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The sweet life: diet sugar concentration influences paracellular glucose absorption

Kathryn R. Napier[1,*], Cromwell Purchase[2], Todd J. McWhorter[1], Susan W. Nicolson[2] and Patricia A. Fleming[1]

[1]*School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA 6150, Australia*
[2]*Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, Republic of South Africa*
**Author for correspondence* (*k.napier@murdoch.edu.au*).

Small birds and bats face strong selection pressure to digest food rapidly in order to reduce digesta mass carried during flight. One mechanism is rapid absorption of a high proportion of glucose via the paracellular pathway (transfer between epithelial cells, not mediated by transporter proteins). Intestinal paracellular permeability to glucose was assessed for two nectarivorous passerines, the Australian New Holland honeyeater (*Phylidonyris novaehollandiae*) and African white-bellied sunbird (*Cinnyris talatala*) by measuring the bioavailability of radiolabelled, passively absorbed L-glucose. Bioavailability was high in both species and increased with diet sugar concentration (honeyeaters, 37 and 81% and sunbirds, 53 and 71% for 250 and 1000 mmol l^{-1} sucrose diets, respectively). We conclude that the relative contribution of paracellular to total glucose absorption increases with greater digesta retention time in the intestine, and paracellular absorption may also be modulated by factors such as intestinal lumen osmolality and interaction with mediated glucose uptake. The dynamic state of paracellular absorption should be taken into account in future studies.

Keywords: paracellular permeability; glucose absorption; sunbird; honeyeater; nectarivore; passerine

1. INTRODUCTION

The paracellular (non-mediated) absorption of glucose in the small intestine accounts for a minimal degree (approx. 5%) of total glucose uptake in non-flying mammals (reviewed by McWhorter 2005). Birds and flying mammals, however, have significantly shorter small intestines and less small intestinal surface area than non-flying mammals of a similar size, equating to a greater than 50% reduction in intestinal volume (Caviedes-Vidal *et al.* 2007). As the energetic costs of flight increase with the load carried, a decrease in the mass of digesta carried is advantageous; yet these animals need to somehow satisfy relatively high energy needs with reduced absorptive surface area (Caviedes-Vidal *et al.* 2007). Data presented for birds (Karasov & Cork 1994; Caviedes-Vidal & Karasov 1996; Levey & Cipollini 1996; Afik *et al.* 1997; McWhorter *et al.* 2006) and bats (Tracy *et al.* 2007) suggest that the enhanced intestinal paracellular absorption of water-soluble nutrients such as glucose and amino acids may compensate for the reduction in intestinal absorptive surface area (Caviedes-Vidal *et al.* 2007). Paracellular absorption provides a non-saturable absorptive process that automatically compensates for acute changes in dietary nutrient concentrations and, in contrast to the mediated routes of absorption, the rate varies linearly with solute concentration (Ferraris 2001). Nectar-feeding birds, with their simple diets, high metabolic demands and extremely rapid behavioural responses to changes in diet energy density (Fleming *et al.* 2004*a*,*b*; McWhorter *et al.* 2006), may therefore be excellent models to study the regulation and mechanisms of nutrient absorption and epithelial permeability.

Along with the Neotropical hummingbirds (Trochilidae), the passerine Australasian honeyeaters (Meliphagidae) and African/Asian sunbirds make up the three major radiations of nectarivorous birds (Nicolson & Fleming 2003*b*). Convergence in diet has led to the evolution of many similar physiological traits between passerines and hummingbirds (Nicolson & Fleming 2003*b*). For example, all three groups exhibit compensatory feeding, whereby food intake is adjusted with diet sugar concentration to maintain constant rates of energy intake (Nicolson & Fleming 2003*a*; Schondube & Martinez del Rio 2003; Fleming *et al.* 2008). Novel data presented by McWhorter *et al.* (2006) suggest that food energy density has an effect on paracellular glucose uptake in hummingbirds. L-glucose is a biologically inert isomer of D-glucose that is absorbed only via non-mediated mechanisms (Karasov & Cork 1994; Chang *et al.* 2004). McWhorter *et al.* (2006) found that L-glucose bioavailability, the fraction (f) of an oral dose absorbed into the systemic circulation, varies with food sugar concentration, which is inversely related to digesta retention time in hummingbirds (Lopez-Calleja *et al.* 1997; McWhorter *et al.* 2006). Our aim was to further investigate the effects of food energy density and intake rate on the bioavailability of radiolabelled L-glucose, at two dietary sugar concentrations (250 and 1000 mmol l^{-1} sucrose) in the New Holland honeyeater (Meliphagidae) and the white-bellied sunbird (Nectariniidae). Based on the patterns indicated for broadtailed hummingbirds (McWhorter *et al.* 2006), we hypothesized that there would be extensive absorption of orally ingested radiolabelled L-glucose in both species, indicative of significant non-mediated glucose uptake, and that L-glucose bioavailability would increase with diet sugar concentration due to increased digesta retention time. Selective pressure due to their common nectar diet may result in similar mechanisms of intestinal carbohydrate absorption between these three nectarivore lineages.

2. MATERIAL AND METHODS

Seven New Holland honeyeaters (*Phylidonyris novaehollandiae*, body mass 22.41±0.58 s.e.m. g) and seven white-bellied sunbirds (*Cinnyris talatala*, 8.07±0.17 s.e.m. g) were captured in Murdoch, Western Australia, and Pretoria, South Africa, respectively, by mist netting. Routine animal husbandry, maintenance diets,

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Table 1. Parameters used to determine bioavailability (f) of [^{3}H]-L-glucose in honeyeaters and [^{14}C]-L-glucose in sunbirds, at two diet sucrose concentrations (250 and 1000 mmol l^{-1}) (Values are means ± s.e.m. ($n=7$). Statistical significance determined by repeated-measures ANOVA, with significant values ($p \leq 0.05$) in italic.)

parameter	New Holland honeyeater			white-bellied sunbird		
	250 mmol l^{-1}	1000 mmol l^{-1}	comparison of treatment effect	250 mmol l^{-1}	1000 mmol l^{-1}	comparison of treatment effect
drinking rate (ml min^{-1})	58.46 ± 6.48	18.91 ± 1.31	***$p<0.001$***	40.4 ± 3.09	13.22 ± 1.12	***$p<0.001$***
intake rate, I (dpm min^{-1})	122 000 ± 15000	27 000 ± 3000	***$p<0.001$***	41 400 ± 4500	20 800 ± 1500	***$p=0.005$***
steady-state plasma, P (dpm mg^{-1} of plasma)	538.8 ± 157.9	252.9 ± 29.2	$p=0.081$	360.7 ± 45. 8	250.5 ± 23.0	$p=0.094$
comparison of mono- and bi-exponential models	$F<1.51$, $p>0.255$	$F<3.09$, $p>0.053$		$F<0.41$, $p>0.615$	$F<2.63$, $p>0.092$	
elimination constant, K_{el} (min^{-1})	0.0526 ± 0.0024	0.0523 ± 0.0024	$p=0.110$	0.0369 ± 0.0021	0.0364 ± 0.0039	$p=0.922$
probe distribution space, S (mg of plasma)	1796 ± 435	1641 ± 203	$p=0.602$	1666 ± 175	1666 ± 159	$p=0.984$
bioavailability, f (%)	36.9 ± 8.0	81.2 ± 12.1	***$p=0.003$***	52.7 ± 5.4	71.4 ± 8.5	***$p=0.023$***

experimental housing and additional pharmacokinetic methodology are detailed in electronic supplementary material A.

The fractional absorption (bioavailability) of L-glucose was measured using [^{14}C] and [^{3}H] radiolabelled L-glucose, administered orally and by intramuscular (IM) injection to each bird in separate experiments. To vary food intake rate, birds received two different diets (250 and 1000 mmol l^{-1} sucrose solutions) in separate feeding experiments. Both the order of trials and treatments given were randomly assigned, and followed published protocol (McWhorter *et al.* 2006). Bioavailability (f) was calculated as:

$$f = (P \cdot S \cdot K_{el})/I$$

where P is the steady-state feeding concentration of radiolabelled L-glucose in plasma (dpm mg^{-1} of plasma); S is the probe distribution space of radiolabelled L-glucose in plasma (mg of plasma); K_{el} is the elimination rate constant for the removal of radiolabelled L-glucose from plasma and its excretion in urine (min^{-1}); and I is the ingestion rate of radiolabelled L-glucose (dpm min^{-1}; Karasov & Cork 1994; McWhorter *et al.* 2006).

3. RESULTS

Birds drank approximately three times the volume of the dilute diet (250 mmol l^{-1} sucrose) compared with the more concentrated diet (1000 mmol l^{-1}, table 1). The mean steady-state concentration of radiolabelled L-glucose in plasma (P) was relatively high in both species on both diets, indicating significant absorption of the labelled probe; diet treatment did not have a significant effect on P (table 1).

The elimination of [^{14}C]-L-glucose after IM injection did not fit a bi-exponential model significantly better than a mono-exponential model for all individual birds of both species (table 1), indicating single compartment elimination kinetics. Diet treatment did not have a significant effect on the elimination rate constant K_{el} (min^{-1}) or distribution space S (mg plasma) in either species (table 1). It appears that elimination is quicker in honeyeaters when the half-time to elimination ($T_{1/2}=0.693/K_{el}$) is compared with sunbirds; $T_{1/2}$ in theory should scale with mass and be longer in the heavier honeyeater (Gibaldi & Perrier 1982). The value of K_{el} for L-glucose is dependent upon renal function (i.e. glomerular filtration rate), which may differ from values predicted from body size of our study species.

Bioavailability of L-glucose was significantly greater for both species when feeding on the more concentrated diet (repeated-measures ANOVA; honeyeaters: $F_{1,6}=21.73$, $p=0.003$; sunbirds: $F_{1,6}=9.22$, $p=0.023$; table 1). There was no significant interspecific difference in bioavailability on either diet concentration (one-way ANOVA; 250 mmol l^{-1} sucrose: $F_{1,12}=2.69$, $p=0.127$; 1000 mmol l^{-1}: $F_{1,12}=0.43$, $p=0.523$).

4. DISCUSSION

We found extensive absorption of orally ingested radiolabelled L-glucose in the New Holland honeyeater and the white-bellied sunbird (table 1), which is indicative of significant non-mediated (paracellular) glucose uptake. L-glucose bioavailability increases significantly with diet concentration in both honeyeaters and sunbirds, confirming the pattern suggested for broadtailed hummingbirds (table 2, McWhorter *et al.* 2006). Like hummingbirds (Schondube & Martinez del Rio 2003), New Holland honeyeaters have high D-glucose apparent assimilation efficiency

Table 2. Bioavailability (*f*) of radiolabelled L-glucose absorbed via the paracellular route in different avian species. *Experimental diet concentration estimated from data provided by authors.

species	natural diet	experimental diet	experimental diet type	bioavailability, *f* (%)	reference
Colinus virginianus (northern bobwhite quail)	insectivorous/ granivorous	62% D-glucose (wt/wt)	dry powder	92±7	Levey & Cipollini (1996)
Dendroica coronata (yellow-rumped warbler)	omnivorous	655 mmol l^{-1} D-glucose*	agar gel	91±23	Afik *et al.* (1997)
Passer domesticus (house sparrow)	granivorous	~1900 mmol l^{-1} D-glucose* (61.5% wt/wt dry matter)	wet mash	80±7	Caviedes-Vidal & Karasov (1996)
Trichoglossus haematodus (rainbow lorikeet)	nectarivorous	400 mmol l^{-1} D-glucose	liquid	80±6	Karasov & Cork (1994)
Selasphorus platycercus (broadtailed hummingbird)	nectarivorous	292 mmol l^{-1} sucrose 876 mmol l^{-1} sucrose	liquid	49 74	McWhorter *et al.* (2006)
Phylidonyris novaehollandiae (New Holland honeyeater)	nectarivorous	250 mmol l^{-1} sucrose 1000 mmol l^{-1} sucrose	liquid	37±8 81±12	present study
Cinnyris talatala (white-bellied sunbird)	nectarivorous	250 mmol l^{-1} sucrose 1000 mmol l^{-1} sucrose	liquid	53±5 71±9	present study

(99.8±0.05% s.e.m. (*n*=16); T. J. McWhorter & P. A. Fleming 2006, unpublished data) that is independent of diet concentration. D-glucose assimilation efficiency by white-bellied sunbirds has not yet been measured, but we predict that it will be similarly high based on the measurements in the congeneric lesser double-collared sunbird, *Cinnyris chalybeus* (97.9%) (Lotz & Nicolson 1996). As L-glucose bioavailability increases with diet concentration while that of D-glucose does not change measurably, the nutritional significance of paracellular uptake (i.e. relative contribution to total carbohydrate absorption indicated by the ratio of L-glucose to D-glucose bioavailability, typically measured by a multiple blood-sampling pharmacokinetic procedure) must also increase with sugar concentration (McWhorter *et al.* 2006). Single values of bioavailability are usually reported in the literature for birds (table 2), although paracellular absorption is clearly a highly dynamic process; furthermore, any interspecific comparison needs to account for diet sugar concentration. For example, the nectarivorous rainbow lorikeet apparently absorbs a similar fraction of radiolabelled L-glucose to the granivorous house sparrow, but the comparative significance of this observation is unclear as the sparrows were presented with a glucose diet approximately five times greater in sugar concentration (table 2).

The relationship between L-glucose bioavailability and sugar concentration is most likely due to the positive correlation between digesta retention time (i.e. contact time with absorptive surfaces in the intestine) and diet energy density as shown in hummingbirds (Lopez-Calleja *et al.* 1997). Another possibility, which is not mutually exclusive, is that mediated nutrient uptake enhances the uptake by the paracellular pathway, either through increased water absorption via the process of solvent drag or modulation of paracellular permeability; the mechanisms by which epithelial permeability might be regulated in response to the presence of luminal nutrients are poorly understood (reviewed by Chediack *et al.* 2003). Understanding why paracellular nutrient uptake changes with diet energy density will require disentangling the effects of digesta retention time, osmolality and mediated nutrient transport on paracellular permeability. The dynamic state of paracellular absorption should be taken into account for future studies. This study reveals new understanding of nutrient absorption in these volant animals and emphasizes that digestive physiology may be a significant determinant of feeding behaviour.

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