five times body mass per 12 h were not unusual (Fig. 1).

Discussion

Hummingbirds vary their food intake rate in response to sugar concentration (Fig. 1). This behavioral response allows the exploration of their physiological responses to an ingested water load that can be made to vary severalfold. Our results indicate that both fractional water turnover rate and water flux increased linearly with water ingestion in hummingbirds. A simple mass-balance model suggested that the majority of ingested water is absorbed in the gastrointestinal tract and hence must be processed by the kidneys. However, water absorption was variable and did not appear to be correlated with food or water intake. Our results do not lend support to the hypothesis of Beuchat et al. (1990) that the bulk of dietary water passes through the intestine quickly and that relatively little is absorbed.

The conclusion that the majority of water ingested by hummingbirds is absorbed in the gastrointestinal tract depends on the use of a simple mass-balance model. Because our main conclusion relies on this model, the first section of this discussion examines the validity of its assumptions. The conclusion that there is almost complete absorption of ingested water in hummingbirds leads to intriguing consequences. We examine these in subsequent sections of this discussion. Specifically, we discuss the consequences of our conclusion for field doubly labeled water studies, renal function and rates of energy acquisition of nectar-feeding animals.

Assumptions of the water mass-balance model

Our mass-balance model relies on several assumptions. First, the model assumes that the total body water of hummingbirds behaves as a single, well-mixed compartment that exhibits simple first-order kinetics. This assumption appears to be satisfied (Fig. 2). The decline in ^3H activity after injection was well described by negative exponential functions and did not exhibit the multi-phasic decrease patterns that characterize systems with poor mixing and/or with more than one compartment (Speakman, 1997). Second, the model assumes steady-state water balance in which water inputs equal water losses. Collins (1981) demonstrated that overnight water losses in nectarivorous Australian honeyeaters were completely recovered within the first hour of foraging. Thus, for the time scale over which our experiments were conducted, it is likely that hummingbirds were in neutral water balance. It is important to point out,

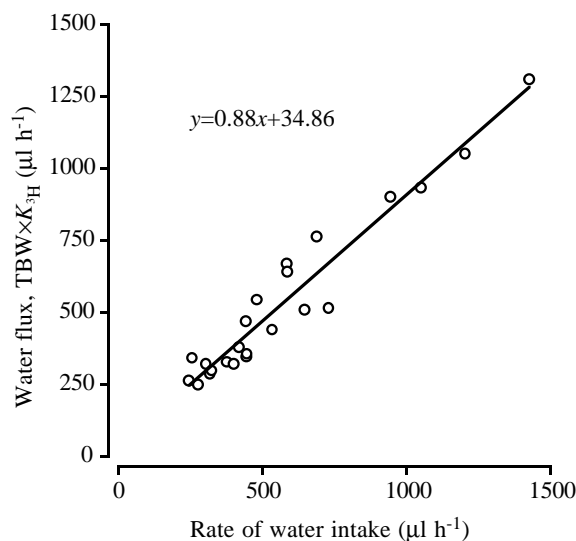


Fig. 3. Relationship between water flux and water intake. Water flux was estimated as the product of fractional water turnover rate ($K_{3\text{H}}$) and total body water (TBW) and increased linearly with water intake ($F_{1,21}=300.52$, $P=0.0001$). The slope of this relationship was significantly less than 1 (0.88 ± 0.05 , slope \pm S.E.M., $t=-2.5$, $P<0.05$). Water flux ranged from 250.82 to $1311.93 \mu\text{l h}^{-1}$. When birds were feeding on the most dilute nectar (292 mmol l^{-1} sucrose), approximately half of their TBW was turning over each hour.

however, that if the assumption of neutral water balance is not met, our model will over- or underestimate fractional water absorption, depending on the hydration status of the animal. A 5% error in TBW will lead to an error of approximately 6% in our estimate of fractional water absorption. In two cases, birds bled severely and went into shock upon injection of marker. In addition to the potential for perturbations of normal physiological processes, the assumption of neutral water balance was probably not satisfied because of body fluid loss. Indeed, the model provided estimates of fractional water absorption that were considerably greater than 1 in these cases. These values were not included in our analyses. Third, the model assumes that metabolic water is produced solely as a product of sugar metabolism and that it is possible to approximate the rate of sugar metabolism by the rate at which sugar is assimilated. Suarez et al. (1990) and Powers (1991) have demonstrated that actively feeding hummingbirds catabolize primarily carbohydrates. Because birds were in mass balance during the experiments, it is likely that only a small fraction of the total sugar assimilated was used to synthesize lipid. In summary, the assumptions of our model are either satisfied by the conditions of our experiments or the deviations from these assumptions are relatively minor.

Two additional lines of evidence provide further support for the idea that hummingbirds absorb most of the water that they ingest: (1) glucose absorption is probably accompanied by a large flux of water uptake, and (2) the intestinal and renal morphology of hummingbirds is consistent with significant water absorption and water processing. The mechanisms of intestinal water absorption in the intestines of hummingbirds are unknown but are probably facilitated by intestinal sugar uptake. Active sugar uptake in the intestine can facilitate water absorption by two mechanisms. First, active glucose uptake increases the rate of Na^+ transport across the brush border, and the Na^+/K^+ pump subsequently ejects this Na^+ across the basolateral membrane into the blood. This increase in the rate of salt absorption leads to water absorption (Wright et al., 1994). Second, Loo et al. (1996) demonstrated that the translocation of each glucose molecule by the intestinal $\text{Na}^+/\text{glucose}$ cotransporter into enterocytes is coupled with the transport of 260 water molecules (approximately 4.8 l of water per mole of glucose). A broad-tailed hummingbird drinking a 584 mmol l^{-1} sucrose solution assimilates on average $5.0 \times 10^{-3} \pm 1.4 \times 10^{-3} \text{ mol}$ (mean \pm S.D., $N=12$) of glucose in 16 h. The uptake of this quantity of glucose in the small intestine could be responsible for the transport of 24.2 ml of water. This amount exceeds the water content in the food by a large margin. Indeed, the amount of water that potentially accompanies glucose as it is absorbed in the intestine can account for the complete absorption of water in the food over the entire range of concentrations at which we have tested hummingbirds ($292\text{--}1168 \text{ mmol l}^{-1}$ glucose; see also O'Rourke et al., 1995, for similar observations in rat intestine). Hummingbirds exhibit the highest rate of carrier-

mediated glucose uptake measured in a vertebrate (Karasov et al., 1986). These high rates may be responsible for the high intestinal 'assimilation' of water apparently exhibited by hummingbirds.

Hummingbird gastrointestinal and renal morphology also support the conclusion that dietary water is largely absorbed. In *S. platycercus*, the diameter of the intestinal lumen decreases dramatically from 1.59 mm at the pyloric-duodenal junction to 0.64 mm at the distal ileum (T. J. McWhorter and C. Martínez del Río, unpublished data). This decrease suggests that digesta volume must decrease distally. Near-complete intestinal water absorption requires hummingbirds to have the capacity to produce copious amounts of dilute urine while feeding, but to avoid losing water and electrolytes while fasting. The kidneys of the three hummingbird species that have been examined appear to be composed largely of unlooped, reptilian-type nephrons and may lack medullary cones (Beuchat et al., 1990; Johnson and Mugaas, 1970). The paucity of long-looped nephrons in their kidneys indicates that they probably have little ability to raise urine osmotic concentrations above those of plasma (Beuchat et al., 1990). Calder and Hiebert (1983) found that the mean osmolalities of cloacal fluid samples obtained from seven hummingbird species in the field were all less than $100 \text{ mosmol kg}^{-1} \text{ H}_2\text{O}$, irrespective of whether the birds inhabited cool montane or arid desert habitats. These values are only one-third of those for typical avian plasma and are comparable with values for the urine of freshwater amphibians and fishes (Calder and Hiebert, 1983). Hummingbird kidneys appear to be well designed for the excretion of extraordinary quantities of dilute urine, suggesting their potential for processing large amounts of ingested water.

Consequences for doubly labeled water studies

Many researchers have hypothesized that water turnover in birds can be used to approximate water intake, given that metabolic water production is taken into account. This assumption has been extended to permit estimation of food intake in nectar-feeding animals by adding the supplementary supposition that ingested water comes only from nectar (von Helversen and Reyer, 1984; Kunz and Nagy, 1988; Powers and Nagy, 1988; Weathers and Stiles, 1989; Tiebout and Nagy, 1991). These hypotheses assume that 100% of ingested water is absorbed in the intestine. Although the tight relationship between fractional water turnover rate (K_{EH}) and water intake indicates that these parameters are indeed closely linked (Fig. 3), we must add the caveat that, if our observation of variable water absorption proves to be a general pattern in nectarivorous animals, these studies may underestimate nectar intake.

Consequences for renal function

Even when feeding on relatively concentrated nectars ($\geq 1200 \text{ mmol l}^{-1}$ sucrose), water intake from food exceeds hummingbird needs unless ambient temperatures are high ($>26^\circ\text{C}$; Calder, 1979). Thus, our results support the

hypothesis that, under most circumstances, hummingbirds are diuretic (Calder and Hiebert, 1983). Because the study of renal processes in birds has emphasized dehydration over diuresis, our current understanding of the role of the avian kidney in water conservation is more highly developed than our understanding of its role in water disposal (Braun, 1993). Thus, our discussion of the effects of high water loads on hummingbird renal function is, of necessity, speculative.

It is believed that glomerular filtration rates (GFR) are more variable and more responsive to water status in birds than in mammals (Williams et al., 1991; Dantzler, 1992). However, the form of the relationship between water load and GFR has not been described for birds. We hypothesize that GFR is high and well-regulated in hummingbirds. To our knowledge, the magnitude of GFR has not been quantified in hummingbirds. An allometric regression derived from several bird species can be used to provide an educated guess for the GFR of a 3.3 g broad-tailed hummingbird (Calder and Braun, 1983). This value (1.8 ml h^{-1}) is higher by approximately 50 % than the average water intake of a broad-tailed hummingbird fed on a 292 mmol l^{-1} sucrose solution ($1.2 \pm 0.1 \text{ ml h}^{-1}$, mean \pm S.E.M., $N=4$). In the field, however, hummingbirds can encounter and feed on nectars with sugar concentrations as low as 6.7 % sucrose equivalents (196 mmol l^{-1}) (Gryj et al., 1990). At this sugar concentration, the predicted volumetric intake of water (1.7 ml h^{-1} , from Fig. 1) closely approaches the estimated GFR.

Consequences for rates of energy intake

Water ingestion and its subsequent absorption in the intestine have the potential consequences of constraining the energy intake of an animal and exceeding the limits of its renal function. Fig. 1 illustrates the general observation that nectar-eating birds modulate volumetric food consumption in response to food energy density (see also Downs, 1997; López-Calleja et al., 1997). The higher ingestion rates that accompany low sugar concentrations in food compensate for potential losses in energy intake resulting from the lower quality of the food. However, for some species, this compensation is only partial: birds feeding on food with a low sugar concentration, especially under energetically demanding conditions, can show a decreased energy intake and decreases in body mass (Levey and Martínez del Río, 1999). We speculate that the ability of hummingbirds to feed on nectar with a low sucrose concentration is limited by the ability of these birds to dispose of absorbed water.

Although it appears that hummingbirds can ingest prodigious amounts of water with impunity, this ability does not appear to be the norm among terrestrial vertebrates. In humans, rats, domestic pigeons and gray parrots, over-ingestion of water is accompanied by 'water intoxication' (Lumeji and Westerhof, 1988; Gebel et al., 1989; Gevaert et al., 1991; De León et al., 1994). Water over-ingestion leads to negative effects because it can be accompanied by plasma dilution, hyponatremia (low plasma Na^+ level) and rupture of erythrocytes resulting from osmotic swelling (Faenestil, 1977).

Water intoxication is often the result when excessive water intake and absorption overwhelm healthy, intact osmoregulatory processes (Gebel et al., 1989). How do hummingbirds cope with what can be best characterized as natural, voluntary polydipsia? On the basis of our experiments, hummingbirds do not restrict water absorption across the intestine as hypothesized by Beuchat et al. (1990). Thus, hummingbird kidneys must be especially well suited to dispose of the large volume of water absorbed by their gastrointestinal tract. Digestive and renal function appear to be inextricably linked in hummingbirds.

Energy acquisition and water availability and use are closely interrelated in birds (Ricklefs, 1996). Ecological physiologists have emphasized low water availability as a constraint on energy use, on microhabitat selection and on activity patterns (Goldstein and Nagy, 1985; Ricklefs et al., 1986; Kam et al., 1987; Wolf and Walsberg, 1996; Wolf et al., 1996). Our research emphasizes a novel aspect of the water/energy interaction: water overabundance in food. We speculate that the rate at which hummingbird kidneys can process water may impose limits to energy intake. Although hummingbird kidneys appear well suited to excrete large volumes of dilute urine (Calder and Hiebert, 1983; Beuchat et al., 1990), rates of energy assimilation in hummingbirds may be constrained by excess water elimination when these birds are feeding on nectars with a low sugar concentration.

We are grateful to Casey Cotant for his assistance in data collection. Previous versions of this manuscript benefited from the critical comments of Eldon Braun, Bill Calder, Shelli Dubay, Doug Levey, Andrea McWhorter, Diane O'Brien, John Pappenheimer, Blair Wolf and two anonymous referees. This research was supported by NSF (IBN-9258505 to C.M.R.). This paper is dedicated to the memory of Vaughan H. Shoemaker – friend, mentor and pioneer in the area of amphibian osmoregulation.

References

- Baker, H. G. (1975). Sugar concentrations in nectars from hummingbird flowers. *Biotropica* **7**, 37–41.
- Baker, H. G. (1977). Non-sugar chemical constituents of nectar. *Apidologie* **8**, 349–356.
- Baker, H. G. and Baker, I. (1983). Floral nectar constituents in relation to pollinator type. In *Handbook of Pollination Biology* (ed. C. E. Jones and R. J. Little), pp. 117–141. New York: Scientific & Academic.
- Beuchat, C. A., Calder, W. A. III and Braun, E. J. (1990). The integration of osmoregulation and energy balance in hummingbirds. *Physiol. Zool.* **63**, 1059–1081.
- Braun, E. J. (1993). Renal function in birds. In *New Insights in Vertebrate Kidney Function* (ed. J. A. Brown, R. J. Balment and J. C. Rankin), pp. 167–188. Cambridge: Cambridge University Press.
- Brice, A. T. and Grau, C. R. (1989). Hummingbird nutrition: development of a purified diet for long-term maintenance. *Zoo Biol.* **8**, 233–237.
- Calder, W. A. III (1975). Factors in the energy budget of mountain

- hummingbirds. In *Perspectives of Biophysical Ecology* (ed. D. M. Gates and R. Schmerl), pp. 431–441. New York: Springer.
- Calder, W. A. III** (1979). On the temperature-dependency of optimal nectar concentrations for birds. *J. Theor. Biol.* **78**, 185–196.
- Calder, W. A. III** (1994). When do hummingbirds use torpor in nature? *Physiol. Zool.* **67**, 1051–1076.
- Calder, W. A. III and Braun, E. J.** (1983). Scaling of osmotic regulation in mammals and birds. *Am. J. Physiol.* **244**, R601–R606.
- Calder, W. A. III and Hiebert, S. M.** (1983). Nectar feeding, diuresis and electrolyte replacement of hummingbirds. *Physiol. Zool.* **56**, 325–334.
- Collins, B. G.** (1981). Nectar intake and water balance for two species of Australian Honeyeater, *Lichmera indistincta* and *Acanthorhynchus superciliosus*. *Physiol. Zool.* **54**, 1–13.
- Dantzler, W. H.** (1992). Comparative aspects of renal function. In *The Kidney, Physiology and Pathophysiology* (ed. D. W. Seldin and G. Giebisch), pp. 885–942. New York: Raven Press.
- De León, J., Verghese, C., Tracy, J., Jossianen, R. C. and Simpson, G. M.** (1994). Polydipsia and water intoxication in psychiatric patients: A review of the epidemiological literature. *Biol. Psychiat.* **35**, 408–419.
- Downs, C. T.** (1997). Sugar digestion efficiencies of Gurney's sugarbirds, malachite sunbirds and black sunbirds. *Physiol. Zool.* **70**, 93–99.
- Faenestil, D. D.** (1977). Hyposmolar syndromes. In *Disturbances of Body Fluid Osmolality* (ed. T. E. Andreoli, J. J. Grantham and F. C. Rector), pp. 267–284. Bethesda, MD: American Physiological Society.
- Gebel, F., Meng, H., Michot, F. and Truniger, B.** (1989). Psychogenic water intoxication. *J. Suisse Med.* **119**, 169–177.
- Gevaert, D., Nelis, J. and Veraenghe, B.** (1991). Plasma chemistry and urine analysis in induced polyuria in Racing pigeons *Columba livia*. *Avian Path.* **20**, 379–386.
- Goldstein, D. L. and Bradshaw, S. D.** (1998). Regulation of water and sodium balance in the field by Australian Honeyeaters (Aves: Meliphagidae). *Physiol. Zool.* **71**, 214–225.
- Goldstein, D. L. and Nagy, K. A.** (1985). Resource utilization by desert quail: time and energy, food and water. *Ecology* **66**, 378–387.
- Grant, K. A. and Grant, V.** (1968). Reciprocal evolution of hummingbirds and plants. In *Hummingbirds and Their Flowers* (ed. K. A. Grant and V. Grant), pp. 86–95. New York, London: Columbia University Press.
- Gryll, E., Martínez del Río, C. and Baker, I.** (1990). Avian pollination and nectar use in *Combretum fruticosum* (Loefl.). *Biotropica* **22**, 266–271.
- Hainsworth, F. R.** (1978). Feeding models of costs and benefits in energy regulation. *Am. Zool.* **18**, 701–714.
- Hainsworth, F. R.** (1981). Energy regulation in hummingbirds. *Am. Scient.* **69**, 420–429.
- Hainsworth, F. R. and Wolf, L. L.** (1983). Models and evidence for feeding control of energy. *Am. Zool.* **23**, 261–272.
- Johnson, O. W. and Mugaas, J. N.** (1970). Some histological features of avian kidneys. *Am. J. Anat.* **127**, 423–436.
- Kam, M., Degen, A. and Nagy, K. A.** (1987). Seasonal energy, water and food consumption of Negev chukars and sand-partridges. *Ecology* **68**, 1029–1037.
- Karasov, W. H. and Cork, S. J.** (1994). Glucose absorption by a nectarivorous bird: the passive pathway is paramount. *Am. J. Physiol.* **267**, G18–G26.
- Karasov, W. H., Phan, D., Diamond, J. M. and Carpenter, F. L.** (1986). Food passage and intestinal nutrient absorption in hummingbirds. *Auk* **103**, 453–464.
- Kunz, T. H. and Nagy, K. A.** (1988). Methods of energy budget analysis. In *Ecological and Behavioral Methods for the Study of Bats* (ed. T. H. Kunz), pp. 277–302. Washington, London: Smithsonian Institution Press.
- Levey, D. J. and Martínez del Río, C.** (1999). Test, rejection and reformulation of a chemical reactor-based model of gut function in a fruit-eating bird. *Physiol. Zool.* **72**, 369–383.
- Loo, D. D. F., Zeuthen, T., Chandy, G. and Wright, E. M.** (1996). Cotransport of water by the Na⁺/glucose cotransporter. *Proc. Natl. Acad. Sci. USA* **93**, 13367–13370.
- López-Calleja, M. V., Bozinovic, F. and Martínez del Río, C.** (1997). Effects of sugar concentration on hummingbird feeding and energy use. *Comp. Biochem. Physiol.* **118A**, 1291–1299.
- Lumeij, J. T. and Westerhof, I.** (1988). The use of water deprivation tests for the diagnosis of psychogenic polydipsia in a socially deprived African gray parrot *Psittacus erithacus*. *Avian Path.* **17**, 875–878.
- Martínez del Río, C.** (1990). Dietary, phylogenetic and ecological correlates of intestinal sucrase and maltase activity in birds. *Physiol. Zool.* **63**, 987–1011.
- Motulsky, H. J. and Ransnas, L. A.** (1987). Fitting curves to data using nonlinear regression: a practical and nonmathematical review. *FASEB J.* **1**, 365–374.
- Nicholson, S. W.** (1998). The importance of osmosis in nectar secretion and its consumption by insects. *Am. Zool.* **38**, 418–425.
- O'Rourke, M., Shi, X., Gisolfi, C. and Schedl, H.** (1995). Effect of absorption of D-glucose and water on paracellular transport in rat duodenum–jejunum. *Am. J. Med. Sci.* **309**, 146–151.
- Powell, D. W.** (1987). Intestinal water and electrolyte transport. In *Physiology of the Gastrointestinal Tract*, second edition (ed. L. R. Johnson), pp. 1267–1305. New York: Raven Press.
- Powers, D. R.** (1991). Diurnal variation in mass, metabolic rate and respiratory quotient in Anna's hummingbirds. *Physiol. Zool.* **64**, 850–870.
- Powers, D. R. and Conley, T. M.** (1994). Field metabolic rate and food consumption of two sympatric hummingbird species in southeastern Arizona. *Condor* **96**, 141–150.
- Powers, D. R. and Nagy, K. A.** (1988). Field metabolic rate and food consumption by free-living Anna's hummingbirds *Calypte anna*. *Physiol. Zool.* **61**, 500–506.
- Ricklefs, R. E.** (1996). Avian energetics, ecology and evolution. In *Avian Energetics and Nutritional Ecology* (ed. C. Carey), pp. 1–30. New York: Chapman & Hall.
- Ricklefs, R. E., Roby, D. D. and Williams, J. B.** (1986). Daily energy expenditure of adult Leach's Storm-petrels during the nesting cycle. *Physiol. Zool.* **59**, 649–660.
- Rooke, I. J., Bradshaw, S. D. and Langworthy, R. A.** (1983). Aspects of water, electrolyte and carbohydrate physiology of the silveryeye (*Zosterops lateralis*). *Aust. J. Zool.* **31**, 695–704.
- Schmidt-Nielsen, K.** (1964). *Desert Animals: Physiological Problems of Heat and Water*. Oxford: Oxford University Press.
- Skadhauge, E.** (1981). Osmoregulation in birds. *Zoophysiology*, vol. 12 (ed. W. S. Hoar, B. Hoelldobler, K. Johansen, H. Langer and G. Somero), pp. 1–203. New York: Springer.
- Speakman, J. R.** (1997). *Doubly Labelled Water: Theory and Practice*. London: Chapman & Hall.
- Suarez, R. K., Lighton, J. R. B., Moyes, C. D., Brown, G. S. and Hochachka, P. W.** (1990). Fuel selection in rufous hummingbirds:

- ecological implications of metabolic biochemistry. *Proc. Natl. Acad. Sci. USA* **87**, 9207–9210.
- Tiebout, H. M. and Nagy, K. A.** (1991). Validation of the doubly labeled water method ($^3\text{HH}^{18}\text{O}$) for measuring water flux and CO_2 production in the tropical hummingbird *Amazilia saucerottii*. *Physiol. Zool.* **64**, 362–374.
- von Helversen, O. and Reyer, H. U.** (1984). Nectar intake and energy expenditure in a flower visiting bat. *Oecologia* **63**, 178–184.
- Weathers, W. W. and Stiles, F. G.** (1989). Energetics and water balance in free-living tropical hummingbirds. *Condor* **91**, 324–331.
- Williams, J. B.** (1993). Energetics of incubation in free-living orange-breasted sunbirds in South Africa. *Condor* **95**, 115–126.
- Williams, J. B. and Nagy, K. A.** (1984). Daily energy expenditure of savannah sparrows: comparison of time–energy budget and doubly labeled water estimates. *Auk* **101**, 221–229.
- Williams, J. B., Pacelli, M. M. and Braun, E. J.** (1991). The effect of water deprivation on renal function in conscious unrestrained Gambel's quail. *Physiol. Zool.* **64**, 1200–1216.
- Wolf, B. O. and Walsberg, G. E.** (1996). Thermal effects of radiation and wind on a small birds and implications for microsite selection. *Ecology* **77**, 2228–2236.
- Wolf, B. O., Wooden, K. M. and Walsberg, G. E.** (1996). The use of thermal refugia by two small desert birds. *Condor* **98**, 424–428.
- Wright, E. M., Hirayama, B. A., Loo, D. D. F., Turk, E. and Hager, K.** (1994). Intestinal sugar transport. In *Physiology of the Gastrointestinal Tract*, third edition (ed. L. R. Johnson), pp. 1751–1772. New York: Raven Press.