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Small intestinal epithelial permeability to water-soluble nutrients higher in passerine birds than in rodents

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Abstract

In the small intestine transcellular and paracellular pathways are implicated in water-soluble nutrient absorption. In small birds the paracellular pathway is quantitatively important while transcellular pathway is much more important in terrestrial mammals. However, there is not a clear understanding of the mechanistic underpinnings of the differences among taxa. This study was aimed to test the hypothesis that paracellular permeability in perfused intestinal segments is higher in passerine birds than rodents. We performed in situ intestinal perfusions on individuals of three species of passerine birds (*Passer domesticus*, *Taeniopygia guttata* and *Furnarius rufus*) and two species of rodents (*Mus musculus* and *Meriones ungiculatus*). Using radio-labelled molecules, we measured the uptake of two nutrients absorbed by paracellular and transcellular pathways (L-proline and 3–O–methyl–D-glucose) and one carbohydrate that has no mediated transport (L-arabinose). Birds exhibited ~2 to ~3 times higher L-arabinose clearance per cm^2 epithelium than rodents. Moreover, paracellular absorption accounted for proportionally more of 3–O–methyl–D-glucose and L-proline absorption in birds than in rodents. These differences could be explained by differences in intestinal permeability and not by other factors such as increased retention time or higher intestinal nominal surface area. Furthermore, analysis of our results and all other existing data on birds, bats and rodents shows that insectivorous species (one bird, two bats and a rodent) had only 30% of the clearance of L-arabinose of non-insectivorous species. This result may be explained by weaker natural selection for high paracellular permeability in animal- than in plant-consumers. Animal-consumers absorb less sugar and more amino acids, whose smaller molecular size allow them to traverse the paracellular pathway more extensively and faster than glucose.

KEYWORDS
birds, intestinal absorption, intestinal perfusion, L-arabinose, paracellular pathway, rodents, water-soluble nutrients

1 | INTRODUCTION

Water-soluble nutrients are absorbed in the small intestine via transcellular and paracellular pathways. In the former case, membrane transporters on the apical side of enterocytes translocate nutrients from the intestinal lumen into the cytosol, and then export of nutrients to the bloodstream is likewise achieved by transporter-mediated translocation across the basolateral membrane (Schultz & Curran, 1970). In contrast, the paracellular pathway is not transporter mediated; water-soluble compounds

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reach the basolateral space by traversing by diffusion or solvent drag through tight junctions (TJ) formed by adjacent enterocytes (Pappenheimer & Reiss, 1987).

A large body of work, recently summarized by Price, Brun, Caviedes-Vidal, and Karasov (2015), indicates that the paracellular pathway is quantitatively important in nutrient absorption in flying vertebrates. Experiments in intact small birds and bats using paracellular probes (hydrophilic probes that do not interact with intestinal mediated absorption mechanisms) have shown that up to 60%–90% of orally ingested glucose and amino acids are absorbed by this pathway, whereas the transcellular pathway is much more important in terrestrial mammals (Price et al., 2015). This high reliance on paracellular absorption, along with lower nominal intestinal surface area (area of the smooth-bore tube) in flyers compared with non-flyers, has been hypothesized to derive from an evolutionary pressure to reduce digesta mass in flying animals (Caviedes-Vidal et al., 2007) while having as a trade-off greater exposure to water-soluble toxins (Diamond, 1991).

There are theoretical reasons why differences in whole-animal absorption of paracellular probes should correspond to differences in permeability per unit area intestine (Chediack, Caviedes-Vidal, Fasulo, Yamin, & Karasov, 2003), but outright testing of this idea has mainly been done in comparisons of bats *vs.* rodents (Brun et al., 2014). Except for one study involving pigeons (Lavin et al., 2007), a broad comparison of clearance of a paracellular probe molecule in avian *vs.* mammalian small intestine has not been performed. The primary goal of this study was to test the hypothesis of enhanced paracellular permeability in birds compared with non-flyers using perfused intestinal segments ("in situ") of passerine birds and rodents. A comparison with in situ perfused intestinal segments can control for other factors that might influence absorption of paracellular probes at the whole animal level such as increased retention time or increased nominal surface area for passive absorption. We predict that in our intestinal perfusion experiments the birds would absorb more of the paracellular probe L-arabinose than the rodents per cm^2 nominal intestine, and paracellular absorption will account for a higher percentage of 3OMD-glucose and L-proline absorption in birds compared with rodents.

2 | MATERIALS AND METHODS

2.1 | Animals

We selected for study three passerine species that differ in natural diet: Red ovenbirds (*Furnarius rufus*), which are strict insectivores (Fraga, 1980), House sparrows (*Passser domesticus*), which are mainly granivores but take some insects when seasonally available (Anderson, 2006), and Zebra finches (*Taeniopygia guttata*), which are specialist granivores (Zann, 1996). In this study, we have included two rodent species for direct comparative purposes: granivorous Mongolian gerbils (*Meriones unguiculatus*) and omnivorous laboratory mice (*Mus musculus*). Together, these species represent a range of body masses with overlap between the birds and rodents, which thus help to control for any effect of body mass (Caviedes-Vidal et al., 2007) and improves coherence across different studies.

Zebra finches were purchased in San Luis, Argentina and House sparrows and Red ovenbirds were captured on the campus of Universidad Nacional de San Luis (UNSL), San Luis. House sparrows and Zebra finches were housed in cages indoors under constant environmental conditions (25 ± 1°C, relative humidity of 50 ± 10%) on a photoperiod of 14:10 (L:D) with water and food ad libitum (alpist and millet seeds, vitamins and minerals). Red ovenbirds were used on the same day of capture in order not to alter their eating habits. Experiments with rodents were performed using adult mice (*M. musculus*) from the strain C57 provided by the animal facility of the UNSL and Mongolian gerbils (*M. unguiculatus*) were purchased in San Luis. They were held in cages with ad libitum food (respectively, standard laboratory chow and mix of seeds supplemented with vitamins; Table 1) and water at constant environmental conditions (24 ± 1°C, relative humidity of 35 ± 3%), on a photoperiod of 13:11 (L:D). All animal procedures adhered to institutional animal use regulations and approved animal use protocols by the Animal Care and Use Committee of the UNSL, protocol number B212/15. Captured animals were approved by the Environmental Office of the State of San Luis, resolution number 75-PBD-2015.

TABLE 1 Animal attributes. Values represent means ± 1 SEM

Perfused Animals	*Passer domesticus*	*Taeniopygia guttata*	*Furnarius rufus*	*Mus musculus*	*Meriones unguiculatus*
N (male/female)	6/4	3/5	5/5	6/3	5/5
Body mass (g)	24.1 ± 0.38	14.1 ± 0.45	48.8 ± 1.64	23.2 ± 0.81	33.2 ± 0.46
Small intestine length (cm)	13.1 ± 0.20	12.4 ± 0.20	16.6 ± 0.23	29.1 ± 0.94	27.5 ± 1.52
Small intestine circumference (cm^2)	0.6 ± 0.02	0.5 ± 0.02	0.9 ± 0.02	0.7 ± 0.02	1.1 ± 0.03
References	This study	This study	This study	This study	This study
Diet	Omnivore (Anderson, 2006)	Granivore (Zann, 1996)	Insectivore (Fraga, 1980)	Omnivore	Granivore (Chen and Li 2000)

2.2 | Recirculating intestinal perfusions

In perfusions in situ we measured absorption of (a) the carbohydrate L-arabinose [relative molecular mass (M_r) = 150.1], a neutral non-metabolized paracellular probe that does not interact with intestinal nutrient mediated absorption mechanisms (Chediack, Caviedes-Vidal, Karasov, & Pestchanker, 2001; Lavin et al., 2007), (b) 3–O–methyl–D-glucose (3OMD-glucose; M_r = 194.2), a non-metabolized analogue of D-glucose (M_r = 180.2) and (c) L-proline (M_r = 115.1) which, like D-glucose, is absorbed by both transcellular and paracellular pathways/mechanisms.

To examine tissue-level absorption, we used the same protocol described in Brun et al. (2014). The animals were anesthetized with isoflurane (1%–5%) (Isoflurane, Scott-Argentina S.A., Buenos Aires, Argentina) and oxygen was delivered by a vapourizer (Surgivet/Anesco Isotec 4 N° series: W621107, Smiths Medical PM, Norwell, MA, USA). The body temperature during the procedure was maintained using a heating pad (Deltaphase Isothermal Pad, Braintree Scientific, Braintree, MA, USA). The abdominal cavity was opened with a peritoneal incision and proximal and distal ends of the intestine were identified. In birds, which have shorter intestines, both ends were cannulated in order to perfuse as much of the intestine as possible. In rodents, an average of 11.9 ± 0.76 cm was perfused beginning from the proximal section. The intestine was flushed with a prewarmed saline solution (0.95% for birds and 0.9% for rodents) for 15 min to remove digesta. The saline solution was then evacuated using air. Then the animal was perfused with the test perfusate using a perfusion pump (Watson-Marlow Alitea 400, Watson-Marlow Fluid Technology Group Argentina, Buenos Aires, Argentina) for 2 hours at 1 ml/min. During perfusion, the perfusate returned to a reservoir and was continuously recirculated. The test perfusion solutions for birds and rodents are detailed in Table 2 (Sigma–Aldrich, Sigma–Aldrich de Argentina S.A., Buenos Aires, Argentina). The solutions were labelled with radioactive isotopes that were recirculated in separate experiments that differ in the type of radioisotopes added to the solutions. Each solution was labelled with a trace amount of [1–^{14}C]–L-arabinose, in some cases together with a trace amount of [methyl–^{3}H]–3–O–methyl–D-glucose and in others with [2,3–^{3}H]–L-proline (Perkin Elmer, Waltham, MA, USA). The perfusate was weighed carefully before and after the perfusion. Subsamples (50 μl) of the perfusate collected before and after the perfusion were counted using 1 ml Ultima Gold TM scintillation cocktail (Perkin Elmer, Waltham, MA, USA) in 8 ml glass scintillation vials with a dual scintillation counter (Wallac 1409 DSA, Perkin Elmer Argentina S.R.L., Buenos Aires, Argentina). At the end of the surgery the animal was euthanized with isoflurane at 5%. The intestine was dissected out and the length and circumference of the perfused segment was measured.

TABLE 2 Composition of the perfusion solutions for birds and rodents based on a phosphate 1.2 mM buffer (pH = 7)

	Birds (mM)	Rodents (mM)
L-arabinose	10	10
L-proline	10	10
D-glucose	10	10
Mannitol	130	80
$NaHCO_3$	5	5
KCl	2.5	2.5
$CaCl_2$	1	1
NaCl	85	85
$MgSO_4$	1	1
Total mOsm/L	~350	~300

Absorption of each probe was calculated from the decrease in total radioactivity during the experiment measured by dual-isotope liquid scintillation technique, and was normalized by dividing by the duration (min) of the perfusion and by nominal surface area (/cm) of the perfused section of intestine. To calculate arabinose clearance (μl min^{-1} cm^{-2}), we divided arabinose absorption rate by $[(C_{initial}-C_{final})/\ln(C_{initial}/C_{final})]$, where C is probe concentration (Sadowski & Meddings, 1993). Using L-arabinose absorption at 10 mM as a proxy for the portion of L-proline or 3OMD-glucose absorption at 10 mM that is paracellular, we calculated the percent of absorption that was paracellular absorption as 100 × (arabinose absorption)/(proline or 3OMD-glucose absorption). The portion that was transcellular was taken to equal 100%—percent paracellular.

2.3 | Statistics

Statistical analyses were conducted with SPSS version 23 and results are expressed as means ± 1 SEM. In intestinal perfusions, differences among species in absorption, clearance and paracellular/transcellular percentage were determined using One-way ANOVA with post hoc Tukey's tests. The *F*-values of these and other analyses of variance are presented in the text with the relevant degrees of freedom as subscripts. Normality of data was checked by Shapiro–Wilk test, and homoscedasticity by Levene's test. Significance level was determined at $\alpha = 0.05$.

3 | RESULTS

3.1 | Intestinal permeability

Clearance of L-arabinose varied significantly among species ($F_{4,42}$ = 27.1, $p < 0.001$; Figure 1) and the birds exhibited ~2 to ~3 times higher L-arabinose clearance per square centimetre nominal surface area than the rodents. Interspecific comparisons showed significant differences among the bird species ($p < 0.05$), while no differences among rodent species were detected ($p > 0.4$). L-arabinose clearance was lower in *F. rufus* than in the other two species of birds, whereas it was higher than that of *M. unguiculatus* ($p < 0.05$), and no significant differences were found with *M. musculus* ($p = 0.072$).

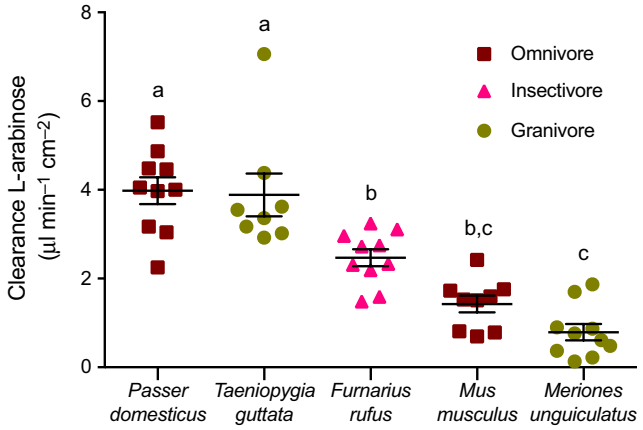

FIGURE 1 Clearance of L-arabinose. Individual values obtained in a 2-hr intestinal luminal perfusion of *P. domesticus*, *T. guttata*, *F. rufus*, *M. musculus*, *M. unguiculatus*. Lines represent the mean ± 1 SEM; groups of points that share letters indicate no statistically significant difference $p > 0.05$)

Absorption of 3OMD-glucose did not differ significantly among species ($F_{4,18} = 1.89$, $p = 0.16$; Figure 2(a)). In contrast, the absorption of L-proline varied significantly between species ($F_{4,19} = 5.40$, $p < 0.01$; Figure 2(c)). The granivore rodent *M. unguiculatus* exhibited significantly lower L-proline absorption than *P. domesticus* and *F. rufus* ($p < 0.05$) but not significantly different from the rest of the species tested (i.e., *T. guttata* and *M. musculus*, $p > 0.05$). All three bird species and *M. musculus* absorbed L-proline similarly ($p > 0.05$).

The percentages of 3OMD-glucose and L-proline absorption that were estimated to be paracellular were higher in birds than in rodents (respectively, $F_{4,18} = 43.4$, $p < 0.001$ and $F_{4,19} = 13.2$, $p < 0.001$; Figure 2(b,d)). In pairwise statistical comparisons for 3OMD-glucose, birds had significantly higher percentages of paracellular/(transcellular + paracellular) than did rodents ($p < 0.05$ for all comparisons). Also between birds, *P. domesticus* and *T. guttata* presented significantly higher percentages than *F. rufus* ($p < 0.05$). However, among the rodents, no significant differences were detected ($p > 0.9$). In some cases, values significantly exceeded 100%

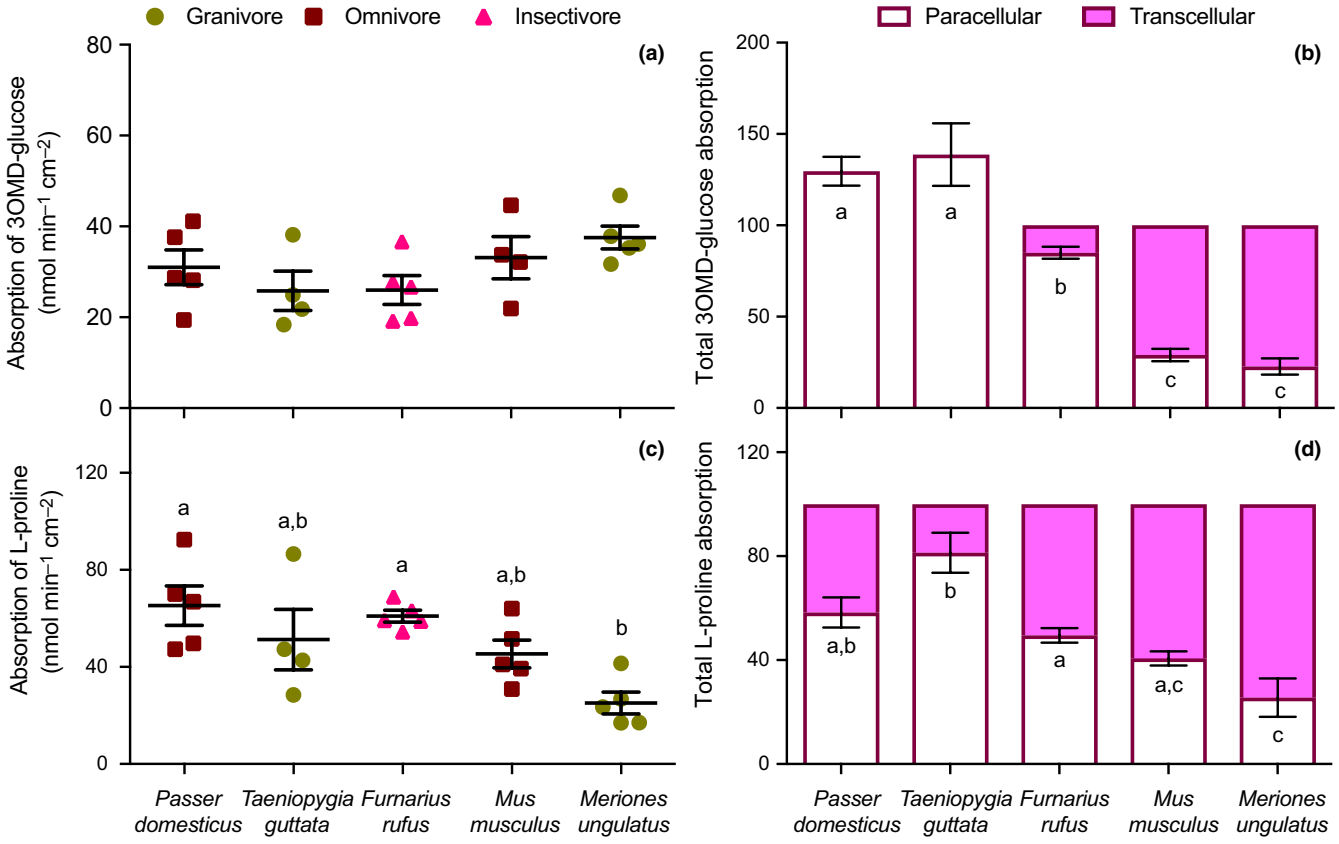

FIGURE 2 Intestinal absorption and pathway (paracellular *vs.* transcellular) used by 3OMD-glucose and L-proline. Absorption of 3OMD-glucose (a) and L-proline (c) in a 2 hr intestinal luminal perfusion. Sample sizes for 3OMD-glucose were as follows: *P. domesticus* $n = 5$, *T. guttata* $n = 4$, *F. rufus* $n = 5$, *M. musculus* $n = 4$, *M. unguiculatus* $n = 5$. For L-proline were as follows: *P. domesticus* $n = 5$, *T. guttata* $n = 4$, *F. rufus* $n = 5$, *M. musculus* $n = 5$, *M. unguiculatus* $n = 5$. Apparent percent absorption by the paracellular and transcellular pathways of 3OMD-glucose (b) and L-proline (d) based on the absorption of L-arabinose. Values for paracellular absorption exceed 100%, likely because of size differences among, 3OMD-glucose ($M_r = 194$) and L-arabinose ($M_r = 150$; see Discussion). Data are means ± 1 SEM; bars that share letters indicate no statistically significant difference ($p > 0.05$)

TABLE 3 L-arabinose clearance, 3OMD-glucose and L-proline absorption values (mean ± 1 SEM) of insectivorous (I), omnivorous (O) and (G) granivorous bird, bat and rodent species from this intestinal perfusion study and literature

				3OMD-glucose			L-proline			
Species	Body mass (g)	Diet	Clearence L-arabinose (μ min^{-1} cm^{-2})	Perfusate concentration (mM)	Absorption (nmol min^{-1} cm^{-2})	% paracellular	Perfusate concentration (mM)	Absorption (nmol min^{-1} cm^{-2})	% paracellular	References
Birds										
Passer domesticus	24.1 ± 0.38	O	3.9 ± 0.30	10	31.0 ± 3.84	129.6 ± 7.86	10	65.2 ± 8.15	58.3 ± 5.77	This study
Taeniopygia guttata	14.1 ± 0.45	G	3.8 ± 0.48	10	25.8 ± 4.32	138.6 ± 17.11	10	51.2 ± 12.43	81.3 ± 7.69	This study
Furnarius rufus	48.8 ± 1.64	I	2.4 ± 0.19	10	25.9 ± 3.17	84.9 ± 3.30	10	60.9 ± 2.44	49.5 ± 2.77	This study
Columba livia	~300	O	16.9 ± 2.43	-	-	-	-	-	-	Lavin et al., 2007
Bats										
Tadarida brasiliensis	15.6 ± 0.26	I	1.9 ± 0.08	10	18.8 ± 1.2	109.0 ± 6.2	10	44.4 ± 3.2	44.2 ± 2.1	Price et al., 2013;
Myotis lucifugus	7.8 ± 0.2	I	2.8 ± 0.72	10	33.5 ± 14	97.1 ± 45.7	10	47.0 ± 5.0	42.5 ± 10	Price et al., 2014;
Artibeus lituratus	61.0 ± 1.4	F	16.0 ± 5.78	10	67.0 ± 22.0	158.0 ± 33.33	-	-	-	Brun et al., 2014;
Sturnira lilium	21.8 ± 0.7	F	14.2 ± 3.15	10	59.0 ± 11.6	185.7 ± 28.57	-	-	-	Brun et al., 2014;
Carollia perspicillata	16 ± 0.7	F	8.9 ± 0.83	-	-	-	-	-	-	Brun et al., 2014;
Rodents										
Meriones unguiculatus	33.2 ± 0.46	G	0.7 ± 0.18	10	37.54 ± 2.52	22.72 ± 4.40	10	25.13 ± 4.50	25.55 ± 7.41	This study
Mus musculus	23.2 ± 0.81	O	1.4 ± 0.19	10	33.12 ± 4.65	29.0 ± 3.41	10	45.34 ± 5.72	40.65 ± 2.73	This study
Mus musculus	34.5 ± 3.2	O	1.7 ± 0.28	10	72 ± 4.8	24.7 ± 3.80	-	-	-	Brun et al., 2014;
Akodon montensis	37.4 ± 3.4	O	4.2 ± 0.85	10	78.8 ± 9.60	57.1 ± 9.52	-	-	-	Brun et al., 2014;
Rattus norvegicus	502 ± 48	O	1.4 ± 0.32	10	62.5 ± 3.9	22.6 ± 4.6	-	-	-	Brun et al., 2014;
Rattus norvegicus	325-500	O	4.8 ± 0.5	-	-	-	-	-	-	Lavin et al., 2007
Onychomys leucogaster	38.1 ± 3.4	I	1.2 ± 0.12	-	-	-	10	58.1 ± 10.85	22.2 ± 1.01	Price et al., 2014;
Peromyscus leucopus	22.4 ± 0.76	I	0.9 ± 0.11	10	48.3 ± 4.8	21.2 ± 1.94	10	47.6 ± 6.2	21.1 ± 3.64	Price et al., 2014

in birds (3OMD-glucose, *P. domesticus* $p < 0.001$ and *T. guttata* $p = 0.004$). These likely happened because of the molecular size differences between probes used to estimate the paracellular contribution to absorption (see Discussion). For L-proline, *P. domesticus* and *T. guttata* had the highest paracellular/total percentages of L-proline and no differences were detected between them ($p = 0.069$), whereas *M. unguiculatus* had the lowest paracellular/(paracellular + transcellular) percentage, which was similar to that for *M. musculus* ($p > 0.05$). However, paracellular/(transcellular + paracellular) percentages of L-proline did not differ significantly between *M. musculus*, *P. domesticus* and *F. rufus* ($p > 0.05$).

The analysis of our results and all the existing other data from intestinal perfusions in birds, bats and rodents species showed that volant species (birds + bats; $n = 9$ species) exceeded rodents ($n = 6$ species; species averages used for the 2 mouse and 2 rat measurements) by an average of four times in arabinose absorption (i.e., clearance) per cm^2 small intestine ($F_{1,12} = 9.28$, $p = 0.01$) (Table 3). The four insectivorous species that include one species of birds, two species of bats and one rodent species had average arabinose clearance only 30% that of non-insectivorous species ($F_{1,12} = 5.79$, $p = 0.033$).

4 | DISCUSSION

In agreement with our hypothesis, the three bird species exhibited ~2- to ~3-fold higher clearance at tissue level of L-arabinose than the two rodent species in the intestinal perfusions. These findings are in accordance with those of Lavin et al. (2007), and provide support for the previously described differences in L-arabinose absorption between intact birds and rodents (Afik, McWilliams, & Karasov, 1997; Chang, Chediack, Caviedes-Vidal, & Karasov, 2004; Karasov & Cork, 1994; Karasov et al., 2012; Levey & Cipollini, 1996; McWhorter, Bakken, Karasov, & Martínez del Rio, 2006). Moreover, these differences in L-arabinose absorption could be explained by differences in intestinal permeability, and not merely by differences in gut retention time, intestine size or other factors. Because birds had higher clearance of the paracellular probes but did not differ markedly from rodents in the total absorption of either 3OMD-glucose or L-proline, the estimates of the percentage of absorption that was paracellular were higher in birds than rodents for both 3OMD-glucose and L-proline (Figure 2b,d). Values for percentage of paracellular absorption of 3OMD-glucose in birds sometimes exceeded 100%, but this is likely due to either or both of two explanations: (a) our inaccuracy in correcting for how the size sieving effect that occurs in this pathway (Camenisch, Folkers, & van de Waterbeemd, 1996) differs between arabinose and the nutrients and (b) possible depressing effect of anaesthesia (Uhing & Kimura, 1995), which might affect mediated nutrient absorption more than passive paracellular absorption. Isolated preparations and cell cultures (Pauletti, Okumu, & Borchardt, 1997) and typical mammalian laboratory models (Delahunty & Hollander, 1987) as well as bird (Chediack et al., 2003) and bat species (Fasulo et al., 2013; Price, Brun, Fasulo, Karasov, & Caviedes-Vidal, 2013) have been used to verify the size-sieving effect. The inverse relationship between molecule size and epithelial paracellular absorption confounds simple comparisons of absorption of different-sized molecules relative to the size of L-arabinose, which has $M_r = 150$. Therefore, our calculations of relative paracellular absorption of nutrients are possibly overestimated for 3OMD-glucose ($M_r = 194$) and at the same time underestimated for L-proline ($M_r = 115$). Apparently higher absorption of L-arabinose than 3OMD-glucose has been observed previously in some bat species in both intestinal perfusions (Table 3) and in intact animals (Brun et al., 2014; Karasov et al., 2012; Price et al., 2013).

Our findings comparing three avian flying species and two rodent species are consistent with those comparing mammalian flying species (i.e., bats) and rodents (Table 3), as suggested in a review of bats and non-flying mammals (Price et al., 2015). Additionally, within each taxon, insectivorous species that digest mainly protein seem to have lower values of arabinose clearance. Thus, insectivorous species (i.e., one bird, two bats and a rodent) only had 30% of the clearance of L-arabinose of the non-insectivorous species (Table 3). This may make functional sense, because the amino acid products of protein digestion have smaller molecular masses than the monosaccharide products of carbohydrate digestion (e.g., D-glucose) and therefore have higher paracellular clearance across the intestinal epithelium. Studies in bats and rodents have shown that smaller paracellular probes (such as creatinine, which models the paracellular absorption of amino acids) are absorbed to a much greater extent than larger, glucose-sized paracellular probes (Dominguez & Pomerene, 1945; Lundholm & Svedmyr, 1963; Pappenheimer, 1990; Price, Rott, Caviedes-Vidal, & Karasov, 2014). It is plausible that there has been weaker natural selection for high tight junction paracellular permeability in carnivores and insectivores compared to herbivores and omnivores because of the higher clearance of the smaller amino acid hydrolytic products of protein as compared to carbohydrate. Within this scenario, differences among species in reliance on paracellular absorption would be consistent with the idea of a tradeoff between the putative benefit of high intestinal paracellular permeability, which is an additional low cost pathway for nutrient absorption, and a putative cost—greater exposure to water-soluble toxins (Diamond, 1991).

Our results of this investigation show a higher clearance of L-arabinose in the three species of bird than in the two species of rodents giving support to our hypothesis that birds would have higher intestinal permeability to paracellular restricted probes. Additionally, our data support that paracellular permeability accounts for the vast majority of total 3OMD-glucose and L-proline intestinal absorption in birds when compared to rodents.

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CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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