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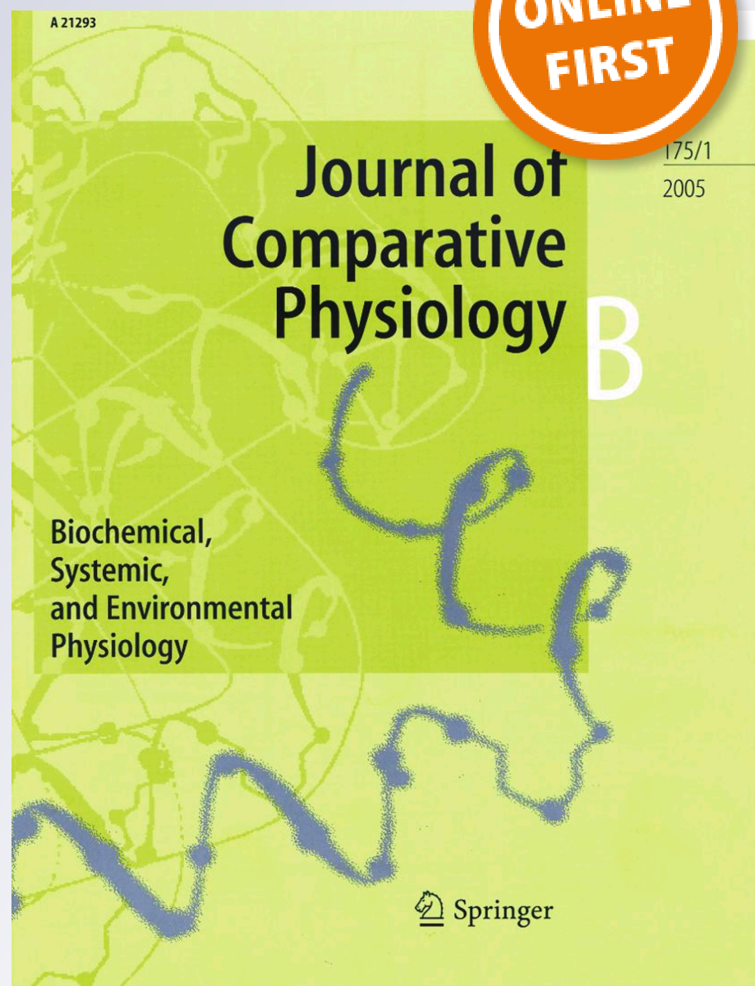

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ORIGINAL PAPER

The capacity for paracellular absorption in the insectivorous bat *Tadarida brasiliensis*

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Abstract Water-soluble nutrients are absorbed by the small intestine via transcellular and paracellular processes. The capacity for paracellular absorption seems greater in fliers than in nonfliers, although that conclusion rests mainly on a comparison of flying birds and nonflying mammals because only two frugivorous bat species have been studied. Furthermore, the bats studied so far were relatively large (>85 g, compared with most bat species which are <20 g) and were not insectivores (like about 70 % of bat species). We studied the small (11 g) insectivorous bat *Tadarida brasiliensis* and tested the prediction that the capacity for paracellular absorption would be as high as in the other bat and avian species studied so far, well above that in terrestrial, nonflying mammals. Using standard pharmacokinetic technique, we measured the extent of absorption (fractional absorption = f) of inert carbohydrate probes: L-arabinose (MM = 150.13) absorbed exclusively by paracellular route and 3OMD-glucose (MM = 194) absorbed both paracellularly and transcellularly. As predicted, the capacity of paracellular absorption in this insectivorous bat was high (L-arabinose $f = 1.03 \pm 0.14$) as in other frugivorous bats and small birds. Absorption of 3OMD-glucose was also complete ($f = 1.09 \pm 0.17$), but >80 % was accounted for by paracellular absorption. We conclude that passive paracellular absorption of molecules of the size of amino acids and glucose is extensive in this bat and, generally in bats, significantly higher than that in nonflying mammals, although the exact extent can be somewhat lower or higher depending on molecule size, polarity and charge.

Keywords Intestine · L-arabinose · Mediated absorption · Passive absorption · 3-*O*-methyl-D-glucose

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Introduction

Water-soluble (hydrosoluble) nutrients such as glucose, amino acids and some vitamins are absorbed in the small intestine mainly via the transcellular and paracellular pathways. Transcellular absorption is primarily mediated by membrane-bound transporter proteins that take up hydrosoluble nutrients from the gut lumen into the enterocyte across the apical membrane, and hasten their exit from enterocyte to blood across the basolateral membrane.

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Paracellular absorption involves movement of hydrosoluble solutes through the tight junctions (TJs) adjoining cells by diffusion or by the process of solvent drag (Pappenheimer and Reiss 1987). This passive route is quantitatively important in some species, accounting for the majority of glucose absorption in four avian species (Chang and Karasov 2004; Karasov et al. 2012; McWhorter et al. 2010) and two bat species (Caviedes-Vidal et al. 2008; Tracy et al. 2007). Enhancement of passive absorption might reduce costs of absorption if it replaces reliance on transporters (saving synthesis costs) and/or active transport (requiring ATP), but the primary hypothesized adaptive value is that it is an important complement in mediating absorption in species with less absorptive surface area, as in fliers with reduced gut size such as small birds and bats (Caviedes-Vidal et al. 2007). The capacity for paracellular absorption is significantly greater in fliers than in nonfliers, although conclusion rests mainly on a comparison of flying birds and nonflying mammals, because only two bat species have been studied (Caviedes-Vidal et al. 2007). An opposing cost of enhanced passive absorption might be greater exposure to water-soluble plant secondary metabolites (Karasov et al. 2012).

The majority of bat species are small (<20 g; Simmons and Conway 2003) but the two published studies on paracellular absorption in bats were on species larger than 80 g (Caviedes-Vidal et al. 2008; Tracy et al. 2007). Also, about 70 % of bat species are insectivorous (Jones and Rydell 2003), but published studies of paracellular absorption in birds (13 species), bats (2 species) and eutherian nonfliers (13 species) used almost entirely omnivores, frugivores, nectarivores, and granivores. Other features of digestive biochemistry in bats, such as hydrolytic enzyme capacity, vary with dietary substrate (Schondube et al. 2001). Only one species whose primary dietary substrate is protein, the carnivore, *Felis domesticus,* has been studied for paracellular absorption (Bijlsma et al. 1995). We do not know whether the difference between fliers and nonfliers occurs for all types of feeders or mainly those that eat carbohydrate-rich diets. We expect the flier–nonflier difference to occur also in primarily protein consumers because amino acids are primary water-soluble monomers absorbed following breakdown of protein, and thus could be absorbed by the paracellular pathway as is glucose in species that eat carbohydrate-rich diets. An enlarged comparative dataset including insectivorous/carnivorous as well as frugivorous/nectarivorous bats varying in body size will ultimately permit the most robust, phylogenetically informed test of the hypothesis that increased intestinal paracellular absorption has evolved as a compensation for smaller intestinal size in flying vertebrates.

This study is the first to measure the capacity for paracellular absorption in an insectivorous bat species. We predicted that this capacity would be as high as in the other bat and avian species studied so far, well above that in terrestrial, nonflying mammals. To measure paracellular absorption (measured as fractional absorption, or bioavailability), we used a standard pharmacokinetic protocol used in other studies. We measured absorption of neutral, water-soluble compounds L-arabinose (MM = 150.13 Da) and 3-*O*-methyl-D-glucose (3OMD-glucose; MM = 194). L-Arabinose is a metabolically inert carbohydrate that lacks affinity for intestinal mediated uptake mechanisms and is therefore absorbed exclusively by paracellular route (Lavin 2007). 3OMD-glucose is a nonmetabolizable analog of D-glucose that is absorbed both paracellularly and transcellularly via the D-glucose-mediated Na^+-coupled transporter. We chose these probe molecules for the following reasons. To our knowledge, there is no nonmetabolizable analog of amino acids that lack affinity for amino acid transporters (e.g., like L-glucose in the case of D-glucose transport), and L-arabinose is intermediate in size between amino acids and D-glucose. The similarity in size is important because both free diffusion and permeation through the paracellular space are influenced by molecule size (Chediack et al. 2003; Hamilton et al. 1987). (In Discussion, we also consider the influence of molecule charge on paracellular permeation). We used 3OMD-glucose because preliminary experiments with the nonmetabolizable actively transported amino acid analog, α-methylaminoisobutyric acid (MeAIB, 117.1 Da) (Hopfer 1987) suggested that it was not immediately cleared from the body. Also, our use of 3OMD-glucose allows easy comparison with other species that have been studied. Simultaneous comparison of the extent of absorption of compounds absorbed only passively (L-arabinose) and both actively and passively (3OMD-glucose) provides the best perspective on the nutritional significance of the passive route of absorption.

Materials and methods

Animal experiments

Experiments were conducted on 19 adult bats *T. brasiliensis* (mean mass ± SEM: 10.94 ± 0.91 g) held in cages at the campus of the Universidad de San Luis (Argentina). Bats were brought into the laboratory immediately after capture, and experiments were conducted the same day. All animal procedures adhered to institutional animal use regulations and approved animal use protocols (Animal Care and Use Committee Universidad Nacional de San Luis).

Measurement of absorption

Fractional absorption, or bioavailability, of test compounds ("probes") was determined using standard pharmacokinetic

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procedures in which probe molecules were administered orally or by injection, followed by serial blood sampling and analysis (Caviedes-Vidal et al. 2008). Two sets of experiments were conducted, one of them measuring probes with radiolabeled compounds and the other with HPLC fluorescence. The former, compared with the latter, approach yielded better data for pharmacokinetic analysis because measurement by liquid scintillation was more sensitive than by HPLC fluorescence, whose values late in the serial collections sometimes were at the limit of detection. Nonetheless, the HPLC data provided some independent verification of the findings with liquid scintillation, and so we present those data also.

Radiolabeled measurement of probes

Probes were administered to animals either by intraperitoneal injection ($n = 5$) or by oral gavage ($n = 6$). Oral doses of the following probes were administered in an isosmotic solution (55.8 ± 3.52 µl or approximately 0.05 % of body mass) that also contained 50 mM NaCl, 100 mM 3-*O*-methyl-D-glucose and 100 mM L-arabinose and [^{3}H]3OMD-glucose (~0.6 µCi/g animal) and L-[^{14}C]arabinose (~0.4 µCi/g animal). Injected doses of the same solutions were delivered in a sterile isosmotic solution (51.8 ± 1.57 µl or 0.5 % of body mass). A series of small blood samples (≤9 µl) were taken from the superficial veins from wings and legs at 5.9 ± 0.8, 11 ± 0.4, 30 ± 0.4, 59 ± 0.8, 90 ± 0.5, and 152 ± 1.0 min post-administration. A background blood sample was also taken before administration of probes. Blood plasma was separated from cells using standard heparinized hemo-capillary tubes (Fisher Scientific, Pittsburg, PA, USA) and a micro-hematocrit centrifuge (Cavour VT-1224 Ind. Argentina). Plasma mass was determined to be 0.1 mg and samples were counted in Ultima Gold (Perkin Elmer IN Waltham, USA) by two channel liquid scintillation (Wallac 1409 DS Liquid Scintillation Counter) with corrections for variable quenching and spill between channels.

HPLC analysis

In another set of experiments, the same solutions (above) were administered to eight bats (4 each by intraperitoneal injection and oral gavage), followed by serial blood sampling. Plasma was separated and then deproteinized by filtration with Nanosep 30K omega molecular weight cutoff centrifuge filters (Pall Corporation, East Hills, NY, USA) with the addition of 100 µl ultrapure water (18.3 MΩ resistance, Barnstead Easy Pure UF System) and centrifuged at 10,000*g* for 20 min, followed by a rinse step with an additional 50 µl water at 100,000*g* for 20 min. Deproteinized samples were dried at 50 °C for 18 h and then stored at −20 °C until analysis. We performed a derivatization reaction on the dried samples, which makes sugars detectable by fluorescence detection after separation for liquid chromatography (HPLC), following Anumula method (1994). Briefly samples were hydrated with 50 µl of 1 % sodium acetate solution and mixed with 50 µl of anthranilic acid reagent solution. The anthranilic acid reagent consisted of 30 mg ml^{-1} anthranilic acid and 20 mg ml^{-1} of sodium cyanoborohydride dissolved in a solution with: 4 % sodium acetate trihydrated and 2 % boric acid in methanol. To develop the reaction, samples were heated during 8 h at 65 °C in tightly closed screw-cap glass autosampler vials. After cooling to ambient temperature, 1 ml of HPLC solvent 1 (see below) was added to vials. Carbohydrate derivatives were separated at 25 °C on an Inertsil ODS-3 reversed phase HPLC column (4.5 mm × 150 mm, GL Sciences INC, Japan) and using a flow rate of 1 ml min^{-1}. Solvent 1 consisted of 0.2 % 1-butylamine, 0.5 % phosphoric acid, and 1 % tetrahydrofuran in ultrapure water (18.3 MΩ resistances, Barnstead Easy Pure UF System) and solvent 2 consisted of equal parts of solvent 1 and HPLC grade acetonitrile. Anthranilic acid and sodium cyanoborohydride were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile and tetrahydrofuran HPLC grade were obtained from Sintorgan (Argentina), 1-butylamine and phosphoric acid from Anedra (Anedra S.A. Buenos Aires, Argentina).

The HPLC system consisted of a Beckman automated binary system with a pump (model 126), an autosampler (model 507) and an interface (model 406). Derivatives of carbohydrate probes in samples and standard solutions were detected with fluorescence Detector Gilson Model 121 (Gilson, Inc) with an excitation filter of 305–395 nm and emission filter of 450 nm (band pass = 40 nm).

Pharmacokinetic calculations

For each compound, the concentration in each plasma sample at time t was normalized to the weight of each sample (C_t, dpm g^{-1} plasma) and to the administered dose (dpm), and plotted against sampling time since the compound was administered either orally or by injection. The resultant values have units of g^{-1} plasma. 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 0 up to infinity time (∞). Following typical procedures in pharmacokinetics, the area from $t = 0$ to $t = x$ min (when the final blood sample was taken) was calculated using the trapezoidal rule. The area from $t = x$ min to $t = \infty$ was calculated as $AUC^{x \to \infty} = C_t$ (at $t = x$)/k, where k is a rate constant which can be determined for each bat in each experiment based on the terminal portion of its absorption curve. The total $AUC^{0 \to \infty}$ was obtained

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by summing the two areas. Fractional absorption (*f*), or bioavailability, for each compound was estimated based on the ratio between the area under the plasma concentration versus time curve for oral administration experiments (AUC_{oral}, in units of mg min g plasma^{-1}) and injection experiments (AUC_{inj}):

$$\text{Fractional absorption}(f) = AUC_{oral}/AUC_{inj} \quad (1)$$

This method of calculating *f* is favored because it makes no major assumptions about compartments or kinetics. Fractional absorption estimates how much of the ingested probe was absorbed into the animal's system. The calculations of AUCs and their statistical comparison (below) were performed based on data for individuals, although data shown in figures are mean values. Apparent fractional absorptions for each individual bat could not be calculated because individuals were not administered probes by both routes of administration. However, mean and variance of *f* for the population of bats was estimated based on the mean and variance for each route of administration as described in (Stuart and Ord 1994). This method assumes that the gavage and injection data were independent, which was the case because different sets of bats were used.

Statistical analysis

Numerical data are presented as mean ± SEM (n = number of animals). AUCs, terminal slopes, and intercepts for the two compounds, measured within each bat, were analyzed by repeated measures analysis of variance (ANOVA). Comparisons of *f* for different probe molecules measured in different species were performed by two-way ANOVA on values of *f* that were arcsin-square root transformed. Comparisons of $\log_{10}$ (intestinal area) between bats and nonflying mammals were performed by analysis of covariance (ANCOVA) with $\log_{10}$ body mass as the covariate. The *F* values of these ANOVAs are presented in the text with the relevant degrees of freedom as subscripts. Statistical significance was accepted for $\alpha < 0.05$.

Results

Following injection, plasma levels of probes peaked earlier than following oral administration for both L-arabinose (Fig. 1a) and 3OMD-glucose (Fig. 1b), as might be expected, and subsequently declined in log-linear fashion (insets, Figs. A' and B'). Within individuals, L-arabinose declined more steeply (slope = -0.0162 ± 0.0015 min^{-1}) than did 3OMD-glucose (slope = -0.0136 ± 0.0012; repeated measures ANOVA $F_{1,9} = 13.5$, $P = 0.005$), but there was no significant difference in slopes according to mode of administration ($F_{1,9} = 1.92$, $P = 0.2$). Slower elimination is expected for 3OMD-glucose than for L-arabinose because the former is actively absorbed in the kidney and thus remains in the body circulation longer. The zero-time intercepts of the injection plots, which correspond to the inverse of distribution space (assuming a 1-compartment model), did not differ significantly between L-arabinose and 3OMD-glucose ($F_{1,9} = 2.5$, $P = 0.19$, implying very similar distribution spaces.

Total amounts in plasma over time following either oral administration or injection correspond to areas under the curves (AUCs). Within individuals, AUC did not differ significantly for 3OMD-glucose versus L-arabinose in the injection trials (respectively, 9.16 ± 1.81 vs. 9.45 ± 1.07, $n = 5$ bats) or in the oral administration trials (respectively, 9.94 ± 1.27 vs. 10.75 ± 1.49, $n = 6$ bats), based on repeated measures ANOVA ($F_{1,9} = 1.60$, $P > 0.2$). Moreover, these data indicated no significant difference in AUCs according to the mode of administration ($F_{1,9} = 0.34$, $P > 0.5$) and no significant interaction ($F_{1,9} = 0.37$, $P > 0.5$). As would be expected, then, based on the AUC means and variances, the population estimates

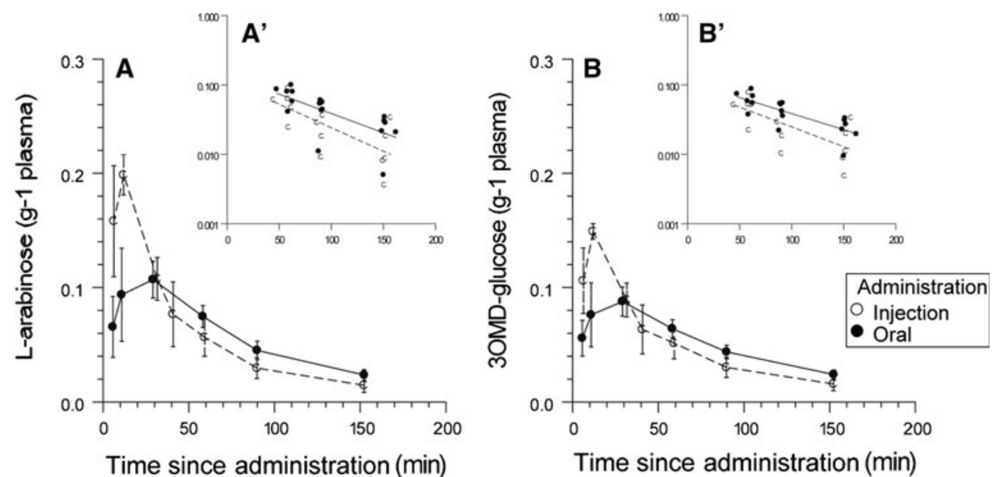

Fig. 1 Levels in plasma of (A) [14C]L-arabinose, (B) [3H]3OMD-glucose in bats following injection (*unfilled symbols*, *dashed line*, $n = 5$) or oral administration (*filled symbols*, *solid lines* $n = 6$). Values were normalized to doses. *Bars* represent SE. The *insets A'* and *B'* show the respective semi-log plots of the *terminal curves* **a** and **b**

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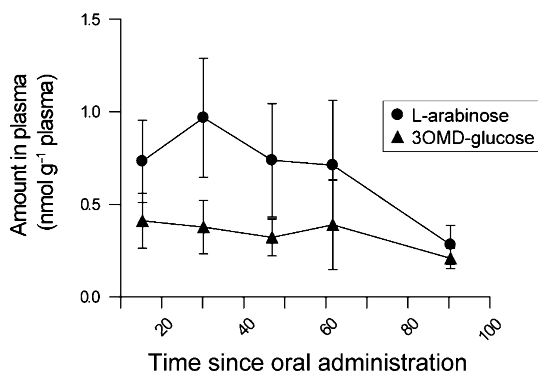

Fig. 2 Levels measured in plasma by HPLC of L-arabinose (*filled circles*) and 3OMD-glucose (*filled triangles*) in four bats following oral administration of the same amounts of each probe. *Bars* represent SE

of fractional absorption (*f*) were essentially unity: 1.03 ± 0.14 for L-arabinose and 1.09 ± 0.17 for 3OMD-glucose. Neither of these differed significantly from 1.0 ($P > 0.5$), implying that absorption was essentially complete for both probes.

Our more limited measurements using HPLC confirmed the impression from the isotope data that the bats absorbed L-arabinose and 3OMD-glucose to a similar extent. When four bats were orally administered solutions with the same nanomolar amounts of the two probes, plasma L-arabinose levels tended to be higher than those of 3OMD-glucose ($F_{1,23} = 3.04$, $P = 0.095$), and both probes declined over sampling time ($F_{1,23} = 7.94$, $P < 0.01$) (Fig. 2).

Discussion

We found a very high capacity for paracellular absorption in insectivorous *T. brasiliensis* bats, as we predicted. Evidence for this comes from comparisons of the [^{14}C]L-arabinose AUCs following oral and injection administration, and comparisons of absorption of L-arabinose and 3OMD-glucose by both liquid scintillation and HPLC fluorescence. We discuss these points in the sections below, and finish with a comparison of our data on this small insectivorous bat with those in larger frugivorous bats and nonflying mammals.

High capacity for paracellular absorption based on analysis of AUCs

We found no significant difference in AUCs between oral and injection administration of radiolabeled probes, implying complete absorption of both passively absorbed L-arabinose ($f = 1.03 \pm 0.14$) and 3OMD-glucose ($f = 1.09 \pm 0.17$), which is absorbed by both active, mediated processes and the passive, paracellular route (Fig. 1). Similar, quantitative absorption of L-arabinose was also observed in small birds (*Onychognathus tristramii*, $f = 0.98 \pm 0.07$; *Pycnonotus xanthopygos*, $f = 1.10 \pm 0.15$), who also absorbed all 3OMD-glucose (respectively, 0.95 ± 0.08 and 0.80 ± 0.19; Karasov et al. 2012), as occurs also in most mammals tested (Table 1).

In the present study, we measured fractional absorption using two different groups of bats, one group for oral administration and the other for injection. We did this to minimize stress on bats that would otherwise have to be retained in captivity for at least a week for a second measurement. The resulting population-based estimate of mean *f* should yield the same value as a mean calculated based on dual measurements (oral and injection) in many individuals, but the latter approach allows additional kinds of analyses that we could not perform, such as estimates mean and variance of apparent rates of absorption (e.g., as in Karasov et al. 2012; Tracy et al. 2007).

High capacity for paracellular absorption based on comparison of L-arabinose and 3OMD-glucose

Active transport of D-glucose provides an excellent mechanism to absorb remaining glucose in the gut even at very low concentrations. Hence, there is the expectation that most glucose will be absorbed, which is what we observed. But, the extent to which glucose is absorbed actively via intestinal SGLT1 and other transporters versus passively via paracellular absorption differs among animals. The relative extent of passive glucose absorption has been effectively studied in avian and mammalian species by measuring simultaneously the absorption of D-glucose (or its nonmetabolizable analog 3OMD-glucose) and the absorption of a water-soluble probe whose absorption is not mediated by any membrane transporter. For example, based on simultaneous measures with D-glucose and either L-glucose (the stereoisomer not actively transported) or L-arabinose (also not absorbed by a mediated mechanism, Lavin et al. 2007), rats (Uhing and Kimura 1995), wild rodents (Karasov et al. 2012), dogs (Lane et al. 1999; Pencek et al. 2002), and humans (Fine et al. 1993) absorbed 3–10 times more D-glucose or its analog 3OMD-glucose than passive probes, implying that the majority (66–90 %) of glucose absorption was mediated. But, our measurements of L-arabinose and 3OMD-glucose in plasma of *T. brasiliensis* measured both radiometrically (Fig. 1) and chemically (Fig. 2) following oral administration of both probes, showed similar, high absorption of both L-arabinose and 3OMD-glucose. Our results supported the notion that these compounds are absorbed to a similar extent and perhaps at the same rate as in this insectivorous bat. Comparison of both probes in the present study implies that a high proportion of 3OMD-glucose is paracellular.

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To estimate how much absorption of 3OMD-glucose was passive in *Tadarida brasiliensis*, we directly compared AUCs and fractional absorptions (*f*s). But, the comparisons should be corrected for the small difference in MW between 3OMD-glucose and L-arabinose. Because diffusion in water declines with $MW^{1/2}$ (Smulders and Wright 1971), each value of L-arabinose absorption should be decreased by 12 % $(=100 \times [194^{1/2} - 150^{1/2}]/194^{1/2})$. Assuming that the absorption of 3OMD-glucose represents the sum of passive + mediated absorption, a direct comparison of the ratio of the amounts absorbed (molecule size-adjusted $f_{\text{L-arabinose}}/f_{\text{3OMD-glucose}}$) indicates the proportion of 3OMD-glucose absorption that occurs via the passive pathway. The AUCs presented in Results, even after this adjustment, would still indicate that at least 80 % of 3OMD-glucose uptake was nonmediated. In rodents, the corrected ratio of $f_{\text{L-arabinose}}/f_{\text{3OMD-glucose}}$ was 0.34 (Karasov et al. 2012). We thus conclude that in *Tadarida brasiliensis*, the paracellular component of hydrosoluble nutrient absorption is of greater nutritional significance than in nonflying mammals.

Although in this analysis we focused on glucose absorption, we expect a somewhat similar pattern for absorption of amino acid(s) and peptides. It is clear from these findings that passive paracellular absorption of neutral (uncharged) molecules of the size of amino acids and glucose is extensive in this bat. Permeation by charged amino acids and peptides may differ because the paracellular path(s), formed by interlacing claudin proteins, is thought to be lined with negative charges (Colegio et al. 2002; Fanning et al. 1999) that impart cation selectivity, in addition to being molecule size selective. In house sparrows, which also have a high capacity for paracellular absorption, a dipeptide with a MW of approximately 225 Da might be expected to have a passive fractional absorption of around 0.25 (Chediack et al. 2003). For charged D-dipeptides of this size, the fractional absorption of a positively charged dipeptide (serine-lysine, MW = 233 Da) was 0.30 ± 0.05, significantly higher than that for a negatively charged dipeptide (serine-aspartic, MW = 220 Da), 0.17 ± 0.03 (Chediack et al. 2006). Thus, relatively high paracellular absorption of water-soluble molecules might be somewhat increased or decreased, depending on charge. However, in laboratory rats, in contrast, charge had relatively little effect on peptide fractional absorption (He et al. 1996). These kinds of experiments should be undertaken in bats. Moreover, the relative importance of the paracellular route should be studied at different solute concentrations, because at low levels of amino acid(s) passive absorption declines and transporters with high affinity become relatively more important (Ferraris 1994). Indeed, under these conditions differential expression of transporters for essential amino acids, as occurs to some extent in laboratory mice on low protein diet (Ferraris 1994), could be an important intestinal absorptive adjustment, whereas paracellular absorption likely would be less selective in this regard. But, in any event, we expect that in small bats consuming diets primarily composed of protein and fat the paracellular pathway would provide a rapid and energetically cheap route for absorption of amino acids and peptides that would complement other transporter-mediated and/or active routes of absorption.

Differences in paracellular absorption: a comparative perspective between mammalian fliers and nonfliers

Tadarida brasiliensis absorbed more than double the amount of the paracellular probe L-arabinose than the nonflying mammals we have studied, and two other bat species absorbed more than double the amount of the probe L-rhamnose than the nonflying mammals (Table 1). The data set assembled can be used to illustrate several points. First, paracellular absorption in bats and nonflying mammals declines with increasing MW size of the paracellular probe [two-way ANOVA on arcsin (square root (*f*)); $F_{2,1} = 6.2$, $P = 0.014$] and differs according to taxon ($F_{1,12} = 7.38$, $P = 0.019$). Post hoc comparisons of the least square means showed that *f* for L-arabinose significantly exceeded that for L-rhamnose and lactose/cellobiose (P's < 0.05), and the latter two did not differ significantly ($P > 0.5$). Second, *f* for bats was significantly higher than for the nonflying mammals. Based on the three bat species studied so far, bats join birds (Lavin and Karasov 2008) in having significantly higher capacity for paracellular absorption than similar-sized nonflying mammals. The primary hypothesized adaptive value is that this is an important complement to mediate absorption in species with less absorptive surface area, as in fliers with reduced gut size such as small birds and bats (Caviedes-Vidal et al. 2008). Indeed, even in the small data set in Table 1 the intestinal nominal surface area is significantly lower in the bats than in the rodents (by ANCOVA, $F_{1,2} = 49.5$, $P = 0.02$).

It appears that reduced intestinal size (Caviedes-Vidal et al. 2007) and elevated intestinal paracellular absorption together constituted part of the set of adjustments (Maina 2000) that evolved in actively flying vertebrates. The high and extensive absorption might be a shared feature in bats generally.

The difference between bats and nonflying mammals does not occur for intestinal absorption generally. Members of both groups absorb nearly all the 3OMD-glucose provided orally (Table 1). Mediated (including active) absorption of water-soluble nutrients works in parallel with passive, paracellular absorption. The similarity in total

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Table 1 Fractional absorption in small mammals (<500 g)

Common name	Scientific name	Body mass (g)	Intestinal nominal area (cm^2)[a]	Fractional absorptions measured for different probes				Reference(s) for fractional absorptions
				L-arabinose	L-rhamnose	L-lactulose or cellobiose	3-*O*-methyl-D-glucose	
Free-tailed Brazilian bat	*Tadarida brasiliensis*	11	8	1			1	This study
Great fruit-eating bat	*Artibeus literatus*	70	28		0.90	0.1	0.96	Caviedes-Vidal et al. (2008)
Egyptian fruit bat	*Rousettus aegyptiacus*	125	33.3		0.62	0.22	0.91	Tracy et al. (2007)
Laboratory mouse	*Mus musculus*	30	28	0.21		0.13	0.98	Unpublished
Common spiny mouse	*Acomys cahirinus*	55		0.42		0.05	0.95	Karasov et al. (2012)
Laboratory rat	*Rattus norvegicus*	300	119	0.34	0.134	0.09	0.93	Lavin et al. (2007)
Common marmoset	*Callithrix jacchus*	370			0.3	0.17	0.83	McWhorter and Karasov (2007)

[a] Small intestine nominal surface areas from Caviedes-Vidal et al. (2007) and unpublished data

absorption of a nutrient, such as glucose, which is absorbed by both mediated/transcellular and passive/paracellular processes versus the difference in absorption of strictly paracellular probes underscores that the groups rely on different pathways and processes to achieve the same net effect—nearly complete absorption of nutrients such as glucose and amino acids.

Even though the mechanisms of the increased paracellular absorption are unknown in some species, at least three explanations could be included. One possible mechanism is that the effective pore size of the tight junction between enterocytes, caused by differences in claudins or other proteins that create the sieving effect, will increase paracellular permeation over certain ranges of molecules (Chediack et al. 2003). The decline in fractional absorption with increasing probe size in both bats species and in nonflying mammals (Table 1) suggests no major difference in effective pore size. Second, increase in water flux across the tight junction will increase solute permeation by increased solvent drag (Pappenheimer 1990; Pappenheimer and Reiss 1987). We have no data of water flux in bats to compare with other mammals. Third, increased villous area per unit intestinal nominal surface area might be associated with more cell junctions across which paracellular transport occurs, if villous surface areas are increased mainly by increase in number of similar-sized enterocytes. There is some evidence that bats have increased villous area per unit intestinal nominal surface area.

The ratio of villous area relative to nominal area is also called the surface enlargement factor (SEF). It has been measured by a number of investigators using a variety of methods, and its measurement seems to be sensitive to the particular method (Snipes et al. 1994). Three groups used uniform methods that allow a comparison of bats(s) with nonflying mammal(s), and all three sets of comparisons indicate that bats have greater SEFs; Barry (1976) includes species from both groups, as do two studies by Mayhew (Makanya 1997; Mayhew and Middleton 1985) and two by our group (Caviedes-Vidal et al. 2008; Lavin 2007). Clearly, more intestinal morphometry data are needed, especially to determine whether enterocyte sizes on the villi are similar or different between fliers and nonfliers, and thus whether these differences in SEF translate to differences in number of tight junctions.

In conclusion, this investigation of the only insectivore and the smallest bat yet studied found that *T. brasiliensis* shares with two other bat species (both frugivores) a high capacity for paracellular absorption of water-soluble nutrients. When considered in relation to other data on nonflying mammals (Table 1), and the previous comparison of small birds with nonflying mammals (Caviedes-Vidal et al. 2007), extensive paracellular absorption of water-soluble chemicals seems to be a phenomenon of vertebrate fliers. These new data suggest that this pattern occurs in other types of feeders (insectivorous) and not only in carbohydrate-rich food eaters. But, the comparative data set is still relatively small. An enlarged comparative dataset including insectivorous/carnivorous as well as frugivorous/nectarivorous bats varying in body size and absorbing different-sized, and -charged water-soluble compounds will ultimately permit the most robust, phylogenetically informed test of the hypothesis that increased intestinal paracellular absorption has evolved as a compensation for smaller intestinal size in flying vertebrates.

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