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Does sugar content matter? Blood plasma glucose levels in an occasional and a specialist avian nectarivore

Minke Witteveen, Mark Brown, Colleen T. Downs *

School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa

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ABSTRACT

Nectar composition within a plant pollinator group can be variable, and bird pollinated plants can be segregated into two groups based on their adaptations to either a specialist or an occasional bird pollination system. Specialist nectarivores rely primarily on nectar for their energy requirements, while occasional nectarivores meet their energy requirements from nectar as well as from seeds, fruit and insects. Avian blood plasma glucose concentration (P_{Glu}) is generally high compared with mammals. It is also affected by a range of factors including species, gender, age, ambient temperature, feeding pattern, reproductive status, circadian rhythm and moult status, among others. We examined whether sugar content affected P_{Glu} of two avian nectarivores, a specialist nectarivore the Amethyst Sunbird *Chalcomitra amethystina*, and an occasional nectarivore the Cape White-eye *Zosterops virens*, when fed sucrose–hexose sugar solution diets of varying concentrations (5%–35%). Both species regulated P_{Glu} within a range which was affected by sampling time (fed or fasted) and not dietary sugar concentration. The range in mean P_{Glu} was broader in Amethyst Sunbirds (11.52–16.51 mmol/L) compared with Cape White-eyes (14.33–15.85 mmol/L). This suggests that these birds are not constrained by dietary sugar concentration with regard to P_{Glu} regulation, and consequently selective pressure on plants for their nectar characteristics is due to reasons other than glucose regulation.

1. Introduction

Nectar is one of the most common floral rewards offered by plants, and it is used by a wide range of pollinators (Simpson and Neff, 1981). Although originally thought to be a simple sugar solution, nectar has since been shown to be able to contain a variety of free amino acids, vitamins and lipids dissolved or suspended in an aqueous sugar solution made up of up to three different sugars: monosaccharides glucose and fructose and the disaccharide sucrose (Simpson and Neff, 1981; Brandenburg et al., 2009). Nectar can be highly variable in its composition due to environmental variability, and is pollinator specific (Brandenburg et al., 2009).

Nectar composition within a plant pollinator guild can be variable, as can be seen in nectar produced by plants pollinated by birds. Bird pollinated plants can be segregated into two groups based on their adaptations to a bird pollination system (Johnson and Nicolson, 2008). Plants adapted to specialist avian nectarivores produce a small volume (~10–30 μL) of concentrated (~15–25% w/w) sucrose dominant (~40–60%) nectar while plants adapted to occasional avian nectarivores produce a large volume (~40–100 μL) of dilute (~8–12% w/w) hexose dominant (~0–5% sucrose) nectar (Johnson and Nicolson, 2008). Specialist nectarivores (e.g. hummingbirds and sunbirds) are birds which rely primarily on nectar for their energy requirements, while occasional nectarivores (e.g. bulbuls and white-eyes) meet their energy requirements from nectar as well as from seeds, fruit and insects (Johnson and Nicolson, 2008; Brown et al., 2010b).

The nectar composition of bird pollinated plants has long been thought to be due to the selective pressure of avian pollinator preferences (Lotz and Schondube, 2006; Chalcoff et al., 2008; Medina-Tapia et al., 2012). Avian preferences are often due to physiological processes, mainly digestive, but sensorial physiological processes such as taste have also been seen to potentially influence preferences (Lotz and Schondube, 2006; Chalcoff et al., 2008; Medina-Tapia et al., 2012). The presence or absence of the enzyme sucrase has been an important factor that has influenced avian preferences and shows the importance of nectar composition (Brandenburg et al., 2009; Brown et al., 2012). Sucrase is the enzyme that hydrolyses sucrose into glucose and fructose which are then absorbed in the gastrointestinal tract (Martinez del Rio, 1990; Braun and Sweazea, 2008). The presence and activity of sucrase have been documented in Cape White-eyes (Bizaaré et al., 2012) while Amethyst Sunbirds have a high assimilation efficiency of sucrose indicating the presence and high activity of sucrase (Downs, 1997).

Blood plasma glucose concentration (P_{Glu}) in birds is regulated at a level averaged at twice that maintained in mammals of similar body mass (Braun and Sweazea, 2008; Polakof et al., 2011). If such high P_{Glu} were maintained in mammals, a hyperglycaemic-induced increase in reactive oxygen species would cause oxidative stress leading to impaired cell function and tissue damage (King and Loeken, 2004). Birds, on the other hand, are able to maintain such concentrations and

* Corresponding author.
E-mail address: downs@ukzn.ac.za (C.T. Downs).

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mitigate the physiological consequences (Smith et al., 2011). There are two hypotheses as to how they are able to do this: 1) due to the high turnover rate of red blood cells in birds (21 days vs. 120 days in humans) they are able to avoid glycation, a symptom of glucose toxicity (Hargrove, 2005) and 2) endogenous and dietary antioxidant pathways are able to minimise oxidative stress (Smith et al., 2011). However, the physiological significance of these comparatively high P_{Glu} is currently undetermined (Scanes and Braun, 2013).

P_{Glu} in birds can vary according to different factors including: species (Braun and Sweazea, 2008; Polakof et al., 2011); gender (Kern et al., 2005); age (Villegas et al., 2002; Prinzinger and Misovic, 2010); ambient temperature (Downs et al., 2010); feeding pattern (Jenni and Jenni-Eiermann, 1996); reproductive status (Gayathri et al., 2004); circadian rhythm (Frelin, 1974; Jenni and Jenni-Eiermann, 1996) and moult status (Driver, 1981), among others (Lill, 2011). Despite this variation in P_{Glu}, in some species it is under strict homeostatic control and is regulated around a set point whereas in other species the range around the set point is broad (Scanes and Braun, 2013). In birds, the antagonistic relationship of the glucagon–insulin pair does not function to regulate P_{Glu} as it does in mammals (Polakof et al., 2011). As a result, high P_{Glu} could be attributed to a low insulin:glucagon ratio also causing avian metabolism to be controlled by glucagon levels, but the ultimate cause of high P_{Glu} is uncertain (Polakof et al., 2011; Scanes and Braun, 2013).

The challenge of P_{Glu} regulation is expected to be compounded in nectarivores, with a diet of sugar-rich nectar (Beuchat and Chong, 1998). This is evidenced by the high fasting and fed P_{Glu} in hummingbirds (Beuchat and Chong, 1998). However, as specialist nectarivores, hummingbirds feed primarily on concentrated nectar (Johnson and Nicolson, 2008). The question is would they be able to maintain high P_{Glu} on a varied diet? Occasional nectarivores naturally ingest a range of sugar concentrations through nectar as well as fruit (Lotz and Schondube, 2006). How does this affect their ability to regulate P_{Glu}? Consequently, we examined if sugar content affected the P_{Glu} of two nectarivores, a specialist the Amethyst Sunbird *Chalcomitra amethystina*, and an occasional nectarivore the Cape White-eye *Zosterops virens*, when fed sucrose–hexose sugar solution diets of varying concentrations (5%–35%). We hypothesised that P_{Glu} in these nectarivores was affected by increased dietary sugar concentration.

2. Material and methods

2.1. Study species

Cape White-eyes (*Z. virens*) are small (~13.5 g) occasional nectarivores that feed on a variety of fruit, insects and nectar (Franke et al., 1998; Hockey et al., 2005). These birds are known to be important pollinators of a number of genera including *Aloe* (Johnson et al., 2006; Botes et al., 2008; Symes et al., 2008); *Erythrina* (M. Brown, unpublished data); *Kniphofia* (Brown et al., 2011); *Salvia* (Wester and Claβen-Bockhoff, 2006) and *Schotia* spp. (M. Brown, unpublished data).

Amethyst Sunbirds (*C. amethystina*) are small (~15 g) specialist nectarivores which feed primarily on floral nectar but have been seen to feed occasionally on insects (Hockey et al., 2005). They, too, are known to be important pollinators of a number of genera including *Aloe* (Botes et al., 2008); *Erythrina* (Bruneau, 1997); *Kniphofia* (Brown et al., 2011); *Protea* (Hockey et al., 2005) and *Strelitzia* spp. (Frost and Frost, 1981).

2.2. Bird capture and maintenance

Capture was done under the authority of permits from Ezemvelo KZN Wildlife and trials with ethical clearance from the University of KwaZulu-Natal. Seven adult non-breeding Cape White-eyes (11.27 ± 0.24 g) were captured during March 2011 on the Pietermaritzburg campus of the University of KwaZulu-Natal (UKZN) (29°38′S, 30°24′E). Four non-breeding adult and three juvenile Amethyst Sunbirds (13.55 ± 0.19 g) were caught at Riley Crescent, Howick (29°28′S, 30°13′E), Sakabula Estate, Howick (29°52′S, 30°23′E) and Shiraz Villas, Pietermaritzburg (29°37′S, 30°26′E) during May 2012 using Ecotone® (Ecotone, Gdynia, Poland) mist nets. Birds were kept in separate cages (45 × 45 × 40 cm) in a constant environment room (25 °C; 12L: 12D photocycle) at the Animal House, School of Life Sciences, UKZN, Pietermaritzburg campus. Cape White-eyes were fed a maintenance diet of fresh fruit (apples, pears, bananas, pawpaws and oranges) supplemented with Aviplus Softbill Mynah Pellets (AviProducts, Durban, South Africa), as well as fruit flies and water ad libitum. Amethyst Sunbirds were fed a maintenance diet of ~20% sucrose, fructose, and glucose (2:1:1) sugar solution with Ensure nutrient supplement (Abbott Laboratories, Hoofddorp, The Netherlands) as well as fruit flies ad libitum.

2.3. Blood glucose trials

To determine the extent to which Amethyst Sunbirds and Cape White-eyes regulate their P_{Glu} they were given sucrose–glucose–fructose (2:1:1) solutions of varying concentrations (5%, 10%, 15%, 20%, 25% and 35%) and P_{Glu} was measured at each of these concentrations. Food was removed overnight prior to a trial day and the trial began when the lights came on at 06:00 h. For the extent of the trial day, 06:00 h to 18:00 h, birds were fed one of the six sugar solutions, offered ad libitum from 50 mL nectar feeders.

After 9 h of feeding, i.e. at 15:00 h, a drop of blood was collected on an Accu-Chek® Performa Blood Glucose Test Strip (Roche Diagnostics, Randburg, South Africa) from the brachial vein after venipuncture with a 29 gauge syringe needle. The strip was immediately inserted into the Accu-Chek® Performa Blood Glucose Meter (Roche Diagnostics, Randburg, South Africa) and P_{Glu} measured. At the end of the day, i.e. at 18:00 h, the nectar feeders were removed and birds were starved overnight until 07:30 h the following morning when P_{Glu} was again measured taking a drop of blood from the other wing. Trials were run once a week to allow birds six days of maintenance diet to recover between trials.

As a response to stress, glucocorticoids such as corticosterone are released, which can cause an increase in P_{Glu} (Warne et al., 2009). However, a study done by Fokidis et al. (2011) showed that handling stress and stress of being in captivity did not significantly increase P_{Glu}. Regardless, handling was kept to a minimum with birds being caught, placed in a cloth bag and processed within 30 min.

2.4. Statistical analyses

Intraspecific P_{Glu} were analysed against sugar solution concentration for the two sampling times using a Generalised Linear Model Repeated Measures Analysis of Variance (GLM RMANOVA), followed by a post-hoc Tukey HSD test. All data were analysed using STATISTICA 7 (StatSoft, Tulsa, OK, USA). All results reported are mean ± standard error.

3. Results

3.1. Body mass

Body mass of Cape White-eyes did not differ significantly in the morning (06:00 h) before feeding between the respective trials (RMANOVA: $F_{5,\ 30} = 0.563$, $P = 0.727$; Fig. 1; mean ± SE range 11.06 ± 0.25–11.30 ± 0.37 g), nor at 15:00 h (RMANOVA: $F_{5,\ 30} = 2.034$, $P = 0.102$; Fig. 1; mean ± SE range 11.24 ± 0.41–11.76 ± 0.46 g), nor the following morning at 07:30 h after the trials (RMANOVA: $F_{5,\ 30} = 1.164$, $P = 0.350$; Fig. 1; mean ± SE range 10.57 ± 0.25–10.91 ± 0.41 g).

In contrast body mass of Amethyst Sunbirds did differ significantly in the morning (06:00 h) before feeding between the respective trials