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Paracellular absorption in laboratory mice: Molecule size-dependent but low capacity

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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 lower in nonfliers than in fliers, although that conclusion rests largely on a comparison of relatively larger nonflying mammals (>155 g) and relatively smaller flying birds (<155 g). We report on paracellular absorption in laboratory mice, the smallest nonflying mammal species studied to date. Using a standard pharmacokinetic technique, we measured the extent of absorption (fractional absorption = f) of inert carbohydrate probes: L-arabinose (M_r = 150.13 Da) and cellobiose (342.3) that are absorbed exclusively by the paracellular route, and 3-*O*-methyl D-glucose (3OMD-glucose) (M_r = 194) absorbed both paracellularly and transcellularly. f was measured accurately in urine collection trials of 5–10 h duration. Absorption of 3OMD-glucose by mice was essentially complete ($f = 0.95 \pm 0.07$) and much higher than that for L-arabinose ($f = 0.21 \pm 0.02$), indicating that in mice, like other nonflying mammals, >80% of glucose is absorbed by mediated process(es) rather than the passive, paracellular route. As in all other vertebrates, absorption of cellobiose ($f = 0.13 \pm 0.02$) was even lower than that for L-arabinose, suggesting an equivalent molecular size cut-off for flying and nonflying animals and thus a comparable effective TJ aperture. An important ecological implication is that smaller water-soluble plant secondary metabolites that have been shown to be absorbed by the paracellular path in cell culture, such as phenolics and alkaloids, might be absorbed in substantial amounts by bats and small birds relative to nonflying mammals such as mice.

1. 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. Paracellular absorption involves movement of hydrosoluble solutes through the tight junctions (TJ's) 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, 2004b; McWhorter et al., 2009; Karasov et al., 2012) and three bat species (Tracy et al., 2007; Caviedes-Vidal et al., 2008; Fasulo et al., 2012). In contrast, based on simultaneous measurements 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 3-*O*-methyl-D-glucose (3OMD-glucose) than paracellular probes, implying that the majority (66–90%) of glucose absorption was mediated.

Greater reliance on paracellular 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 to mediated 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). An opposing cost of enhanced paracellular absorption might be

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greater exposure to water-soluble plant secondary metabolites (Karasov et al., 2012). However, that cost is somewhat mitigated because the paracellular route is molecule-size selective in nonflying mammals (Hamilton et al., 1987; He et al., 1998), birds (Chediack et al., 2003; Karasov, 2011), and bats (Tracy et al., 2007; Caviedes-Vidal et al., 2008). Permeation by water-soluble neutral probe molecules that are not actively transported diminishes considerably as molecule M_r approaches 400 Da (Karasov, 2011), which corresponds to a molecular radius of about 5 Å (Chediack et al., 2003).

The capacity for paracellular absorption is significantly lower in nonfliers than in fliers (Caviedes-Vidal et al., 2007), although that analysis was based largely on a comparison of relatively larger nonflying mammals (10 of 11 species > 155 g) and relatively smaller flying birds (6 of 10 species < 155 g). In this study we add substantially to the comparative data set on nonflying mammals in the smaller size range by focusing on the smallest nonflying mammal species studied to date, the laboratory mouse (*Mus musculus*).

Laboratory mice have been used extensively for studies of transcellular, mediated intestinal absorption of solutes (*e.g.*, *in vitro* studies Diamond and Karasov, 1984; Gouyon et al., 2003) but we are aware of only two reports regarding paracellular absorption. Pappenheimer (1990) found that absorption by intact mice of creatinine ($M_r = 113$ Da) was 65%, and we previously reported that absorption by mice of L-rhamnose ($M_r = 164.2$ Da) was 19% (Caviedes-Vidal et al., 2007). The difference hints at the expected effect of molecule size on paracellular permeation, but in this study we extend this analysis by measuring the effect of molecule size within a population of mice using uniform methodology.

We predicted the laboratory mouse would have relatively low capacity for paracellular absorption. This capacity was assessed by measuring absorption by intact animals of two relatively inert, neutral water-soluble probes that do not interact with intestinal nutrient transporters, L-arabinose (molecular weight $M_r = 150.1$ Da) and cellobiose ($M_r = 342.3$) (Lavin et al., 2007). As a comparison, we also measured absorption of 3OMD-glucose ($M_r = 194.2$), which is a nonmetabolized analogue of D-glucose that is passively absorbed through the paracellular space but also transported across the enterocyte membrane by glucose transporters, including SGLT1. Using these probes we tested three specific predictions: (1) absorption of L-arabinose would exceed that of cellobiose, because absorption of hydrosoluble probes will decrease with increasing molecular size; (2) absorption of the D-glucose analogue (3OMD-glucose) would be complete in mice, but (3) considerably higher than that of the two paracellular probe molecules.

2. Methods

2.1. Animals and their maintenance

Experiments in San Luis were conducted on adult male and female mice (*M. musculus*) (28.3 ± 0.5 g; mean ± SEM). They were held in individual cages at relatively constant environmental temperature (24 ± 1.5 °C), relative humidity of (35 ± 3%), and with a lighting schedule of 12:12 h light: dark. Animals had access *ad libitum* to rat chow (Cargill SACI, Saladillo, Buenos Aires, Argentina) and water. All procedures adhered to institutional animal use regulation and approved animal use protocols (Institutional Animal Care and Use Committee IACUC number protocol: B-39/07- Universidad Nacional de San Luis). A limited number of mice (strain ICR from Harlan, Indianapolis) were studied at the University of Wisconsin—Madison under protocol A01441, and their holding conditions were similar.

2.2. Test probe molecules

Carbohydrates were purchased from Sigma Chemicals (St. Louis, MO, USA): L-arabinose ($M_r = 150.1$), 3OMD-glucose ($M_r = 194.2$), and cellobiose ($M_r = 342.3$). Radiolabeled ^{14}C-L-arabinose and ^{3}H-L-arabinose were purchased from Moravek (Brea, CA, USA) and American Radiolabeled Chemicals (St. Louis, USA), respectively.

2.3. Fractional absorption of probes

As a measure of passive, paracellular absorption, we used standard methods from pharmacokinetics to measure the whole-organism fractional absorption of water-soluble compounds. As described in more detail below, probe molecules were injected and also administered orally with a gavage needle to intact animals, usually in separate experiments, and urine samples were serially collected and analyzed for the probe molecules by liquid scintillation or by HPLC. We collected urine rather than blood out of concern for the animals' welfare, because in such small mice urine collection seems less traumatic than repeated blood sampling *via* puncture of the retro-orbital region or of the saphenous vein (Hem et al., 1998) or tail vein. Because the probes are essentially nonmetabolizable, they are recovered in urine following kidney filtration. Fractional absorption (f) was calculated by comparing recoveries post-oral administration with those post-injection (which accounts for any differential recovery). This simple pharmacokinetic method does not require assumptions about pool sizes (*e.g.*, 1 or 2 pools) or kinetics (*e.g.*, 1st order) (Welling, 1986). As reported in the Results section, recoveries of carbohydrate probes were uniformly high, as in our previous studies with rats (Lavin et al., 2007) and marmosets (McWhorter and Karasov, 2007).

Food was withheld during the animals' 12-hour normal inactive period just preceding each trial, but mice were provided with *ad libitum* access to D-glucose solution (10% w/w) as a source of calories during measurement trials, which were performed during their normal activity period (starting at 6 pm and ending at 8 am on the next day). Also, the mice were transferred to metabolic cages with wire bottoms and a tray beneath from which to collect clean urine samples. At the beginning of a trial, mice were orally dosed at 3% body mass with an isosmotic solution containing L-arabinose (40 mM), cellobiose (100 mM) and 3OMD-glucose (50 mM). Oral dose solutions also contained NaCl (~55 mM). NaCl was included in the solution to balance osmolality with plasma (approx. 300 mOsm). Inclusion of Na^+ also provides an essential ion for Na^+-coupled D-glucose absorption, although it is not strictly necessary in this kind of whole-animal study because animals would still absorb nearly all glucose even if the diet is low in Na^+ because additional Na^+ is secreted into the intestinal lumen together with bicarbonate and diffuses from blood (Brody, 1999). In a separate experimental trial, each animal was typically injected (0.3% of body mass in gluteal muscle) with isosmotic NaCl solution containing L-arabinose (40 mM), cellobiose (100 mM) and 3OMD-glucose (50 mM). Syringes were weighed before and after dosing animals to determine actual dose administered.

After administration of probes, mice were returned to their cages, where they had *ad libitum* access to D-glucose solution (10% w/w). The purpose was to provide rodents with water and some calories and also to make them urinate more (Pappenheimer, 1990). Cages of mice were checked for urine collection beginning 30 min after probe administration and every 30 min thereafter until 6 h, and then at 2-hour intervals thereafter.

In a separate set of trials conducted at the University of Wisconsin, we assessed fractional absorption of L-arabinose using 5 mice that were simultaneously injected (^{3}H, i.p.) and gavaged (^{14}C) with radiolabeled L-arabinose. Testing conditions were similar to those described above except for the solutions: the gavage solution contained only 50 mM glucose with NaCl to balance osmolarity plus tracer amounts of the labeled probe; the injection solution contained only isosmotic NaCl plus tracer amounts of the labeled probe. After urine collection, subsamples of urine were counted using liquid scintillation to calculate probe recovery.

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2.4. HPLC sample analysis

Urine samples were diluted with ultra pure water (18.3 MΩ resistance, Barnstead Easy Pure UF System) and filtrated (Nanosep 30K omega molecular weight cutoff centrifuge filters; Pall Corporation, East Hills, NY, USA) by centrifugation at 4000 *g* (Cavour VT-1224 Ind. Argentina). Filtered samples were dried in a vacuum stove to 50 °C for 18 h and then stored at −20 °C until analysis. Carbohydrate probes were derivatized for high performance liquid chromatography (HPLC) fluorescence detection by reductive amination with anthranilic acid (2-aminobenzoic acid), following Anumula (1994) and Du and Anumula (1998) with minor modifications. 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} sodium cyanoborohydride dissolved in a solution with: 4% sodium acetate, 3% H_2O 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 HPLC grade water (18.3 MΩ resistance, Barnstead Easy Pure UF System) and solvent 2 consisted of equal parts 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), and 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 (bandpass = 40 nm). Limits of detection for all probes in water were 1–2 ng.

2.5. Analysis of data

For each compound, the amount in total urine volume at each sample time *t* was normalized to the respective dose. The values for cumulative proportion of dose were plotted as a function of *t*. The cumulative proportional recovery (*CPR*) post-injection was compared with 1.0 (*i.e.*, total recovery) using the 95% confidence interval. Recoveries post-injection were high but not always complete (*i.e.*, 95% confidence interval excluded 1; see Results section). Consequently, the fractional absorption for orally administered probes was calculated as

$$f = (CPR\ following\ oral\ administration)/(CPR\ following\ injection). \quad (1)$$

Recoveries and fractional absorption were compared among probes within mice by paired *t*-test and repeated-measures analysis of variance (ANOVA). Differences in *f* were confirmed by analyses using arcsin-square root transformations, although values >1 were set at 0.9999.

3. Results

The vast majority of probes that were injected were recovered in urine (Fig. 1). Recoveries at the end of each run were significantly lower than 1.0 for both L-arabinose (0.86 ± 0.03, $n = 5$) and cellobiose (0.88 ± 0.04), and their recoveries were significantly lower compared with 3OMD-glucose (1.00 ± 0.04; $P = 0.031$, repeated measures ANOVA). Although urinary recovery of all the probes injected continued to increase beyond even 9 h, the changes were relatively small. Below, we show that, with appropriate procedures, fractional absorption can be accurately measured using shorter time intervals than 24 h.

The time course for recovery of orally administered probes was very similar to that for injected probes (Fig. 1). Fractional absorptions (*f*s) by each individual of orally administered probes were corrected for incomplete recovery by dividing them by the respective probe recovery post-injection using Eq. (1) in the Methods section. For one individual for whom injection data were not available, we use the means from the other individuals. As predicted, *f* (calculated using cumulative collection at 24 h for $n = 5$ mice) for L-arabinose (0.208 ± 0.024; Fig. 1A) significantly exceeded that for cellobiose (0.126 ± 0.022; Fig. 1B) (paired $t_4 = 2.23$ on arcsin-square root transformed values, $P < 0.025$). Also as predicted, 3OMD-glucose was almost entirely absorbed (0.949 ± 0.068; Fig. 1C), and its *f* was significantly higher than that for both other probes (repeated measures ANOVA $F_{2,8} = 197$, $P < 0.001$).

Because the time course for recovery of orally administered probes was very similar to that for injected probes, we wondered whether *f* might be determined accurately if measured in mice over shorter time periods. Consequently, we organized the data into three time blocks (average 5.75 h, 8.5 h, and 23 h post oral administration), and calculated *f* using the urine values recovered within each time block (Fig. 2A). Repeated measures ANOVA showed that *f* did not vary significantly among time blocks for any of the probes (Fig. 2A; all *P*'s > 0.5). In separate trials, we tested some shorter time collection periods using radiolabeled L-arabinose (Fig. 2B). In those trials, *f* differed significantly among time blocks (repeated measures ANOVA $F_{3,6} = 15.7$, $P = 0.003$), with the value based on collections at 1.5 h significantly lower than all the others ($P = 0.04$), which did not differ significantly from each other (all *P*'s > 0.1). For the longest recovery periods, *f* of L-arabinose measured radiometrically did not differ significantly from that measured by HPLC ($P = 0.14$).

4. Discussion

Measurement of fractional absorption (*f*) of L-arabinose was cross-validated using two measurement methods (HPLC and liquid scintillation) but using the same pharmacokinetics methodology that does not require assumptions about pool sizes (*e.g.*, 1 or 2 pools) or kinetics (*e.g.*, 1st order) (Welling, 1986). Probes selected were metabolically inert, as indicated by their quantitative recovery in urine. We found that *f* of three different probe molecules was accurately determined in urinary collection trials even if trial durations were shorter than the period of time necessary for 100% recovery of an injected dose. Based on data in Fig. 2, we recommend a urinary recovery period in mice of at least 5–6 h post oral administration, but variance of the estimate of *f* probably can be reduced by extending cumulative recovery to at least 10 h.

In the paragraphs below we discuss the data in relation to our three specific predictions: (1) absorption of L-arabinose would exceed that of cellobiose, because the paracellular pathway discriminates according to molecule size; (2) absorption of the D-glucose analogue would be complete in mice, but (3) considerably higher than that in the two paracellular probe molecules.

4.1. Effect of molecule size on absorption

As we expected, the smallest probe L-arabinose was absorbed to a greater extent than the largest cellobiose, because paracellular absorption declines with increasing molecular size of probes owing to the paracellular pathway's sieve qualities (Chediack et al., 2003; Chang and Karasov, 2004a). The permselectivity barrier in the TJ,

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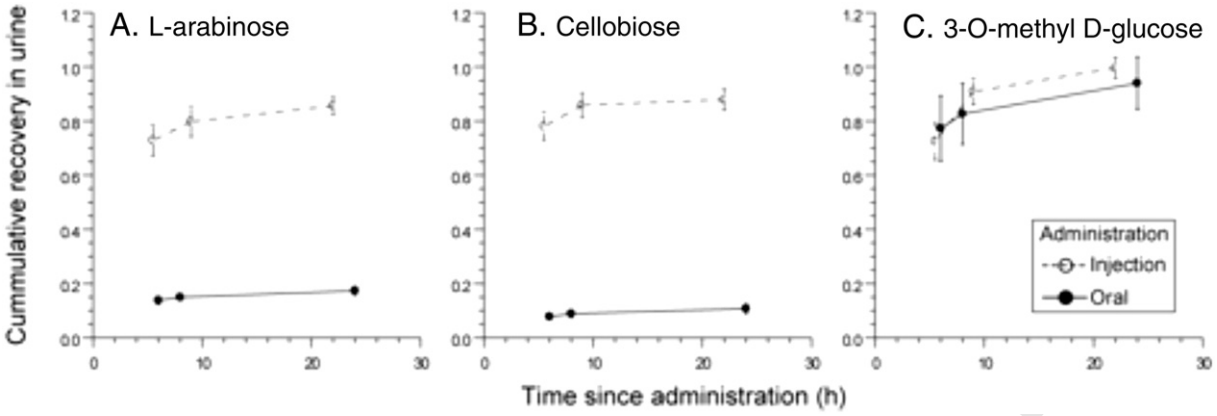

Q2 **Fig. 1.** Urinary elimination of (A) L-arabinose, (B) cellobiose, and (C) 3OMD-glucose as a function of times since administered to laboratory mice. Filled circles and solid lines denote probe in urine following oral administration; unfilled circles and dashed lines represent probe in urine after administration by injection. Error bars are SEM, and sample sizes were 5 mice in each case.

and its "pores" (which may be tortuous channels), are formed by strands of adhesive transmembrane proteins (claudins, occludin, and junctional adhesion molecule [JAM]) that extend into the paracellular space, but how they interact at the molecular and structural level to seal the paracellular cleft and to form pores is still largely unsolved (Krause et al., 2008). The sieving effect on molecule size is also apparent in an analysis of a broader mammalian comparative data set that now includes the mice (Table 1). Paracellular absorption in mammals declines with increasing M_r of the paracellular probe (two-way ANOVA on arcsin (square root (f)); $F_{2,1}=6.2$, $P=0.014$). It also differs significantly between the bats and nonflyers ($F_{1,12}=7.38$, $P=0.019$). However, for larger MW probes (*e.g.* cellobiose or lactulose), the difference between those groups is small, suggesting an equivalent molecular size cut-off for flying and nonflying mammals and thus a comparable effective TJ aperture.

Based on molecular size alone, it could be expected that absorption of L-arabinose should be more extensive than that of rhamnose. Comparing our data with those from Caviedes-Vidal et al. (2007) (who measured rhamnose absorption in mice), we find that L-arabinose absorption was indeed slightly higher than that of rhamnose, although the difference is negligible between these two paracellular markers. The lack of a large difference between the fractional absorptions of the two probes may relate to differences in animal state, age, *etc.*, between studies. Overall, our current and previous (Caviedes-Vidal et al., 2007) results indicate that mouse intestine absorbs a minority of nutrient-sized carbohydrates *via* the paracellular pathway; however, the tight junctions present charge and size selectivity, and our results may not be generalized to all molecules.

Paracellular transport can be modeled as having two components, a system of small pores that are charge and size-selective as well as a second pathway lacking charge or size discrimination that results from discontinuities in the barrier (Anderson and Van Itallie, 2009). The latter pathway is known to be low capacity (Anderson and Van Itallie, 2009; Shen et al., 2011). This explains the size-selectivity of paracellular absorption in our results, as the bulk of absorption likely occurred *via* the size-selective pore pathway. We must be cautious in making any interpretation about the paracellular pore size, because we have to consider that pores may be tortuous channels not necessarily characterized by a single radius, and that the estimation of effective pore size may be more sensitive to small uncertainties in the assumed molecular radii of probes than to experimental variability in the measurement of absorption (Adson et al., 1994).

4.2. Comparison of absorption of paracellular probes with that of 3OMD-glucose

Active transport of D-glucose against a concentration gradient provides an efficient mechanism to absorb the remaining glucose in the

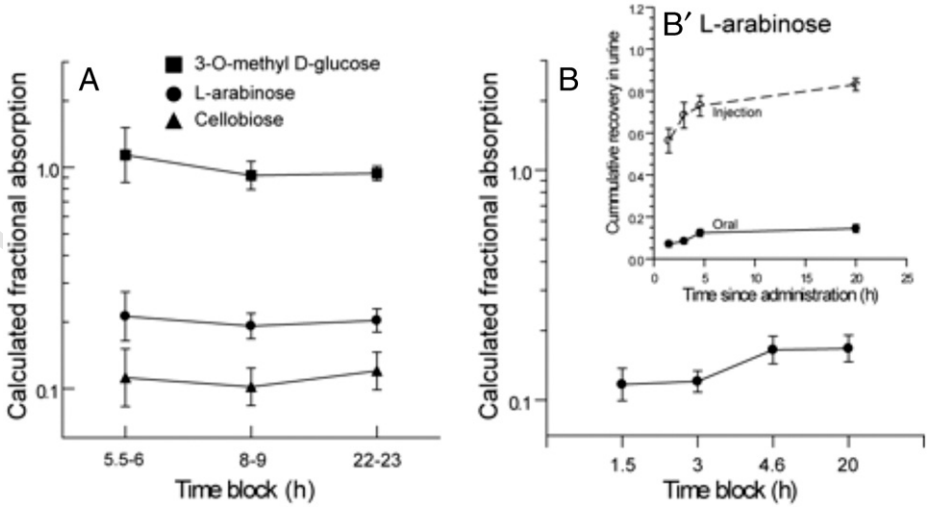

Fig. 2. Fractional absorption (f) of probe molecules calculated based on urine recoveries during different time blocks post administration orally and by injection. (A) Data for 3 probe molecules measured by HPLC in recovery trials from Fig. 1. The values are plotted on a semi-log graph in order to separate the values for L-arabinose and cellobiose. (B) Data for L-arabinose measured by liquid scintillation in recovery trials summarized in the inset (B′).

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

Common name	Scientific name	Body mass (g)	Fractional absorptions measured for different probes				Reference (s)
			L-Arabinose	L-Rhamnose	L-Lactulose or cellobiose	3-*O*-methyl-D-glucose	
Free-tailed Brazilian bat	*Tadarida brasiliensis*	14	1			1	Fasulo et al. (2012)
Great fruit-eating bat	*Artibeus lituratus*	80.6		0.90	0.1	0.96	Caviedes-Vidal et al. (2008)
Egyptian fruit bat	*Rousettus aegyptiacus*	125		0.62	0.22	0.91	Tracy et al. (2007)
Laboratory mouse	*Mus musculus*	28	0.21		0.13	0.95	This study
Common spiny mouse	*Acomys cahirinus*	55	0.42		0.05	0.95	Karasov et al. (2012)
Laboratory rat	*Rattus norvegicus*	300	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)

Note: All experiments included orally dosed nutrients in the gavage trials.

gut even at very low concentrations. Hence, there is the expectation that most glucose will be absorbed, which is what we observed and which occurs in all of the mammals in the comparative data set (Table 1). But, the extent to which glucose (or amino acids) is absorbed actively *via* brush border transporters (*e.g.*, SGLT1) *vs.* passively *via* paracellular absorption differs among animals. The relative extent of paracellular glucose absorption has been effectively studied in avian and mammalian species by measuring simultaneously the absorption of D-glucose (or its nonmetabolizable analogue 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), the two latter paracellular probes can account for the majority (range 50 to >90%) of glucose absorption in four avian species (Chang and Karasov, 2004a, 2004b; McWhorter et al., 2009; Karasov et al., 2012) and in three bat species (Tracy et al., 2007; Caviedes-Vidal et al., 2008; Fasulo et al., 2012). But, in analogous studies in rats (Uhing and Kimura, 1995), wild rodents (Karasov et al., 2012), dogs (Lane et al., 1999), and humans (Fine et al., 1993) L-glucose absorption, and hence paracellular absorption, is quantitatively much less important. Our measurements of much lower L-arabinose absorption than 3OMD-glucose absorption following oral administration of both probes place mice squarely in the latter group.

As a first step to estimate how much absorption of 3OMD-glucose was paracellular in the mice we directly compared AUCs and fractional absorptions (*fs*). But, the comparisons should be corrected for the small difference in MW. 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 paracellular + mediated absorption, the ratio of the amounts absorbed (adjusted L-arabinose/3OMD-glucose) indicates the proportion of 3OMD-glucose absorption that occurs *via* the paracellular pathway. The fractional absorption of L-arabinose after this adjustment would indicate that <20% of 3OMD-glucose absorption was apparently paracellular in the mouse. We expect the same would apply for amino acids.

4.3. Ecological advantage of low paracellular absorption

Due to their low use of paracellular absorption, mice, and other nonflying mammals, do not benefit from its putative advantages, such as reduced costs of absorption *via* diminished reliance on active transport, which requires ATP, and decreased synthesis of transporters. However, it may be important for nonflying mammals to reduce exposure to some plant secondary metabolites by reducing the use of paracellular absorption. A number of studies, mainly using intestinal tissue in cell culture, have demonstrated paracellular absorption of alkaloids (Leahy et al., 1994; Nielsen and Rassing, 2002) and phenolics (Deprez et al., 2001; Konishi et al., 2003a, 2004; Konishi and Kobayashi, 2005; Lafay et al., 2006) in the M_r range 162–460 Da, but there are few measurements in intact animals. Although cell culture studies are useful for demonstrating the potential for absorption, whole animal studies complement them by establishing an absolute capacity that can be interpreted in terms of likely nutritional significance. The survey of whole-animal studies of paracellular absorption (Table 1) supports the notion that smaller water soluble SMs that have been shown to be absorbed by the paracellular path in cell culture, such as nicotine (M_r 162.2; Nielsen and Rassing, 2002), gallic acid (M_r 170.1; Konishi et al., 2003b), caffeine (M_r 194.2; Leahy et al., 1994) and catechin (M_r 290.3; Deprez et al., 2001), might be absorbed in substantial amounts by small bats and birds but not by the nonflying mammals such as mice. Other possible evolutionary and ecological implications of the differences between fliers and nonfliers in paracellular absorption remain to be explored.

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