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RESEARCH ARTICLE

High paracellular nutrient absorption in intact bats is associated with high paracellular permeability in perfused intestinal segments

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ABSTRACT

Water-soluble nutrients are absorbed by the small intestine via transcellular and paracellular mechanisms. Based on a few previous studies, the capacity for paracellular nutrient absorption seems greater in flying mammals than in nonflying mammals, but there has been little investigation of the mechanisms driving this difference. Therefore, we studied three species each of bats (*Artibeus lituratus*, *Sturnira lilium* and *Carollia perspicillata*) and nonflying mammals (*Akodon montensis*, *Mus musculus* and *Rattus norvegicus*). Using standard pharmacokinetic techniques in intact animals, we confirmed the greater paracellular nutrient absorption in the fliers, comparing one species in each group. Then we conducted *in situ* intestinal perfusions on individuals of all species. In both approaches, we measured the absorption of 3OMD-glucose, a nonmetabolizable glucose analog absorbed both paracellularly and transcellularly, as well as L-arabinose, which has no mediated transport. Fractional absorption of L-arabinose was three times higher in the bat (*S. lilium*: 1.2±0.24) than in the rodent (*A. montensis*: 0.35±0.04), whereas fractional absorption of 3OMD-glucose was complete in both species (1.46±0.4 and 0.97±0.12, respectively). In agreement, bats exhibited two to 12 times higher L-arabinose clearance per square centimeter nominal surface area than rodents in intestinal perfusions. Using L-arabinose, we estimated that the contribution of the paracellular pathway to total glucose absorption was higher in all three bats (109–137%) than in the rodents (13–39%). These findings contribute to an emerging picture that reliance on the paracellular pathway for nutrient absorption is much greater in bats relative to nonflying mammals and that this difference is driven by differences in intestinal permeability to nutrient-sized molecules.

KEY WORDS: Intestine, Mediated absorption, Paracellular absorption, Arabinose, Flight, Bats, Intestinal perfusion, *Akodon montensis*, *Sturnira lilium*, *Artibeus lituratus*, *Carollia perspicillata*, *Mus musculus*, *Rattus norvegicus*

INTRODUCTION

Water-soluble nutrients are absorbed at the small intestine via the transcellular and paracellular pathways. The transcellular pathway is mediated by membrane transporters on the apical side of enterocytes that translocate nutrients from the intestinal lumen into the cytosol, and then export of nutrients to the bloodstream is achieved by mediated transport by transporter(s) in the basolateral membrane. In contrast, the paracellular pathway is not transporter mediated; water-soluble compounds reach the basolateral space by traversing tight junctions (formed by adjacent enterocytes) by diffusion or solvent drag (Pappenheimer and Reiss, 1987).

Recent studies suggest that the paracellular pathway may be quantitatively important in nutrient absorption in some vertebrates (He et al., 1998; Karasov et al., 2012; McWhorter, 2005). In particular, paracellular absorption accounts for 60–90% of the glucose absorption in all small birds and bats studied to date (Caviedes-Vidal et al., 2008; Caviedes-Vidal et al., 2007; Chediack et al., 2003; Tracy et al., 2007). In contrast, transporter-mediated absorption is much more important in terrestrial mammals (Lavin and Karasov, 2008; Lavin et al., 2007). Caviedes-Vidal et al. (Caviedes-Vidal et al., 2007) hypothesized that this heavy reliance on paracellular nutrient absorption evolved independently in flying mammals and birds, which have similar daily energy needs compared with nonfliers, probably to compensate for fliers' lower absorptive surface area in smaller and lighter intestines relative to nonfliers. Without the special need for the complementary paracellular absorption pathway, natural selection may favor absorption of water-soluble nutrients by specific apical transporters in order to maintain a better selective barrier against hydrosoluble toxins (Karasov et al., 2012).

Although the capacity for paracellular nutrient absorption has been assessed in a small number of bats and rodents (Caviedes-Vidal et al., 2008; Fasulo et al., 2013a; Fasulo et al., 2013b; Karasov et al., 2012; Pappenheimer, 1990; Price et al., 2013b; Tracy et al., 2007), the mechanistic underpinnings of the differences among taxa have received less attention. Paracellular absorption is measured using nonmetabolized hydrophilic carbohydrate probes that lack affinity for intestinal-mediated uptake mechanisms [e.g. L-arabinose (Lavin et al., 2007)]. Greater absorption of paracellular probes by bats could be due to longer retention time in the intestine, or differences in gastric evacuation (Shilton et al., 1999). Alternatively, bats might have intestines with greater paracellular permeability per unit intestinal area than those of rodents, a feature that could arise from a greater number of tight junctions per unit area or higher permeability of the tight junctions. One approach for assessing these alternatives is to use *in situ* intestinal luminal perfusions, which obviate any effects of differential retention time or gastric emptying. Price et al. (Price et al., 2013a) assessed L-arabinose absorption using intestinal luminal perfusions in an insectivorous bat (*Tadarida brasiliensis*); they concluded that the majority of glucose absorption was paracellular in that species. However, to date, no perfusion

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studies have directly compared bats with nonflying mammals using uniform methodology.

To that end, we conducted *in situ* intestinal luminal perfusions on three bats and three rodents, all of which feed primarily on carbohydrate-rich diets. We selected three frugivorous bats [*Artibeus lituratus* Olfers 1818, *Sturnira lilium* Geoffroy 1810 and *Carollia perspicillata* (Linnaeus 1758)] and an omnivorous rodent from southeastern Brazil (*Akodon montensis* Thomas 1913), and we also performed intestinal perfusions on laboratory mice and rats [*Mus musculus* Linnaeus 1758 and *Rattus norvegicus* (Berkenhout 1769)]. Together, these species represent a range of body masses with overlap between the bats and rodents, which thus helps to control for any effect of body mass (Caviedes-Vidal et al., 2007; Pappenheimer, 1990). In addition, we measured the whole-animal fractional absorption of L-arabinose in the wild rodent and one bat species to expand our survey of measurements in intact animals and to demonstrate connections between tissue-level characteristics and an organismal-level phenomenon [measurements in intact animals were previously made for most of the other species of this study (Caviedes-Vidal et al., 2008; Fasulo et al., 2013b; Lavin et al., 2007)]. In both approaches we used the carbohydrate L-arabinose [relative molecular mass (M_r)=150.1], a neutral nonmetabolized paracellular probe that does not interact with intestinal nutrient transporters, and 3-O-methyl-D-glucose (3OMD-glucose; M_r=194.2), a nonmetabolized analog of D-glucose (M_r=180.2) that is passively absorbed through the paracellular space but also transported across the enterocyte membrane by glucose transporter SLGT1. Based on the hypothesis of Caviedes-Vidal et al. (Caviedes-Vidal et al., 2007), which states that the heavy reliance on paracellular nutrient absorption evolved independently in flying mammals and birds, we predicted that intact bats would absorb more L-arabinose than intact rodents. We also hypothesized that this difference among taxa derives mechanistically from differences in intestinal permeability characteristics. We therefore predicted that in our intestinal perfusion experiments, absorption of 3OMD-glucose would be similarly fast and extensive in all animals, the bats would absorb more L-arabinose than the rodents per square centimeter nominal intestine, and paracellular absorption would account for a higher percentage of glucose absorption in bats compared with rodents.

RESULTS

Intestinal perfusions

L-arabinose clearance varied significantly among species ($F_{5,29}$=28.9, P<0.001; Fig. 1), and the bats exhibited two to 12 times higher L-arabinose clearance per square centimeter nominal surface area than the rodents. In pair-wise statistical comparisons, *A. lituratus* and *S. lilium* had significantly higher L-arabinose clearance than the three rodents (P<0.05). L-arabinose clearance in *C. perspicillata* was significantly higher than that of *R. norvegicus* and *M. musculus* (P<0.05), but was not significantly different from that of *A. montensis* (P=0.072), despite having nearly double the L-arabinose clearance. Among rodents we found no significant differences in L-arabinose clearance (P>0.05).

D-glucose absorption did not differ significantly among the species ($F_{5,30}$=2.31, P=0.069; Fig. 2). Similarly, there were no significant differences among species in 3OMD-glucose absorption ($F_{4,25}$=0.726, P=0.583; Fig. 2). Across all measurements in all species, D-glucose and 3OMD-glucose absorption were correlated ($F_{1,28}$=11.77, r^2=0.29, P=0.002; data not shown).

The percentages of D-glucose and 3OMD-glucose absorption that were estimated to be paracellular differed significantly among species (D-glucose: $F_{5,29}$=36.41, P<0.001; 3OMD-glucose: $F_{4,25}$=77.9, P<0.001; Fig. 3). All bat species had significantly higher paracellular proportions than rodents for both D-glucose and 3OMD-glucose (P<0.05 for all comparisons). Values significantly exceeded 100% in bats in some cases (*S. lilium* 3OMD-glucose, P=0.004, and *A. lituratus* D-glucose and 3OMD-glucose, all P<0.013), likely because of molecular size differences between probes used to estimate the paracellular contribution to absorption (see Discussion).

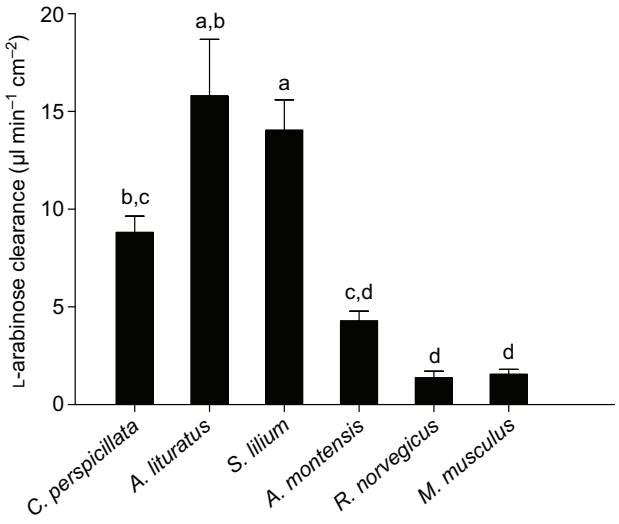

Fig. 1. Clearance of L-arabinose in a 2 h intestinal luminal perfusion of *Carollia perspicillata* (*n*=5), *Artibeus lituratus* (*n*=6), *Sturnira lilium* (*n*=6), *Akodon montensis* (*n*=6), *Rattus norvegicus* (*n*=7) and *Mus musculus* (*n*=6). Data are means ± s.e.m.; bars that share letters indicate no statistically significant difference (P>0.05).

Fractional absorption of probes in intact animals

Fractional absorption (f) of 3OMD-glucose in *A. montensis* was 0.97±0.04 (N=6; not significantly different from unity), and fractional absorption of L-arabinose was significantly lower (0.35±0.05, N=6, P=0.0001; Fig. 4). For *S. lilium*, the fractional absorption of 3OMD-glucose was also complete (1.46±0.48, N=5, not significantly different from unity), as was fractional absorption of L-arabinose (1.2±0.27, N=5; Fig. 4), which was significantly higher than that of the rodent (*A. montensis*; P<0.001).

DISCUSSION

Fractional absorption of probes in intact animals

As we predicted, the fractional absorption of the paracellularly absorbed probe L-arabinose was significantly higher in the bat *S. lilium* than in the rodent *A. montensis*. In both species, 3OMD-glucose was completely absorbed. Fractional absorption of both probes was complete in bats. Values above 1 are likely a result of our experimental design, because different animals were used for gavage and injection trials, instead of measuring both treatments in the same individuals. The difference in L-arabinose absorption between the bat and the rodent is in accordance with the pattern of absorption of nutrient-sized paracellular probes in other small bats, birds and nonflying mammals (Table 1), giving additional support to the hypothesis that high paracellular nutrient absorption is an adaptation for flight (Caviedes-Vidal et al., 2007).

In the bat, we provided animals with oral solutions that lacked Na^+ so that that they would not reject the dose. Although sodium is required for Na^+-coupled D-glucose absorption, it is not required for a whole-animal experiment such as this, because Na^+ is secreted into

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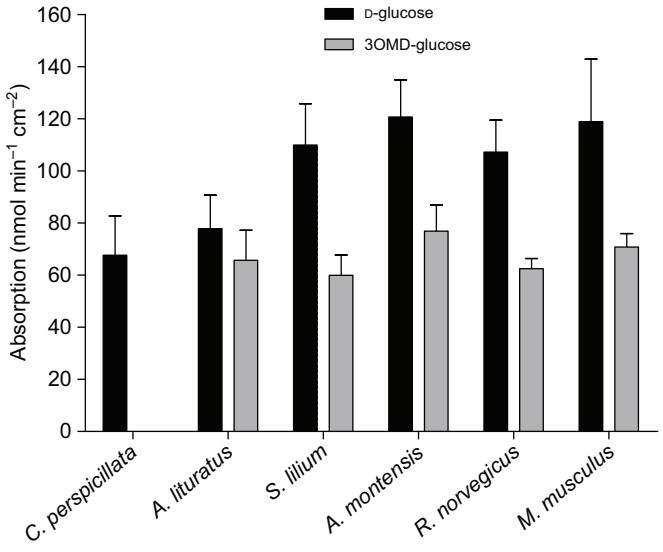

Fig. 2. Absorption of D-glucose and 3OMD-glucose in a 2 h intestinal luminal perfusion in bats and rodents. Data are means ± s.e.m.; there were no statistically significant differences among species for either probe (P>0.05). Sample sizes for D-glucose and 3OMD-glucose, respectively, were: *C. perspicillata*: n=5 and 0; *A. lituratus*: n=6 and 6; *S. lilium*: n=6 and 5; *A. montensis*: n=6 and 6; *R. norvegicus* n=7 and 7; and *M. musculus* n=6 and 6.

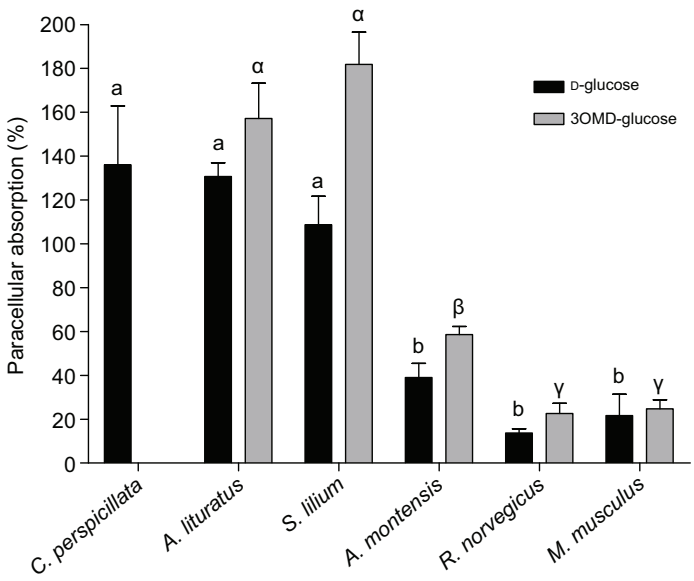

Fig. 3. Apparent percent absorption by the paracellular pathways of D-glucose and 3OMD-glucose based on the absorption of L-arabinose. Data are means ± s.e.m.; similar letters within a series (Latin letters for D-glucose and Greek letters for 3OMD-glucose) indicate no statistically significant difference (P≥0.05). Values for paracellular absorption exceed 100%, likely because of size differences among D-glucose (M_r=180), 3OMD-glucose (M_r=194) and L-arabinose (M_r=150; see Discussion). Sample sizes for D-glucose and 3OMD-glucose, respectively, were as follows: *C. perspicillata*: n=5 and 0; *A. lituratus*: n=6 and 6; *S. lilium*: n=6 and 5; *A. montensis*: n=6 and 6; *R. norvegicus* n=7 and 7; and *M. musculus* n=6 and 6.

the intestinal lumen with bicarbonate and also diffuses from the blood (Brody, 1999). Animals absorb nearly all the glucose in their diets even if those diets are low in Na^+ (Brody, 1999), and in fact, we observed complete absorption of 3OMD-glucose by the bats in this study.

High paracellular nutrient absorption may have consequences in animals that primarily consume fruit, especially fruits rich in secondary metabolites that are potentially toxic, such as those from the families Solanaceae and Piperaceae, which are known to be the main food of *S. lilium* (Mello et al., 2008). The toxin problem may be mitigated by various aspects of bats' behavior and physiology, such as geophagia (Ghanem et al., 2013), efficient tissue-specific detoxification systems, higher rates of toxin biotransformation and higher kidney glomerular filtration rates (Karasov et al., 2012). Karasov et al. (Karasov et al., 2012) demonstrated a high reliance on paracellular nutrient absorption in small birds, and proposed the following trade-off: the energy that birds gain by absorbing nutrients passively at low energetic cost may be offset, at least partially, by the metabolic demands placed on them to biotransform and eliminate concomitantly absorbed secondary metabolites. Our data suggest that the issue of increased toxic burden because of the high paracellular permeability of the intestine may be important for frugivorous bats as well. The need for further research on the mechanisms and implications of the absorption of toxicants, especially water-soluble compounds, is apparent.

Intestinal perfusion experiments

In agreement with our prediction, the three frugivorous bats showed 2- to 12-fold higher L-arabinose clearance than the rodents in our intestinal perfusion experiments. This finding demonstrates that previously described differences in L-arabinose absorption between intact bats and rodents (Caviedes-Vidal et al., 2008; Fasulo et al., 2013a; Fasulo et al., 2013b; Karasov et al., 2012; Lavin, 2007; present study) may be explained by differences in intestinal permeability, and not merely by differences in gut retention time, intestine size or other factors. Although our data indicate a functional difference between bats and rodents at the level of the intestine, the mechanisms driving this difference are uncertain. Our clearance data were calculated per nominal surface area. High L-arabinose clearance in bats could arise from having more tight junctions per nominal surface area (e.g. via longer villi) and/or from having more permeable tight junctions (e.g. via differential expression of particular proteins that form the tight junctions).

Previous studies have suggested that whole-animal paracellular absorption of glucose correlates positively (Pappenheimer, 1990; Pappenheimer, 1998) or negatively (Caviedes-Vidal et al., 2007) with body size. Within bats, our perfusion results might suggest a positive relationship (based on very few data points) between L-arabinose clearance and body size, in that the largest bat (*A. lituratus*) had the greatest L-arabinose clearance while the smallest (*C. perspicillata*) had the lowest. In contrast, the largest rodent (laboratory rat) had the lowest L-arabinose clearance among rodents. Our results are therefore somewhat equivocal as to the effect of body mass on intestinal paracellular permeability to nutrient-sized molecules. We selected bat and rodent species with significant overlap in body mass, such that most of the rodents were bracketed in size by the bats, all of which had much higher L-arabinose clearance than the rodents. Thus, if body mass does have some general effect on L-arabinose clearance, that effect appears small relative to the effect of taxon.

Additionally, it is unknown whether diet is associated with any difference in paracellular permeability of the intestinal epithelium. We tried to minimize any effect of diet by choosing species that all specialize on carbohydrate-based diets. Further diet matching would have been difficult: for example, finding non-flying fruit specialist mammals of the same size range as our bats would have been difficult if not impossible. Thus, it is possible that the high

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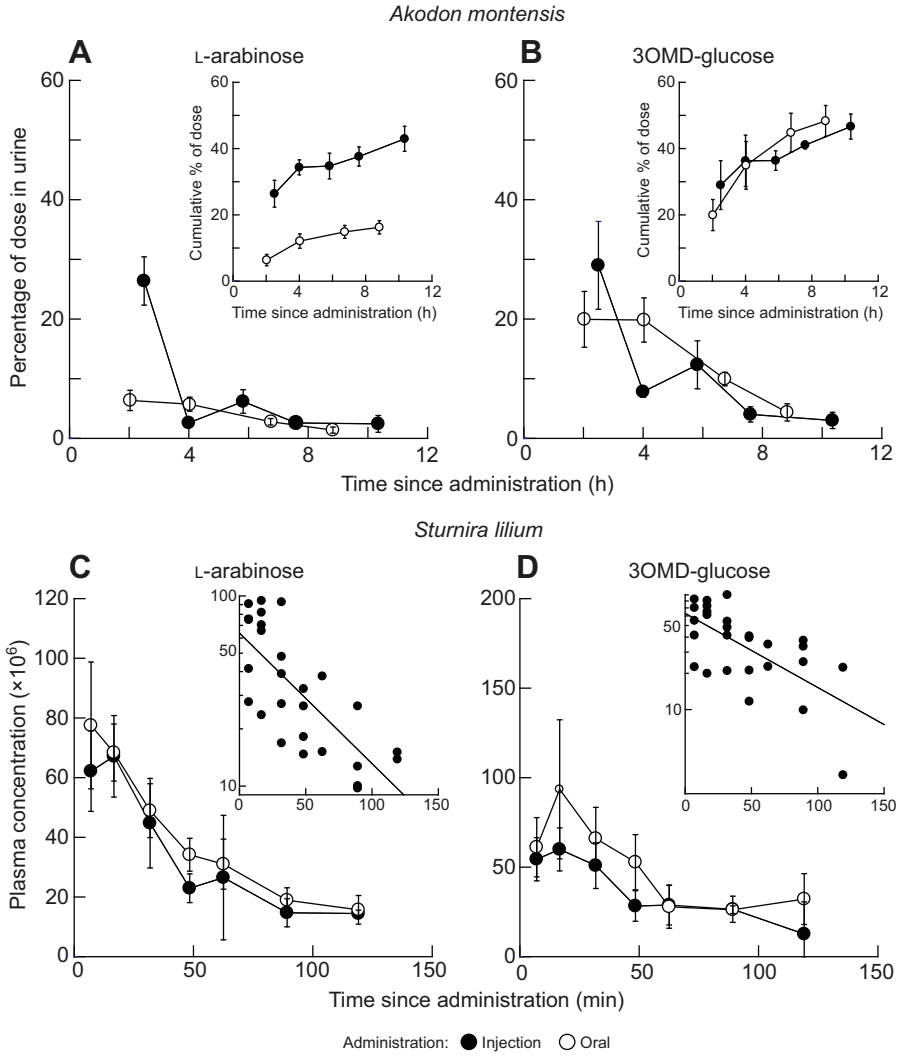

Fig. 4. Urine recovery of probe molecules as a function of time in the rodent *A. montensis*, and plasma concentration of probe molecules as a function of time in the bat *S. lilium*. For *A. montensis* (*n*=8; A,B), urine recovery plots show the percentage of the dose eliminated as a function of time and insets show the cumulative percentage of dose eliminated over the ~10-h trial. Unfilled circles denote the probe in urine following oral administration; filled circles represent the probe in urine after administration by injection. For *S. lilium* (*n*=9; C,D), plasma concentration plots show the concentration normalized to dose (multiplied by 10^6 for ease of display) as a function of time and insets show semi-log plots of plasma values post-injection, fit to a line by linear regression. Unfilled circles denote probe in plasma following oral administration; filled circles represent probe in plasma following injection. Data are means ± s.e.m.

paracellular nutrient absorption we observed in bats relative to rodents is not associated with flight per se, but rather a combination of flight and diet differences between the taxa we tested. Our more immediate concern, however, was demonstration of a link between high paracellular nutrient absorption at the whole-animal level and paracellular permeability characteristics of the intestine. Regardless of any putative dietary association, our results show that the differences among taxa in paracellular nutrient absorption that we have observed in intact animals can be demonstrated at the level of the intestine as well.

Molecule size has an important effect on paracellular absorption, and previous studies in both intact animals and isolated preparations have demonstrated decreasing paracellular probe absorption with increasing probe size (Anderson and Van Itallie, 2009; Chediack et al., 2003; Delahunty and Hollander, 1987; Elia et al., 1987; Lavin et al., 2007; Price et al., 2013a). This size sieving effect makes it difficult to accurately estimate glucose absorption using L-arabinose. Because of its smaller size (M_r=150 versus 180), L-arabinose, compared with D-glucose, can more freely traverse the tight junction, and therefore, our calculations of D-glucose absorption via

Table 1. Fractional absorption of orally dosed carbohydrates in intact small mammals (<100 g)

Common name	Scientific name	Body mass (g)	Fractional absorption measured for various carbohydrate probes			Reference
			L-arabinose (M_r=150)	L-rhamnose (M_r=164)	3-O-methyl-D-glucose (M_r=194)	
Brazilian free-tailed bat	*Tadarida brasiliensis*	11	1.09	–	1.03	Fasulo et al., 2013a
Little yellow-shouldered bat	*Sturnira lilium*	22	1.2	–	1.46	Present study
Great fruit-eating bat	*Artibeus lituratus*	70	–	0.9	0.96	Caviedes-Vidal et al., 2008
Laboratory mouse	*Mus musculus*	28	0.14–0.21	–	0.95	Fasulo et al., 2013b; Price et al., 2013b
Montane grass mouse	*Akodon montensis*	37	0.35	–	0.97	Present study
Common spiny mouse	*Acomys achirinus*	55	0.42	–	0.95	Karasov et al., 2012
Golden spiny mouse	*Acomys russatus*	58	0.37	–	0.95	Karasov et al., 2012

M_r, relative molecular mass.

the paracellular route are overestimated. This explains why we calculate percent paracellular absorption higher than 100% in some cases (in bats); the higher mobility of L-arabinose in crossing tight junctions more than offsets the effect of transporter-mediated absorption of glucose. Higher absorption of L-arabinose than D-glucose has been observed previously in both intestinal perfusions and intact animals (Karasov et al., 2012; Price et al., 2013a). The size sieving effect also explains, in part, why D-glucose (M_r=180) absorption was higher than 3OMD-glucose (M_r=194) absorption. Absorption of D-glucose by transporter-mediated means is also likely higher than 3OMD-glucose because of higher affinity for the glucose transporter (Ikeda et al., 1989; Kimmich, 1981).

Conclusions

This study supports the hypothesis that flying mammals (bats) rely heavily on paracellular nutrient absorption compared with rodents. Furthermore, differences among taxa in their reliance on paracellular absorption are driven by variation in intestinal permeability to nutrient-sized molecules and not merely by differences in gut retention time, intestine size or other factors. These findings are in agreement with other studies, but an enlarged comparative dataset including omnivorous, carnivorous and nectarivorous bats will ultimately permit a more robust, phylogenetically informed test of the hypothesis that increased intestinal paracellular absorption has evolved as compensation for smaller intestinal size in flying vertebrates.

MATERIALS AND METHODS

Animals

Montane grass mice (*Akodon montensis*; body mass: 37.4±3.4 g) are omnivorous cricetid rodents, but their diet consists primarily of plant material (Talamoni et al., 2008). They were captured during the rainy season between October and November 2012 in the Mata do São José (a remnant intact forest 15 km northeast of Rio Claro, São Paulo, Brazil). Montane grass mice were housed individually at the Universidade Estadual Paulista in Rio Claro in standard rodent cages and kept in a temperature-controlled room (22±1°C) with a 12 h:12 h light:dark cycle and access to water and standard laboratory chow (Sogorb, Lapa São Paulo, Brazil) *ad libitum*. Laboratory mice (*Mus musculus*, strain ND4; body mass 34.5±3.2 g) and laboratory rats (*Rattus norvegicus*, strain Sprague-Dawley; 502±48 g) are omnivores with primarily carbohydrate-based diets. Both were obtained from Harlan (Indianapolis, IN, USA) and housed at the University of Wisconsin in similar light and temperature conditions with standard rodent chow (Harlan) and water provided *ad libitum*.

The bats – *Artibeus lituratus* (61±1.4 g), *Sturnira lilium* (21.8±0.7 g), and *Carollia perspicillata* (16±0.7 g) – are fruit and fruit juice specialists of the Phyllostomidae family (Mello et al., 2008; Mello et al., 2004; Passos and Graciolli, 2004). We captured bats with mist nets in November and December 2012 near a cave at the Fazenda da Toca, Itirapina, São Paulo, Brazil (22°11′58″S, 47°44′49″W), and also at the Mata do São José. They were held in large outdoor flight cages in Rio Claro. Bats were provided with fruit (bananas, apples and papayas) and water *ad libitum*.

All experiments were performed during the primary active period of the animals, i.e. nocturnally for all species. Experiments on bats and *A. montensis* were performed at the Universidade Estadual Paulista in Rio Claro, while those on laboratory mice and rats were performed at the University of Wisconsin-Madison with overlapping personnel and using identical protocols except as noted. All animal procedures adhered to institutional animal use regulations and approved animal use protocols (Universidade Estadual Paulista: protocol A1-2013; University of Wisconsin: protocol A01441).

Recirculating intestinal perfusions

The procedure for intestinal perfusions largely followed the protocol of Price et al. (Price et al., 2013a). Animals were anesthetized using isoflurane throughout the experimental procedure (0.8 l min^{-1} oxygen flow, 3.5–4% isoflurane during surgical preparation, 1–2% isoflurane during perfusion). Anesthetized animals were taped to a heating pad (Deltaphase Isothermal Pad, Braintree Scientific Inc., Braintree, MA, USA) that maintained a constant 37°C. A peritoneal incision was made and the intestine was cannulated ~1 cm from the stomach using a rat gavage needle as the cannula, which was secured with suture. An exit cannula was placed as far as possible from the entrance cannula distally, attempting to perfuse as much intestine as possible. The incision site was periodically moistened with physiological saline. Prewarmed saline solution was first flushed through the cannulated segment for 5 min to remove residual digesta. Then, a prewarmed buffer containing the experimental probes (10 mmol l^{-1} D-glucose, tracer amounts of [methyl-^{3}H]-3-O-methyl-D-glucose (3OMD-glucose), 10 mmol l^{-1} L-arabinose, tracer amounts of [1-^{14}C]-L-arabinose, 10 mmol l^{-1} L-proline, 10 mmol l^{-1} lactulose, 100 mmol l^{-1} NaCl, 1.2 mmol l^{-1} $NaHPO_4$, 20 mmol l^{-1} $NaHCO_3$, 5 mmol l^{-1} KCl, 1 mmol l^{-1} $MgSO_4$ and 2 mmol l^{-1} $CaCl_2$, pH 7.4) was passed through the intestinal loop and recirculated using a peristaltic pump for ~2 h (flow rate=1 ml min^{-1}). The perfusion reservoir was kept in a water bath at 37°C and sealed to avoid evaporative water loss. Once the perfusion ended, the perfusate was collected, the perfused section of intestine was removed from the abdomen and the animal was euthanized. The perfused segment of the intestine was measured for length using a caliper and then cut longitudinally and laid flat to measure circumference. The average of three measurements taken along the length of the perfused section was used for further calculations. We calculated the nominal surface area perfused (smooth bore tube) as the product of the length × circumference, and calculated absorption of probes as the decrease in probe amount in the reservoir during the experiment (nmol) divided by the experiment duration (contact time on the intestine; min) and nominal surface area perfused (cm^2). To calculate clearance, we divided absorption rate by $[(C_{initial}–C_{final})/(C_{initial}/C_{final})]$, where C is probe concentration (Sadowski and Meddings, 1993), to correct for changes in concentration over the course of the experiment. Using L-arabinose absorption as a proxy for the portion of glucose absorption that is paracellular, we calculated the percent paracellular absorption as 100×(arabinose absorption)/(glucose absorption). This likely overestimates the proportion of glucose that is paracellular, a point we consider in the Discussion.

The initial perfusion volume was ~12 ml and aliquots were taken at the beginning and end of the perfusion to measure the concentration of probes. The perfusate was weighed pre- and post-perfusion with an analytical scale to determine the change in volume. Radionuclide activity in the samples was measured by scintillation counting (Brazil: Plate Chameleon™V Scintillation Microplate Reader and Plate Chameleon™V Liquid, Hidex, Turku, Finland; USA: Wallac 1414 LSC, PerkinElmer, Waltham, MA, USA, and Ecolume scintillation cocktail, MP Biomedicals, Solon, OH, USA). When using the Plate Chameleon reader, counts were corrected for ^{14}C spill and variable quenching by adding internal standards to replicate samples.

Glucose concentration was measured with commercial kits (Laborlab, Guarulhos São Paulo, Brazil, and Sigma-Aldrich, St Louis, MO, USA); assays were conducted according to the manufacturers' instructions.

Fractional absorption of probes measured *in vivo*

Fractional absorption by intact animals was determined using standard pharmacokinetic procedures. Probes were administered to animals either by intraperitoneal injection or orally (in both cases, *S. lilium* n=5; *A. montensis* n=6). Oral doses were given in a solution (*S. lilium* 59.6±3.6 µl; *A. montensis* 82.1±22.5 µl) that contained 125 mmol l^{-1} NaCl (*A. montensis* only), 50 mmol l^{-1} D-glucose, and tracer amounts of [^{3}H]3OMD-glucose (~0.15 µCi g^{-1} body mass) and [^{14}C] L-arabinose (~0.015 µCi g^{-1} body mass). The dose was administered by oral gavage to the stomach in the grass mice. In the bat, access to the stomach is made difficult by the narrow pharyngeal anatomy of this species. For that reason, we delivered the dose to the mouth in bats, and did not use salt in the gavage solution so that the bats would not reject the dose due to taste. This lack of sodium should not have affected our results, because Na^+ is secreted into the gut with bicarbonate and can diffuse from the blood (Brody, 1999). Intraperitoneal injected doses with the same tracers were delivered in physiological saline (*S. lilium* 26.5±2.7 µl; *A. montensis* 24.1±3.0 µl).

For bats, a series of small blood samples (≤20 µl) was taken from the superficial veins of the wings and legs at ~8, 18, 30, 50, 80 and 120 min post-administration. Plasma was separated using standard heparinized hemo-capillary tubes (Fisher Scientific, Pittsburg, PA, USA) and a microhematocrit centrifuge (Sigma 1-14 Microfuge with microhematocrit rotor 11026). Plasma mass was weighed with an analytical scale and tracer concentration was measured by scintillation counting.

Montane grass mice, after administration of radiolabeled probes, were transferred to metabolic cages with wire bottoms and a funnel underneath to collect urine samples. While in the metabolic chambers, the grass mice were provided sucrose solution (10% w/v) *ad libitum* in order to supply calories and cause them to urinate more (Pappenheimer, 1990). Urine was collected every 1–2 h, and a 10 µl subsample was taken for scintillation counting.

Pharmacokinetic calculations

The procedure for pharmacokinetic calculations followed the protocol of Karasov et al. (Karasov et al., 2012). In plasma sampling, fractional absorption (*f*) was calculated as $(AUC_{totaloral})/(AUC_{totalinj})$, where AUC is the dose-corrected area under the curve of plasma probe concentration versus time. For each compound, the concentration (C_t; dpm g^{-1} plasma) in each plasma sample at time *t* was normalized to the mass of each sample and to the administered dose (dpm), and plotted against time since administration (either orally or by injection). The integration of the area under this curve (AUC_t) represents the amount of compound that has been absorbed from time 0 up to time *t*, whereas AUC_{total} denotes the total amount of compound absorbed from time 0 out to infinity (∞). Following typical procedures in pharmacokinetics, the area from t=0 to t=x (the time of the final blood sample) was calculated using the trapezoidal rule. The area from the final sample time to t=∞ was calculated as $AUC^{x\rightarrow\infty}$=C_t (at t=x)/k, where k is a rate constant that was determined based on the terminal portion of the absorption curve of all bats sampled. The total $AUC^{x\rightarrow\infty}$ was obtained by summing the two areas.

This method of calculating *f* makes no major assumptions about compartments or kinetics and is an estimate of how much of the ingested probe was absorbed into the animal's system. We could not calculate apparent fractional absorption for each individual bat because individuals were not administered probes both by injection and orally. However, the mean and variance of *f* for the population of bats were estimated based on the population of data from each route of administration as described in Stuart and Ord (Stuart and Ord, 1994).

For each probe in the urine collection trials, the amount of the probe collected at each sampling time *t* was normalized to dose and multiplied by 100. Fractional absorption of each probe was determined by dividing the cumulative percentage recovery of each probe following oral gavage by the cumulative percentage recovery following intraperitoneal injection.

Statistical analyses

Statistical analyses were conducted with SYSTAT and results are expressed as means ± 1 s.e.m. In intestinal perfusions, differences among species in absorption, clearance and paracellular proportion were determined using ANOVA with Tukey's *post hoc* tests. We analyzed the correlation between D-glucose and 3OMD-glucose absorption using least-squares regression analysis. Percentages were divided by 10 and then arcsine square root transformed prior to statistical comparisons. Fractional absorption (*f*) values for probes were arcsine-square root transformed prior to statistical comparisons. Differences between species in fractional absorption in intact animals were determined using two-sample *t*-tests. Fractional absorption values differing from the theoretical value of 1 were detected using one-sample *t*-tests. Significance was determined at $\alpha<0.05$.

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Competing interests

The authors declare no competing financial interests.

Author contributions

Conceived and designed experiments: A.B., E.R.P., A.P.C.-N., W.H.K. and E.C.-V. Performed experiments and gathered data: A.B., E.R.P., M.N.G.-F. and G.F.-M. Analyzed data: all. Contributed reagents/materials/analysis tools: W.H.K., E.C.-V. and A.P.C.-N. Wrote the paper: A.B., E.R.P., W.H.K. and E.C.-V.

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