

Drinking problems on a ‘simple’ diet: physiological convergence in nectar-feeding birds

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Running title: Physiology of nectar-feeding birds

Summary

Regulation of energy and water are by necessity closely linked in avian nectarivores, because the easily available sugars in nectar are accompanied by an excess of water but few electrolytes. In general, there is convergence in morphology and physiology between three main lineages of avian nectarivores which have evolved on different continents - the hummingbirds, sunbirds and honeyeaters. These birds show similar dependence of sugar preferences on nectar concentration, high intestinal sucrase activity and rapid absorption of hexoses via mediated and paracellular routes. There are differences, however, in how these lineages deal with energy challenges, as well as processing the large volumes of preformed water ingested in nectar. While hummingbirds rely on varying renal water reabsorption, the passerine nectarivores modulate intestinal water absorption during water loading, thus reducing the impact on the kidneys. Hummingbirds do not generally cope with salt loading, and have renal morphology consistent with their ability to produce copious dilute urine; by contrast, as well as being able to deal with dilute diets, honeyeaters and sunbirds are more than capable of dealing with moderately high levels of added electrolytes. And finally, in response to energy challenge, hummingbirds resort readily to torpor, while the passerines show renal and digestive responses that allow them to deal with short term fasts and rapidly restore energy balance without using torpor. In conclusion, sunbirds and honeyeaters

demonstrate a degree of physiological plasticity in dealing with digestive and renal challenges of their nectar diet, while hummingbirds appear to be more constrained by this diet.

Keywords: dilute nectar, hummingbirds, sunbirds, honeyeaters

Introduction

Nectar has been described as ‘the simplest food on earth’ (Martínez del Rio et al., 2001) but nevertheless may pose significant physiological challenges for animals feeding on it. Since we reviewed the topic a decade ago (Nicolson and Fleming, 2003b), researchers in the Americas, Africa and Australia have made substantial progress towards comparing the physiology of the three main lineages of specialized avian nectarivores: the hummingbirds (Trochilidae), sunbirds (Nectariniidae) and honeyeaters (Meliphagidae) (Fig. 1). Numerous other bird families show varying reliance on nectar (Nicolson and Fleming, 2003b; Symes et al., 2008), but this commentary will focus on how the main radiations deal with the challenges of nectarivory, highlighting both convergence and differences in their responses.



Fig. 1. Specialized avian nectarivores from three lineages. (A) Whitebellied sunbird, *Cinnyris talatala* (photo credit: Rudi van Aarde), (B) New Holland honeyeater, *Phylidonyris novaehollandiae* (Kathryn Napier) and (C) charming hummingbird, *Amazilia decora* (Cole Wolf).

The relatively low concentration of bird nectars (compared to insect nectars: Pyke and Waser, 1981) is the fundamental reason for the physiological problems associated with a nectar diet. Flowers visited by hummingbirds and sunbirds produce nectar averaging 10-30 μ l in volume and 15-25% w/w (0.5 - 0.8 M sucrose equivalents) in concentration (while flowers visited by

generalist birds produce nectars that are even more copious and dilute, Johnson and Nicolson, 2008). Nectar can also be diluted by rainfall events. The challenge of drinking dilute diets is that the birds must handle large volumes of preformed water, necessitating fast transit times (i.e. time for food to pass through the gut; thus minimizing mass gain, which would interfere with flight), and yet maintain high assimilation efficiency, while also warming this large volume of fluid to body temperature. More concentrated nectars are likely to be less problematic, since switching between plants would enable birds to access more dilute nectar and thus balance water intake. The other major challenge is that nectar also contains very little by way of other solutes; nectarivorous birds therefore have to deal with low electrolyte intake as well as low nitrogen, although their nitrogen requirements are relatively low (Tsahar et al., 2006).

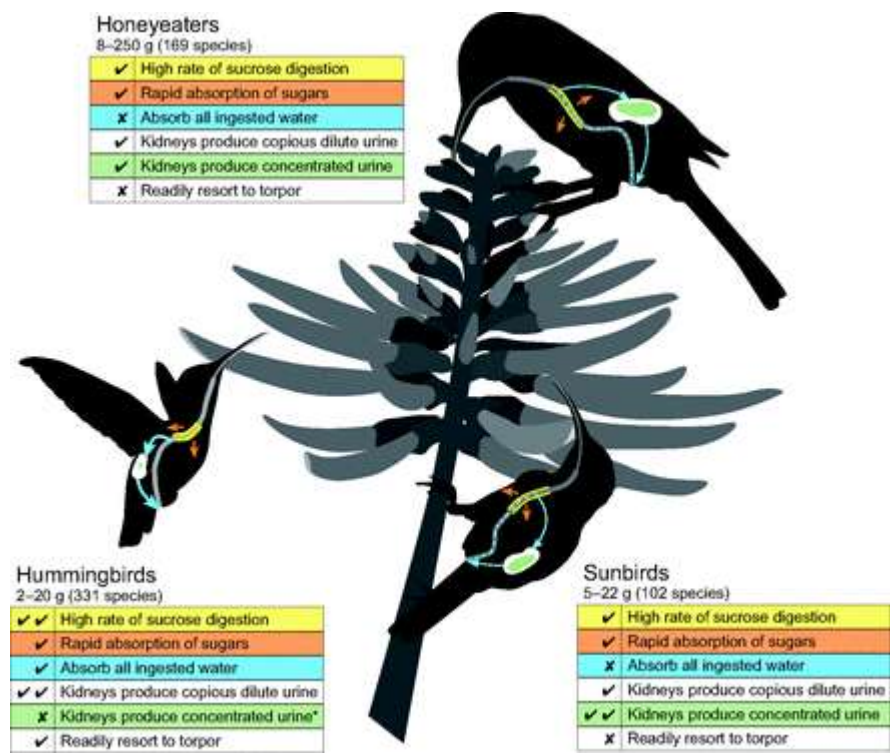


Fig. 2. Some key physiological differences between the three specialist bird lineages. A representative hummingbird, honeyeater and sunbird (note the differences in body size) are shown feeding on nectar of an *Erythrina* species, a major bird-pollinated genus that occurs in the natural range of all three lineages. All lineages show high rates of intestinal sucrose activity (highest for the hummingbirds; thicker yellow lines) and rapid absorption of hexose sugars (orange arrows), but the passerines are capable of shunting water directly through the gut (blue dotted lines) and therefore do not only rely on filtration by the kidneys (blue solid lines). *Hummingbirds show only a small medullary portion to their kidneys (shaded green), which limits their abilities to produce hyperosmotic urine.

It might be predicted that the physiological constraints of a ‘simple’ nectar diet would result in convergence in physiological responses of the different lineages of nectarivorous birds.

However recent findings indicate that this is not always the case and we now know that these birds show interesting differences in response to their nectar diets (Fig. 2). In the first half of this review, we examine some common strategies for dealing with the challenges of a nectar diet. In the second half, we examine available data that indicate differences in how hummingbirds, sunbirds and honeyeaters deal with these challenges.

The drinking process

Nectar-feeding birds show striking convergence in tongue morphology, possessing grooved, bifurcated tongues, with brush or fringed tips (Fleischer et al., 2008). Three decades after initial studies on the mechanics of nectar drinking in birds (Heyneman, 1983; Kingsolver and Daniel, 1983), this field is receiving renewed attention, and not without controversy. Rico-Guevara and Rubega (2011) presented morphological measurements of hummingbird tongues and high-speed videos of drinking that showed that the tongue traps liquid as a result of surface tension: this requires no energy expenditure by the bird and does not involve capillary action, which suggests that viscosity is not important. This finding was disputed by Kim et al. (2012a), who used a modeling approach to differentiate between different modes of nectar drinking, showing that the ‘capillary suction’ mechanism used by hummingbirds, sunbirds and honeyeaters is associated with optimal concentrations in the range 30-50% (1.0-1.8 M sucrose; much higher than natural nectar concentrations). After further theoretical and experimental analysis of drinking in ruby-throated hummingbirds *Archilochus colubris*, Kim et al. (2012b) suggested that fluid trapping (as proposed by Rico-Guevara and Rubega 2011) and capillary suction may be complementary in the nectar loading process.

One hypothesis proposed to explain the low nectar concentrations that have evolved in bird-pollinated plants concerned the compromise between energy content and viscosity (Baker, 1975). Other hypotheses involve discouragement of bees, the water requirements of birds, reduced evaporation in tubular corollas, the osmotic consequences of high hexose levels in nectar, or plant phylogeny; however, the current evidence is not strong for any of these hypotheses (Johnson and Nicolson, 2008). We have demonstrated recently that low viscosities are necessary for efficient nectar uptake by whitebellied sunbirds *Cinnyris talatala* (Köhler et al., 2010a). When the inert polysaccharide Tylose™ was added to artificial nectar to increase its viscosity independently of energy density, sunbirds reduced their licking frequencies and tongue loads at high viscosities, while lick duration increased. The edges of

the tongue coil inward to form two open grooves, and nectars of higher concentration are too viscous for efficient filling of these grooves by capillarity: the tongue loads of birds feeding on 1 M sucrose are half those when feeding on 0.25 M sucrose (Köhler et al., 2010a). At the same temperature and concentration, the viscosities of pure sugar solutions mixed on a percentage mass basis decrease in the order sucrose > glucose > fructose (Heyneman, 1983; Telis et al., 2007). Although there are minimal difference in viscosity between sucrose and hexose solutions over the average concentrations of bird nectars, subtle differences in feeding rate due to viscosity could nevertheless influence dietary sugar preferences in nectarivorous birds. The role of viscosity in sugar preferences warrants investigation.

In addition to concentration, nectar volumes are important for tongue loading. The volume of nectar determines its height in tubular floral corollas, and partial immersion of the tongue is common (Kim et al., 2012b). When honeyeaters and hummingbirds feed from tubular artificial flowers, the amount of nectar loaded per lick increases with greater nectar volumes or shorter flowers, both of which increase the contact between tongue and nectar (Collins, 2008). However, honeyeaters also commonly extract nectar from open, brush-type inflorescences and the effects of inflorescence shape on feeding rates have yet to be investigated.

Sugar preferences and the link to digestion

The simple sugars in nectar are derived from sucrose unloaded from phloem to secretory tissue, and invertase in the nectary or nectar hydrolyses the sucrose to glucose and fructose, either before or after nectar secretion (Heil, 2011). Sucrose can vary from 0-100% of the sugar present, depending on invertase activity, but most nectars are of mixed sugar composition, as shown, for example, in a large survey of plants pollinated by birds and bats (Baker et al., 1998). Extensive data have been collected on nectar sugar compositions and the observed patterns have been variously attributed to plant phylogeny or pollinator preferences, though these may not be mutually exclusive (Nicolson and Thornburg, 2007). Earlier studies suggested a dichotomy between high nectar sucrose in hummingbird-pollinated plants and high hexose levels in nectars of passerine-pollinated plants (Martínez del Rio et al., 1992); however, a more useful distinction is that between high nectar sucrose in flowers pollinated by specialized nectarivores (hummingbirds and sunbirds) and low nectar sucrose in plants

adapted for generalized bird pollination (Johnson and Nicolson, 2008). As yet, few data are available on nectar sugars of Australian plants (Napier et al., 2013).

Nectar-feeding birds are compensatory feeders over a wide range of nectar concentrations (Martínez del Rio et al., 2001; Nicolson and Fleming, 2003a): this means that they adjust their volumetric intake rapidly in response to changes in energy density (Fleming et al., 2004b; Köhler et al., 2008). Sugar concentration consequently has a marked effect on their sugar preferences. Probably because of the rapid transit times and because hexose nectars do not require hydrolysis in order for the sugars to be assimilated, a hexose diet is preferred by both generalist and specialist nectar-feeding birds on dilute diets (for references see Napier et al., 2013).

Interestingly, most species tested to date show a switch over to sucrose preference on concentrated diets (e.g. data for the red wattlebird *Anthochaera carunculata* shown in Fig. 3a). The concentration at which the switch occurs varies between species, and the minimum sugar concentration at which birds show no hexose preference is significantly correlated with intestinal sucrase activity for 11 bird species, even after phylogenetic correction (Fig. 3b) (Napier et al., 2013). Birds with no sucrase (e.g. redwinged starlings *Onychognathus morio*) or relatively low sucrase activity (e.g. rainbow lorikeets *Trichoglossus haematodus*) prefer hexoses at higher sugar concentrations, while birds with the greatest sucrase activity (i.e. hummingbirds, but also sunbirds and honeyeaters) either show no hexose preference or hexose preference on only the most dilute diets (Napier et al., 2013). The sucrose preference at higher sugar concentrations remains more difficult to explain than the hexose preference at low concentrations. There is a range of suggested reasons, including lower osmolality of sucrose than hexose solutions and imprinting from natural conditions (reviewed by Fleming et al., 2008), but the result remains somewhat puzzling. The role of viscosity in the mechanics of drinking may be involved in selection for sucrose over hexose on concentrated diets: although the experimental viscosity of sucrose solutions (Telis et al., 2007) at these high concentrations is higher than that of pure glucose or fructose solutions, mixtures of the hexose sugars may have different properties. Taste may also play a role in sugar type preferences: in humans viscosity affects the perception of flavour (Hollowood et al., 2002), and the broad-billed hummingbird *Cynanthus latirostris* detects fructose at lower concentrations than sucrose or glucose (Medina-Tapia et al., 2012).

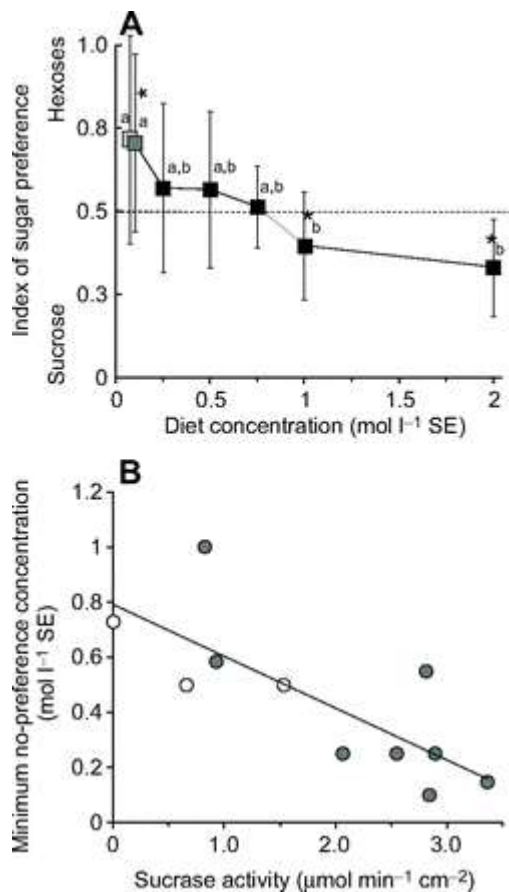


Fig. 3. Sugar preferences and sucrase activity in nectar-feeding birds. (A) Concentration-dependent sugar preferences of red wattlebirds, *Anthochaera carunculata* (Fleming et al., 2008). Birds were offered pairs of sucrose and hexose (fructose + glucose) solutions of varying concentrations from 0.075 to 2 mol l⁻¹ sucrose equivalents (SE). Diets where birds did not achieve energy balance are indicated with increasingly lighter shaded symbols. Values are means \pm 1 s.d. index of sugar preference (i.e. hexose ingested as a proportion of total sugar ingested). Asterisks indicate significant preferences for either hexose or sucrose diets ($*P < 0.05$) and different letters indicate diets that were significantly different from each other in terms of the index of sugar preference (ANOVA and *post hoc* analysis). (B) Relationship between degree of hexose preference (i.e. minimum concentration where no sugar preference was indicated) and standardized intestinal sucrase activity in 11 nectarivore species (Napier et al., 2013). Data are average values for each species. White symbols denote diet generalists and grey symbols nectar specialists.

The methodology of sugar preference tests is important, and the choice offered to the birds must be between equicaloric solutions of sucrose and hexoses (Brown et al., 2008; Fleming et al., 2004c). This is because hexose solutions mixed on a percentage mass basis have only 95% of the energy value of sucrose solutions (Fleming et al., 2004c). This is not inconsequential for the birds. For example, rufous hummingbirds provided with sucrose concentrations in 5% increments up to 45% invariably drink substantially more of high concentrations (Tamm & Gass 1986) and can distinguish concentrations differing by only 1% (Blem *et al.* 2000). The best discrimination occurs between 20 and 30% (the range over which most preference trials have been carried out), when rufous hummingbirds drink about

four times the more concentrated diet compared with the dilute diet (Tamm & Gass 1986; Blem et al. 2000). Consequently many earlier sugar-type preference studies which were carried out using diets close to 20% w/w sugar may require re-visitation for us to be able to compare across a broad range of species.

Absorption of sugars for rapid utilization

Because they need to maintain high metabolic rates (requiring high diet intake) but also need to maintain flight (requiring low mass gain), nectarivorous birds (and bats) have a relatively short gut, associated with a fast transit time (Caviedes-Vidal et al., 2007). Consequently, they need to digest their nectar meals and assimilate the sugars present extremely rapidly. This rapid absorption of sugar in nectarivores has been demonstrated using stable isotope tracing, which takes advantage of the fact that the $^{13}\text{C}:^{12}\text{C}$ signatures of cane sugar and beet sugar are completely different, allowing experimental testing using diet switching. Changing the dietary sugar source and measuring the isotopic composition of expired CO_2 clearly shows the rapid oxidation of ingested sugars during foraging flight in hummingbirds (Welch et al., 2006; Welch and Suarez, 2007). Importantly, direct oxidation of dietary sugar is 16% more efficient than using fat to fuel flight (Suarez et al., 1990).

After enzymatic hydrolysis of sucrose, assimilation of glucose and fructose occurs via two potential routes. Firstly, secondary-active transport requires the input of energy to move the monosaccharides through the intestinal epithelial cells. The sodium-linked glucose transporter SGLT1 transports one glucose molecule together with two Na^+ ions across the apical membrane surface (Drozdowski and Thomson, 2006; Scheepers et al., 2004). The requirement for sodium is evident in whitebellied sunbirds and New Holland honeyeaters *Phylidonyris novaehollandiae*, where intake of very dilute diets increases substantially when sodium is added to these diets (Purchase et al., 2010). Once glucose has entered the intestinal epithelial cells, it can move down its concentration gradient, exiting the cells to enter the blood stream via facilitated diffusion: this is effected by GLUT2 transporters on the basal membrane. Less is known about fructose absorption, which occurs via another member of the glucose transporter family, GLUT5 (McWhorter, 2005). Interestingly, Chen and Welch (2013) have recently demonstrated that hovering hummingbirds are able to utilize fructose and glucose equally; this physiological capacity is lacking in mammals.

The second alternative route is paracellular (i.e. passing *between* the epithelial cells), and this appears to be much more important in birds (and bats) than in non-flying mammals (Caviedes-Vidal et al., 2007; McWhorter, 2005). In avian nectarivores of all main lineages, the paracellular pathway permits rapid and efficient glucose absorption that is modulated by nectar concentration (McWhorter et al., 2006; Napier et al., 2008). The proportion of absorption through the paracellular route is reduced on dilute sugar concentrations: this can possibly be explained by the faster passage rates leading to reduced contact with the absorptive surfaces, and by the reduced intercellular spaces between osmotically-swollen cells. Mediated (or trans-cellular) glucose uptake via the SGLT1 transporter is therefore more important at low sugar concentrations; conversely, the contribution of the paracellular pathway increases at higher sugar concentrations.

All the main nectar-feeding lineages show very high assimilation efficiencies for the simple sugars in nectar, extracting >99% of ingested sugars (Lotz and Schondube, 2006; Napier et al., 2013). Assimilation efficiencies for sucrose are higher in specialist nectarivores than in generalists, which is likely to reflect differences in sucrase activity (Napier et al., 2013). Specialist nectarivores also appear to be capable of maintaining high assimilation efficiency even when the birds are challenged to increase their feeding rate. For example, whitebellied sunbirds maintained high assimilation efficiencies when volumetric intake increased due to low ambient temperatures or feeding on dilute diets (Köhler et al., 2010b).

How do nectarivorous birds deal with low protein intake?

Another important consideration for these birds is that although all essential amino acids are commonly present in floral nectar (Nicolson and Thornburg, 2007), the amount of nitrogen is generally low (~1% of dry matter for nectar, Gartrell, 2000). Furthermore, nectar amino acid composition may not match nitrogen requirements in terms of essential amino acids.

Nitrogen levels found in most floral nectars are generally insufficient to meet the nitrogen requirements of nectarivorous vertebrates (López-Calleja et al., 2003). Supplementing their nectar diet with pollen, insects or spiders is therefore important, especially during seasons when the birds are moulting or breeding. A recent review (Tsahar et al., 2006) concluded that nectarivores and frugivores have nitrogen requirements only ~25% of that of omnivorous birds. Their data set included a large number of hummingbirds but similar data for a range of sunbird and honeyeater species are not yet available.

In the second half of this review, we discuss areas where we have discovered marked differences in how hummingbirds, sunbirds and honeyeaters deal with the physiological challenges of a nectar diet.

Regulation of intestinal water absorption

Consumption of dilute diets by specialized nectarivores leads to extraordinarily high rates of water turnover, about 3-5 times body mass per day (Collins, 1981; McWhorter and Martínez del Rio, 1999; Nicolson and Fleming, 2003a). This raises the issue of how birds dispose of these large water loads. In an influential early review, Beuchat et al. (1990) hypothesized that avian nectarivores might shunt excess water through the intestine to reduce the load on the kidneys. The possible occurrence of such modulation of intestinal water absorption has been tested experimentally by means of pharmacokinetic techniques, using the model developed by McWhorter and Martínez del Rio (1999). Assumptions and limitations of the steady-state pharmacokinetic method are explored in detail by Purchase et al. (2013b) and Napier et al. (2012). Data collected to date suggest that there is no modulation of intestinal water absorption in two hummingbird species (green-backed firecrowns *Sephanoides sephanoides*, Fig. 4a; and broad-tailed hummingbirds *Selasphorus platycercus*; Hartman Bakken and Sabat, 2006; McWhorter and Martínez del Rio, 1999). However, three passerine nectarivores (Palestine sunbird *Cinnyris oseus*, whitebellied sunbird, and New Holland honeyeater) show evidence of modulation of water absorption (Fig. 4b,c,d), absorbing a lower proportion of ingested water on dilute diets compared with concentrated diets (McWhorter et al., 2003; Purchase et al., 2013b). These data therefore support the intestinal shunting hypothesis of Beuchat et al. (1990) for sunbirds and honeyeaters, but not for hummingbirds.

Such modulation of intestinal water absorption has not been demonstrated in any other vertebrate. Freshwater fish and amphibians have similarly high water fluxes (Beuchat et al., 1990; McWhorter et al., 2009); however the route of water ingestion in these animals (gills or skin, respectively) precludes direct comparison of the intestinal mechanisms for handling high water loading with nectarivorous birds. Modulation of water absorption has implications for the efficiency of obtaining nutrients from nectar, requiring the rapid absorption of monosaccharides from a dilute food that is passing rapidly through the

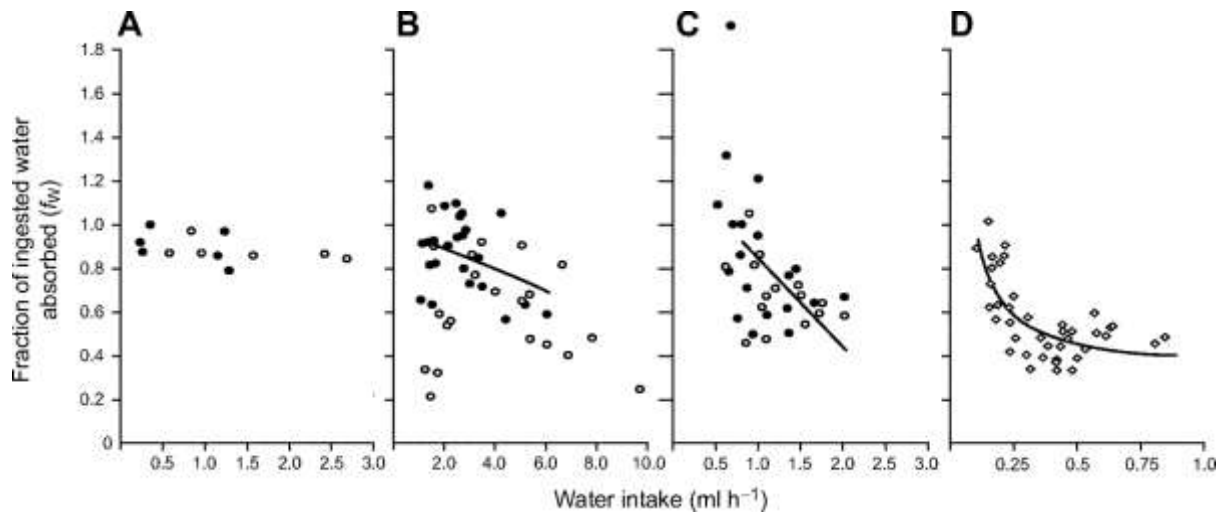


Fig. 4. Fractional water absorption (f_w) in the gastrointestinal tract is reduced with water loading (volume of water ingested) in passerine nectarivores but not hummingbirds. (A) Green-backed firecrewns, *Sephanoides sephaniodes* (Hartman Bakken and Sabat, 2006), (B) New Holland honeyeaters, *Phylidonyris novaehollandiae* (Purchase et al., 2013b), and (C) whitebellied sunbirds, *Cinnyris talatala* (Purchase et al., 2013b) measured at 20°C. Filled circles represent measurements collected in the afternoon and open circles in the morning (only the evening data were significantly correlated for the honeyeaters). Data have also been collected for (D) Palestine sunbirds, *Cinnyris osea*, measured at 30°C (unfilled diamonds) (McWhorter et al., 2003).

intestine, as well as efficient uptake of amino acids and electrolytes present at low concentrations (Beuchat et al., 1990). It is premature to speculate on how intestinal permeability to water is regulated, but the mechanism may involve aquaporin water channels, solvent drag during paracellular absorption of glucose (although absorption via this route is reduced on dilute diets), or water movement associated with mediated glucose transport (McWhorter and Martínez del Rio, 1999).

Kidney structure and function

Because of these differences in intestinal water absorption, we might expect differences in kidney structure and function between avian nectarivores. The avian kidney includes two types of nephrons: those that do not have a loop of Henle ('reptilian-type' or loopless nephrons), and those with a loop of Henle ('mammalian-type' or looped nephrons), the latter making up the medullary part of the kidney and contributing to the ability to concentrate urine (Goldstein and Skadhauge, 2000). Renal morphology of Anna's hummingbird *Calypte anna* - the only hummingbird species examined to date - differs in several significant ways from that of other birds investigated (Beuchat et al., 1999; Casotti et al., 1998). Their kidneys contain very little medullary tissue; 90% of the total volume of the kidneys is cortical tissue, with medulla accounting for only 2% (Casotti et al., 1998). The medullary cones are

small, containing only a few loops of Henle and collecting ducts (Beuchat et al., 1999). The structures that allow the animal to produce urine that is hyperosmotic to plasma are therefore poorly developed or absent. A comprehensive set of analyses in honeyeaters reveals that these patterns are not found across all nectarivorous birds. Honeyeaters, in fact, have kidneys that are well-developed to produce concentrated urine. Primarily-nectarivorous honeyeaters have 4-9% medulla, while primarily-insectivorous honeyeaters have 8-18% medulla (Casotti and Richardson, 1992; Casotti and Richardson, 1993). Importantly, sunbird (e.g. up to 500 ± 40 mOsmol kg^{-1} ; Purchase et al., 2013a) and honeyeater (370 ± 30 mOsmol kg^{-1} ; Purchase et al., 2013a) species can produce urine that is markedly hyperosmotic compared with plasma, surpassing the ability of rufous hummingbirds *Selasphorus rufus*, which become salt loaded when feeding on 0.63 M sucrose diets with even moderate quantities (35 mM) of sodium chloride added (Lotz and Martínez del Rio, 2004). Hummingbirds are therefore specialised for dealing with water loading and retrieval of electrolytes, while honeyeaters and sunbirds are additionally capable of dealing with dehydration and electrolyte loading.

Whole-kidney glomerular filtration rate (GFR; a measure of the rate at which the kidneys filter the plasma) is much more responsive to hydration state in birds than in mammals, decreasing with dehydration or salt loading and increasing with water loading (Goldstein and Skadhauge, 2000). This is due to release of arginine vasotocin (AVT), which acts mainly on GFR, while having a relatively modest effect on the water permeability of the collecting ducts (Goldstein, 2006; Nishimura and Fan, 2003). The reduction of GFR by AVT occurs through a decrease in filtration by reptilian-type nephrons, with mammalian-type nephrons being less affected (Goldstein and Skadhauge, 2000). Plasma AVT concentrations increase with increasing sugar concentration, presumably reflecting a hormonal response to increased plasma osmotic concentration (Gray et al., 2004). Surprisingly, in view of the high variation in water intake, GFR does not vary with water loading for the three avian nectarivore lineages (Goldstein and Bradshaw, 1998; Hartman Bakken and Sabat, 2006; Purchase et al., 2013b), although GFR is greatly reduced at night (see below). The other way to vary urine production is by changes in water reabsorption in the kidney. Hummingbirds (which lack intestinal shunting of preformed water) tend to modulate water reabsorption in the kidneys in relation to water intake (Hartman Bakken and Sabat, 2006); Palestine sunbirds also modulate water reabsorption (McWhorter et al., 2004) but there is no evidence that whitebellied sunbirds and New Holland honeyeaters do so (Purchase et al., 2013b).

The potential role of evaporation

Another possible route for elimination of surplus water is through evaporation. Evaporative water loss (EWL) of Anna's hummingbird is high because of its high mass-specific metabolic rate, but represents a far smaller proportion of daily water loss than in other birds and mammals because of the high excretory losses (Powers, 1992). Estimates of EWL from the difference between water gain and cloacal fluid output have also yielded high values for sunbirds and honeyeaters (Collins, 1981; Fleming and Nicolson, 2003; Lotz and Nicolson, 1999). Nothing is known of the partitioning of evaporation between cutaneous and respiratory routes, but cutaneous EWL is influenced by hydration state in other birds (Williams et al., 2012). Unfortunately the high excretory output of avian nectarivores complicates the direct measurement of EWL, and the pharmacokinetic method has proved unreliable for its estimation (Purchase et al., 2013b). Further research in this area is required to determine the role of evaporation in osmoregulation for the three avian nectarivore lineages.

How do nectarivorous birds deal with low salt intake?

High water fluxes are a problem for salt conservation, and avian nectarivores are very efficient at minimizing electrolyte losses in the cloacal fluid: for example, whitebellied sunbirds feeding on 0.25 M sucrose solutions void cloacal fluid with an osmolality of only 6 mOsmol/kg (Fleming and Nicolson, 2003). Despite similar abilities to produce copious volumes of dilute excreta, extremely dilute sucrose diets devoid of electrolytes cause hummingbirds to stop feeding and go into torpor, whilst honeyeaters and sunbirds suffer decreased plasma sodium levels and are unable to maintain energy balance (Fleming et al., 2004c; Goldstein and Bradshaw, 1998; Lotz and Martínez del Río, 2004). Nectarivores thus appear to be constrained in their intake of dilute diets due to hyponatremia, i.e. low plasma sodium concentration (Fleming and Nicolson, 2003) and the addition of even small amounts of sodium (5-10 mM) to very dilute sucrose diets results in a marked decrease in plasma aldosterone concentration (the active principle of the renin–angiotensin–aldosterone system, stimulating renal reabsorption of sodium) (Fleming et al., 2004a) and enables whitebellied sunbirds and New Holland honeyeaters to increase consumption, up to an extraordinary 8x their body mass daily (Purchase et al., 2010).

The reabsorption of ions from cloacal fluid is likely to also involve the lower intestine. Post-renal modification is important in osmoregulation of birds, and recovery of Na⁺ and K⁺ ions has been demonstrated in hummingbirds, honeyeaters and sunbirds by comparing their concentrations in ureteral urine and cloacal fluid (Lotz and Martínez del Rio, 2004; Purchase et al., 2013a). However, caution is needed in assessing the contribution of the lower intestine to recovery of electrolytes in birds that modulate intestinal water absorption, because the elimination of non-absorbed water will also dilute the urine.

How do nectarivorous birds deal with fasting?

One of the most significant challenges of dealing with a liquid diet and low gut retention time is that when the birds fast (either on a short-term basis between meals, or over longer periods due to inclement weather or overnight), they have little in the way of gut lumen contents to sustain constant energy levels. Variations in patterns of excretion suggest subtle differences in how sunbirds and honeyeaters deal with short-term disturbances. When birds are captured and handled for an intramuscular injection as part of a pharmacokinetic technique (Napier et al., 2012; Purchase et al., 2013b), they may take different lengths of time to return to feed, thereby experiencing a short-term fast. Variations in patterns of excretion following this disturbance suggest that whitebellied sunbirds probably reduce GFR in response to disturbance, but New Holland honeyeaters reduce the frequency of excretion (i.e. store cloacal fluid and reabsorb water in the distal intestine) until they recommence feeding normally.

During the night, nectar-feeding birds are subject to dehydration rather than diuresis. Whitebellied sunbirds (Fleming et al., 2004a) and two honeyeater species (Collins, 1981) show increased water retention over the last few hours of feeding at the end of the day, thus entering their overnight fast with greater total body water content. In all three lineages, GFR is completely shut down in response to water (and energy) deprivation during the overnight fast (Hartman Bakken et al., 2004; Hartman Bakken and Sabat, 2006; Purchase et al., 2013b) (this overnight drop in GFR is much more extreme in nectar-feeding birds than that recorded for song sparrows; Goldstein and Rothschild, 1993). Hormone responses also reflect the osmoregulatory challenge imposed at night: we have recorded elevated concentrations overnight of excreted aldosterone (which stimulates tubular reabsorption of sodium and,

therefore, water retention by the kidneys) coupled with high excreta osmolality (Fleming et al., 2004a).

Disrupted feeding is also likely to influence subsequent energy intake rate. In response to a midday fast (imposed by turning off the lights), rufous hummingbirds are apparently unable to adjust their intake rates and consequently end the day with energy deficits that necessitate the use of torpor (Tooze and Gass, 1985). By contrast, sunbirds and honeyeaters are able to increase their rate of feeding following the fast, implying greater plasticity in their rates of digestion and absorption of sugars (Köhler et al., 2011; Nicolson et al., 2005).

How do birds deal with low ambient temperatures?

Decreased ambient temperatures lead to a substantial increase in metabolic rate for these small endotherms to maintain constant body temperature; this requires integration of intestinal and renal systems. The combination of low temperature and dilute food is particularly stressful because birds have to warm ingested food to body temperature, shown by the increased metabolic rate of hummingbirds consuming dilute nectar in the cold (Lotz et al., 2003). Hummingbirds readily use torpor when their energy balance is threatened, and will enter torpor rather than feeding on energy-dilute or salt-laden diets. However, diurnal diet-induced hypothermia has not been shown for sunbirds or honeyeaters. Sunbirds meet the challenges of low temperature and dilute food by feeding more often and increasing their food intake (Köhler et al., 2010b). Facultative hypothermia in passerine birds such as the malachite sunbird *Nectarinia famosa* (Downs and Brown, 2002) may be a pronounced form of shallow rest-phase hypothermia that evolved in response to fluctuations in energy supply (McKechnie and Lovegrove, 2003).

Conclusions

A wide repertoire of adaptations allows nectarivorous birds to deal with the physical and physiological challenges of their deceptively simple nectar diet. For example, the prioritisation of sugar intake over water intake has recently been confirmed by use of the geometric framework to examine nutrient intake regulation in whitebellied sunbirds (Köhler et al., 2012); when maintained in captivity at 20°C, these birds (body mass 9 g) reach a daily intake target of 2.77 g of sucrose despite a 10-fold range of sugar concentrations (from 0.25

to 2.5 M sucrose, Nicolson and Fleming, 2003a). How these animals maintain such a constant energy and osmotic balance despite this remarkable range of diets has stimulated research across the globe.

While the emphasis here has been on sugars and water, other nectar solutes such as salts, amino acids and secondary metabolites also affect the consumption and processing of nectar diets (Lerch-Henning and Nicolson, 2013). Even though some bird nectars are unexpectedly rich in amino acids (Nicolson, 2007), nectar-feeding birds complement their nectar intake with pollen and arthropods (Stiles, 1995). These electrolytes and nitrogen supplements are important in helping them maintain physiological functions on a nectar diet.

Hummingbirds have the highest mass-specific metabolic rates of any vertebrates (largely due to their diminutive size and hovering flight), fueling their activities through direct catabolism of the nectar sugars they ingest (they also have the highest rates of sucrase activity of any vertebrates). Hummingbirds readily resort to torpor when challenged (e.g. by dilute diet, cold temperatures, or interrupted feeding). In contrast (Fig. 2), the two passerine lineages (sunbirds and honeyeaters) show adaptations for handling both the large water loading associated with dilute nectar diets (modulating water absorption and therefore decreasing renal processing) as well as being capable of producing concentrated excreta. The passerines also show greater plasticity in digestive rate, being capable of increasing energy assimilation in response to acute challenge. Therefore although these lineages show evidence for convergence in adaptations to a nectar diet, there are sufficient differences between the groups to warrant study of each. Future studies addressing these physiological adaptations represent a promising and fruitful research field.

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Author contributions

S.W.N and P.A.F. contributed equally to the project.

Competing interests

No competing interests declared.

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