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Digestive Adaptations of Aerial Lifestyles

Flying vertebrates (birds and bats) are under selective pressure to reduce the size of the gut and the mass of the digesta it carries. Compared with similar-sized nonflying mammals, birds and bats have smaller intestines and shorter retention times. We review evidence that birds and bats have lower spare digestive capacity and partially compensate for smaller intestines with increased paracellular nutrient absorption.

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Due to the need to overcome gravity, flying organisms face substantially increased costs associated with carrying mass compared with nonflyers. The evolutionary result of this selective force is apparent in the morphology of birds and bats; compared with nonflying mammals of similar size, the metabolic and locomotory machinery is enhanced, whereas organs that are not essential to flight are reduced. This phenomenon is illustrated in the hollow bones of birds and reduced hind limbs (119) and brain sizes, particularly in migratory birds (118) and bats (61).

Although the selective pressure to reduce mass has also presumably shaped the evolution of the small intestine in flying vertebrates, the gut must also be able to digest and assimilate foodstuffs required to meet daily energy needs. Bats have higher basal metabolic rates than other mammals (6). Birds (particularly small flying birds) have higher basal and field metabolic rates than mammals (53, 72, 74), which results in higher daily food consumption (73). Birds and bats must therefore find a balance between reducing mass while maintaining enough digestive and absorptive capacity to meet their relatively high daily energy needs. Energetic needs can vary within and across seasons, and birds have been studied extensively as models of phenotypic flexibility, including substantial and rapid changes in gut size in preparation for and in response to migration (9, 29, 52, 68, 69, 84, 85).

In this review, we examine some of the apparent constraints on digestion and absorption in flying vertebrates, and the adaptations they have to mitigate such constraints. We restrict our analysis to vertebrates, but there is evidence that flying insects face similar selection pressures on gut size (122).

Dietary Niche

Birds and bats display a range of diets, including carnivory, insectivory, frugivory, granivory, and in few cases sanguivory. Notably rare is folivory, especially combined with the fermentative digestion of vegetative matter; this stands in contrast to nonflying mammals, many of which consume leafy vegetation and use fermentative digestion with the aid of bacterial communities and specialized organs to house them. Folivory is rare in bats (95); in birds, folivory is restricted to few taxa, including some waterfowl and galliforms (e.g., grouse) (26, 71). Some of these species, such as geese, may be "skimmers," rapidly consuming and egesting food, resulting in a lower assimilation efficiency rather than retaining large amounts of digesta for the prolonged periods of time required for extensive fermentative digestion (49). The enlarged digestive system and long retention time that are associated with fermentative digestion of leaves and twigs are likely disadvantageous for most flying vertebrates due to the extra mass (26, 71). Hoatzins (*Opisthocomus hoazin*), an example of one of the only avian foregut fermenters, are particularly poor flyers (26, 30).

Flyers Have Smaller Small Intestines and Shorter Retention Times

Despite having higher daily energy needs, birds have smaller small intestines compared with nonflying mammals (16, 55, 59) (FIGURE 1A). Small birds (<365 g) have shorter small intestines than nonflying mammals, and birds as a group have 50% smaller nominal surface areas (treating the small intestine as a smooth-bore tube) and 32% smaller small intestinal volumes than nonflying mammals of comparable body mass (55). Interestingly, birds and nonflying mammals have similar small intestinal wet masses (55). The reduced small intestinal size of birds thus does not directly reduce body mass. However, due to the diminished small intestinal volume, the mass of the digesta carried must be substantially reduced.

In view of the diminished volume and nominal surface area of the avian small intestine, birds could potentially compensate by holding ingested food longer in the gut to allow more time for digestion and absorption. However, the opposite occurs by large margins. Birds have mean retention

times that are quite shorter (86% shorter for fluids and 66% shorter for particles) than nonflying mammals (58, 63).

Similarly, bats have shorter small intestines (59) and 25–60% smaller nominal surface areas than nonflying mammals, depending on body size (16) (FIGURE 1). Additionally, anecdotal evidence suggests that bats also have generally shorter retention times that nonflyers (i.e., see data in Refs.13, 50, 54, 58, 93, 103), although passage rate may not differ significantly (50). In summary, both birds and bats have smaller small intestines with smaller nominal surface areas than nonflying mammals, and both groups have shorter retention of digesta than nonflyers. These characteristics reduce the average mass of digesta carried but should constrain the capacity for digestion and absorption. The daily digestive "load" placed on the small intestine, indexed by the ratio of daily energy needs to small intestinal nominal surface area (83), is significantly higher in flyers than in nonflyers by at least double (FIGURE 1B). In fact, the relative deficit in absorptive area is even greater for flyers than this because of the lack of a large intestine in most birds and bats.

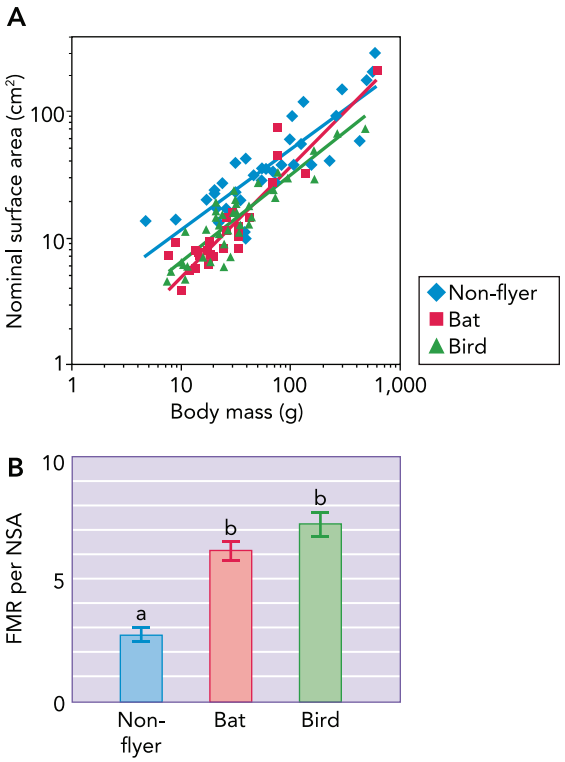

FIGURE 1. **Nominal surface area and daily energy expenditure**
A: nominal surface area of birds, bats, and nonflying mammals. Data are from species weighing <600 g from compilations in Refs. 16, 83, 87 and unpublished observations of A. Brun and E. Caviedes-Vidal. Nominal surface area increases with body mass but at a given body mass is higher in nonflying species. *B*: daily energy expenditure [i.e., field metabolic rate (FMR), kJ/day] divided by nominal surface area. We used animals from *A* and calculated FMR using allometric equations for rodents, eutherian mammals, and birds, as appropriate, from Ref. 72. For bats, we used the allometric equation log(FMR) = 0.7792·log(body mass) + 0.7283, which we derived from the FMR measurements on bats from the several studies cited by Ref. 115. Because both nominal surface area (NSA) and FMR scale with body mass similarly, their ratio was independent of body mass ($P > 0.1$). Different letters above columns signify significant difference in the ratio. Birds and bats have higher daily energy requirements for a given quantity of nominal surface area than nonflying mammals.

Remarkably, these handicaps do not lead to substantially reduced nutrient assimilation. Birds that consume vertebrates, insects, fruit, and possibly seeds have similar utilization efficiencies[1] to mammals and lizards (63, 91). We know of no broad comparison of digestive efficiency in bats and nonflying mammals, so we searched for and compiled studies on digestive efficiency on various diets (FIGURE 2). Bats seem to have similar or even greater digestive efficiency on similar diets than nonflying animals.

Thus, with their relatively short small intestines, birds and bats maintain enough digestive and absorptive capacity to meet equal or higher daily food processing demands compared with nonflying mammals. It is worth considering now how that might occur mechanistically.

Digestion, the breakdown of food into smaller molecules for absorption, is primarily dependent on hydrolytic enzymes that are in the intestinal lumen (of pancreatic origin) or are bound to the apical or "brush border" of enterocytes. Higher rates of brush border digestion in birds and bats could therefore arise from a greater activity of hydrolytic enzymes per unit surface area of small intestine. Birds and bats might also have intestinal morphology that enlarges the surface area (villous amplification) to a greater degree, thus providing more access for the enzymes to the ingested food.

Absorption of the breakdown products of digestion can occur trancellularly via nutrient transporters situated at the apical and basolateral membrane of enterocytes or paracellularly via movement (via diffusion or solvent drag) across the tight junctions that bind adjacent enterocytes (FIGURE 3). One way to increase absorptive capacity could be to increase the density of nutrient transporters in the enterocyte membrane. Increasing villous amplification could also increase the number of enterocytes, and therefore the number of nutrient transporters, per unit nominal surface area. Higher villous amplification would also lead to a higher number of enterocytes, and presumably tight junctions, between enterocytes, and could therefore represent a way to increase paracellular absorption. Paracellular absorption might also be

[1] Utilization efficiency is some measure of what is commonly called digestive efficiency. The efficiencies plotted in FIGURE 2 are a mix of values of dry matter and energy digestibilities, but these measures tend to be close to each other and highly correlated (46).

increased if the tight junctions themselves are somehow more permeable to nutrient-sized molecules. We will examine all of these potential adaptations for birds and bats.

Meeting Digestive Demand With Smaller Intestines

Digestion of most dietary carbohydrates and proteins involves hydrolysis by intestinal brush border enzymes. To compensate for lower nominal surface area, birds and bats might have greater villous amplification that increases mucosal surface area, or alternatively they might pack these enzymes more densely along the brush border.

A comparison of the pigeon and rat found no difference in villous amplification (57). In a larger comparison, however, birds did apparently have more villous amplification than nonflying mammals (~15%), although this is based on measurements from studies employing various methodologies (55). Notably, this number is not enough to compensate for the 50% reduction in nominal surface area of birds compared with mammals. In bats, the data are scarcer, and this is an active area of investigation. Our preliminary comparison of seven bats and eight nonflying mammals using similar methodologies showed a substantially higher villous amplification (75% higher) in bats (123) (Brun A, Caviedes-Vidal E, unpublished observations), and thus bats may be able to compensate for smaller small intestines via higher villous amplification.

Compensation for the reduced mucosal surface area in birds might be found in higher hydrolytic enzyme activities (e.g., greater enzyme density) in flying species. However, a previous comparison of sucrase, maltase, isomaltase, and aminopeptidase-N activities (measured per gram of protein) did not differ significantly among mammals and birds (63). We also compared sucrase, maltase, and aminopeptidase-N activities (per gram wet mass) in birds, bats, and nonflying mammals from studies on species with primarily carbohydrate-based diets, all measured with similar methodology

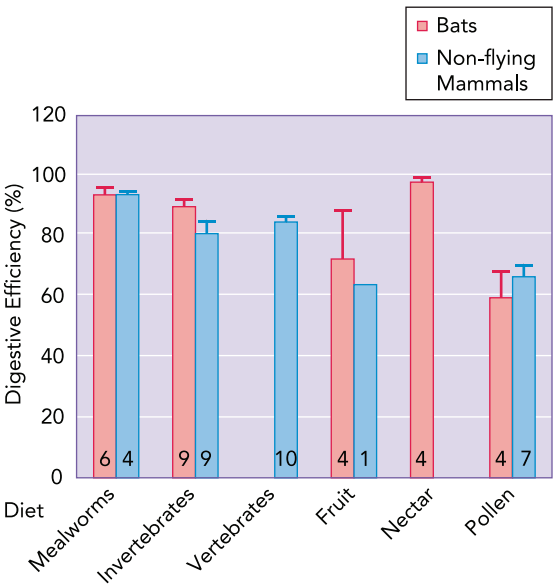

FIGURE 2. **Digestive efficiency (dry matter or energy basis) in bats and nonflying mammals on various diets**
Mealworms are separated from invertebrates only because there was a large enough number of studies with that experimental diet to make a direct comparison. The number of species examined is at the base of each bar. Data are means + SE and are from Refs. 7, 8, 10, 11, 21, 22, 25, 27, 31, 32, 34, 38, 48, 79, 90, 92, 94, 96, 105, 117, 120, 121.

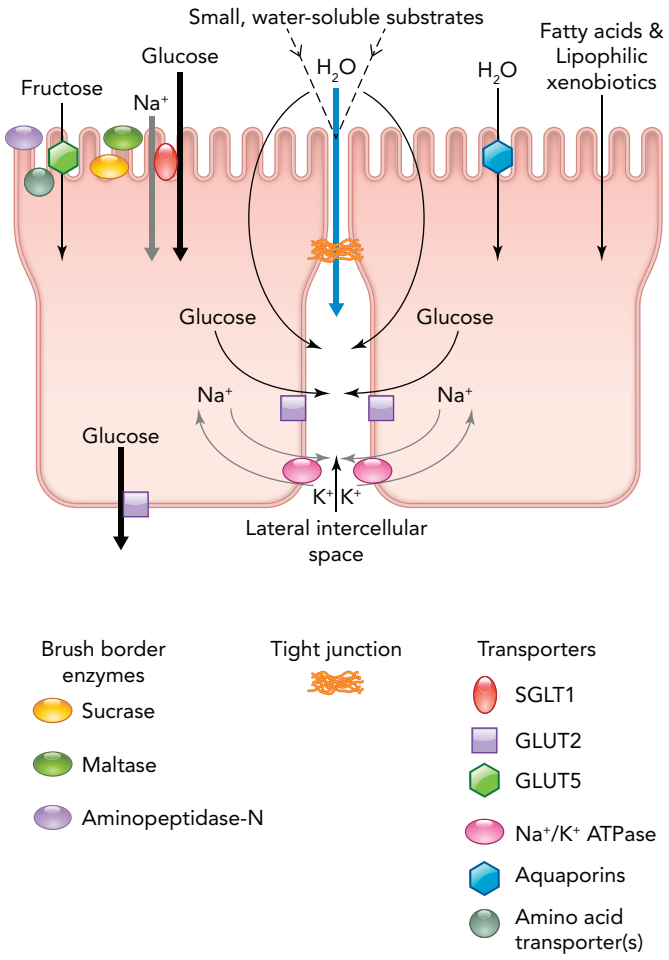

FIGURE 3. **Cartoon illustrating two enterocytes and the mechanisms by which macronutrients are absorbed**
Substrates are hydrolyzed by a variety of brush-border enzymes (e.g., sucrase, maltase, aminopeptidase-N). Water-soluble monomers can move across the epithelium via transporters in the brush border [e.g., SGLT1, fructose transporter (GLUT5), amino acid transporters] but also through the tight junction (composed of a number of interacting protein strands such as claudins, occludin) via diffusion or solvent drag (bulk movement along with absorbed water). Na ions, which move down their electrochemical gradient, are expelled from within the cell by Na-K-ATPase in the basolateral membrane (the exact stochiometries of solute fluxes are not depicted). Lipophilic compounds, such as fatty acids and lipophilic xenobiotic chemicals, can diffuse into and across the cell phospholipid bilayer membranes.

(FIGURE 4). This analysis shows that birds have higher maltase activities [$F(2,26) = 7.4$, $P = 0.003$] than both bats and non flying mammals. Birds had lower sucrase activity than bats [$F(2,19) = 15$, $P = 0.001$], but sucrase activity of nonflyers did not differ from either flying taxon. Aminopeptidase-N activity did not vary significantly among groups [$F(2,19) = 3.2$, $P = 0.06$], although there was a trend for higher activity in the nonflyers. We caution that there have been no studies directly comparing flying and nonflying species, and we have not controlled for specific diet or body mass. But, overall, hydrolytic enzyme activities are similar among taxonomic groups per gram of tissue. Given that birds have similar intestinal wet masses to nonflying mammals (55), they may achieve similar summed enzymatic capacities with their modestly higher villous magnification. Bats also seem to achieve similar digestive capacity with smaller guts via increased villous magnification.

Alternatively, flying species may not need to have similar digestive capacities as nonflying mammals. There is some spare enzymatic capacity in the small intestine that presumably allows animals to rapidly adjust to temporary changes in food consumption (23). Birds and bats may simply have lower spare capacity for digestion.

Meeting Absorptive Demand With Smaller Intestines by Greater Transcellular Absorption

Although there are few studies that address this topic directly, there do not appear to be systematic differences between birds and mammals with regard to maximum mediated glucose uptake rates per cm^2 of small intestine (45). Those measurements were all uptakes measured by the everted sleeve method (43) at saturating substrate concentrations (25–50 mM). When summed over the small intestine, glucose uptakes are significantly ($P < 0.001$) lower in birds than in mammals (FIGURE 5A), and L-proline uptakes tend to be lower, but not significantly ($P = 0.16$; FIGURE 5B). For bats, a similar analysis needs to be conducted, but summed nutrient uptakes were not particularly high or low for a single fruit bat species compared with other mammals (44) (FIGURE 5). Furthermore, in intestinal luminal perfusion studies comparing birds or bats with nonflying mammals, we have failed to find differences between taxonomic groups in uptake of glucose or proline per unit length or nominal surface area (12, 57, 87). Thus the sometimes greater villous amplification ratio discussed above might increase the absorptive surface area, but it does not seem to result in greater mediated absorption in birds and bats. In fact, the low summed glucose uptakes in birds indicate that they have lower mediated glucose uptake due to their smaller small intestines.

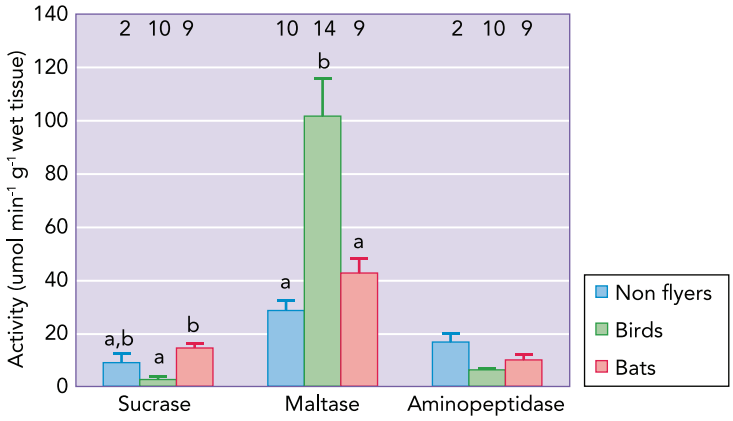

FIGURE 4. **Hydrolytic enzyme activity in birds, bats, and nonflying mammals with primarily carbohydrate-based diets**
Data are means + SE and are from unpublished observations of K. Lessner and W. Karasov and Refs. 51, 89, 97, 98. Sample sizes are listed across at *top*.

Meeting Absorptive Demand With Smaller Intestines via Higher Paracellular Nutrient Absorption

Although glucose and amino acid absorption is traditionally thought to occur transcellularly via apical membrane-bound transporters (e.g., SGLT1), nutrients can also be absorbed paracellularly by passing through the tight junctions that link adjacent enterocytes (80–82) (FIGURE 3). These tight junctions are composed of multiple extracellular protein strands that project from the cells' lateral membranes and form a sieve-like barrier (101). Paracellular absorption has the benefits that absorption rate increases linearly with load (does not involve carriers that can be saturated) and that transport is passive and requires no endogenous metabolic energy source (82), at least not directly (110). However, the tight junctions are selective only for molecule size and charge in both mammals (37, 101) and birds (18, 20), and thus epithelia that are more paracellularly permeable to nutrients are also more permeable to water-soluble dietary toxins (23). It is presumably for this reason that the paracellular permeability to glucose-sized molecules is low in traditional nonflying mammals such as mice, rats, and humans (23, 40).

Previously, we proposed that flying animals compensate for their smaller small intestines by relying more extensively on paracellular absorption of nutrients (16). This hypothesis was supported primarily with data from birds, which show high fractional absorption of paracellular probes (nutrient-sized carbohydrates that do not have affinity for nutrient transporters). Fractional absorption is measured in intact animals using methods that are standard in pharmacology. Small birds in particular absorb the majority of orally dosed

paracellular probes (3, 17–19, 40, 62, 64, 77, 108), whereas absorption of the same probes in small nonflying mammals is much lower (12, 28, 40, 87). This difference declines with increasing body size, such that large birds have similar fractional absorption of paracellular probes as nonflying mammals (16, 56).

We have since extended our measurements to include more birds, several bat species, other small nonflying mammals, and a lizard (12, 14, 27, 28, 67, 75, 76, 78, 87, 88, 107) (Price E, Karasov W, unpublished observations). In FIGURE 6A, we have plotted the 7 bats, 19 birds, 1 lizard, and 18 nonflying mammals for which we could find measurements of the fractional absorption of the following nutrient-sized probes: L-arabinose (M_r 150), L-rhamnose (M_r 164), L-glucose (M_r 180), and mannitol (M_r 182). There is a significant effect of taxonomic group [ANCOVA, $F(2,41) = 34$, $P < 0.0001$] and an interaction between group and body mass [$F(2,41) = 6.3$, $P = 0.004$]. Because the size-sieving effect of tight junctions can affect the absorption of different sized probes, we also present the same results but restricted to measurements of L-arabinose (FIGURE 6B). Similarly, there are significant differences among taxonomic groups [$F(2,17) = 25$, $P < 0.001$] and an interaction between group and body mass [$F(2,17) = 12.5$, $P < 0.001$]. Diet was not a significant factor in either set. In both analyses, nonflying vertebrates have uniformly low absorption of paracellular probes, whereas birds have high absorption of the probes that declines with increasing body mass. This decline in paracellular probe absorption may correspond to the narrowing of the difference in small intestine length between birds and nonflying mammals at increasing body masses (16). Bats have higher absorption of the probes than nonflyers ($P < 0.001$), but there have not been measurements of large bats (>150 g), so it is not yet clear whether fractional absorption also declines with body mass in bats.

These differences in nutrient-sized probe absorption can be demonstrated at the tissue level using perfusion experiments that control for potential confounding factors such as gastric emptying, retention time, intestinal flora, and dietary water. For birds, a study of paracellular probe absorption during recirculating intestinal perfusions in anesthetized animals demonstrated that pigeons had much greater clearance of the probes than the similarly sized rat (57). For mammals, we have collected the results from several recent perfusion studies conducted with uniform methodology to compare bats and nonflyers (12, 86, 87) (Price E, Cruz-Neto A, Caviedes-Vidal E, unpublished observations) (FIGURE 7). Arabinose clearance was higher for bats as a whole [$F(2,7) = 22.8$, $P = 0.003$], although it was particularly high for bats that have carbohydrate-rich diets [$F(2,7) = 13.5$, $P = 0.006$; significant group × diet interaction, $F(2,7) = 9$, $P = 0.024$] (FIGURE 7A).

To estimate the importance of paracellular absorption in glucose uptake for each species, we can use L-arabinose absorption as an estimate of the paracellular portion of glucose absorption (FIGURE 7B). We can see that the proportion of glucose absorption that is paracellular is much higher in bats than in nonflyers [$F(2,7) = 23.9$, $P = 0.0027$], and there is no diet effect (FIGURE 7B). The values for bats sometimes exceed 100%, a phenomenon that is driven by the smaller size of arabinose compared with glucose and the size-sieving effect of the tight junction. Nonetheless, the measurements should be comparable among species and generally indicate that bats rely on the paracellular route for a majority of glucose absorption,

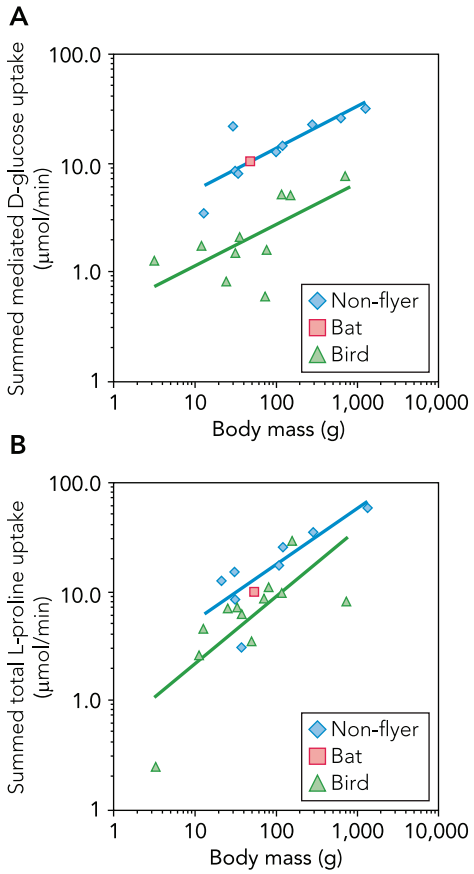

FIGURE 5. Summed uptake over the entire small intestine of nonflying mammals, birds, and one bat species
These are unidirectional uptake rates measured by the everted sleeve method (43) at saturating concentrations (25–50 mM). *A*: mediated D-glucose uptake is significantly higher in nonflyers compared with birds ($P < 0.001$; no significant difference in slope; carnivores/insectivores excluded). *B*: total L-proline uptake tends to be higher in nonflyers compared with birds, but the difference is not significant ($P = 0.16$; no significant difference in slopes; carnivores/insectivores included). Data are from Refs. 2, 15, 35, 39, 41, 42, 44.

whereas nonflying mammals do so much less. This result is not likely driven by differences in transporter kinetic properties, such as K_m, which are similar among species (47). An interesting result is that L-arabinose clearance was higher in frugivorous bats compared with insectivorous bats, even though bats with both diets have high L-arabinose fractional absorption in experiments in intact animals. This may derive from the shorter intestines of the frugivores (Brun A, Caviedes-Vidal E, unpublished observations).

Assessing the importance of paracellular absorption for protein assimilation is made difficult by several factors. First, amino acids vary greatly in size and are generally smaller than glucose. Due to their smaller size, substantial proportions of amino acid absorption may be via the paracellular pathway. In intact animals, the majority of orally dosed creatinine (M_r 113), a paracellular probe comparable in size to proline (M_r 115), is absorbed in rodents, bats, humans, and other mammals (24, 81, 87, 109). In an intestinal perfusion study, the proportion of proline that was absorbed paracellularly (estimated by creatinine) was actually higher in a wild insect-eating rodent (55%) than in an insectivorous bat (41%) in perfusions with 10 mM proline (87). Thus, with small molecules such as amino acids, among-taxa differences in paracellular absorption diminish. As a second complication, the bulk of amino acid absorption may not occur as monomers but rather as di- and tripeptides (1), and we have yet to assess the rate of mediated vs. nonmediated dipeptide absorption in flying species (but see Ref. 20).

In summary, intestinal perfusion experiments confirm the results from intact animals, demonstrate the sieving effect of the tight junction, and indicate greater permeability of the intestinal epithelium of flyers compared with nonflyers, particularly for larger nutrients such as glucose.

Despite having somewhat higher villous amplification, summed mediated glucose uptake is lower in birds than in nonflying mammals. The higher paracellular absorption that we observe in small birds thus seems to represent an evolutionary compensation for this deficit. In bats, it is still unclear to what degree higher villous amplification and summed mediated glucose uptake can offset the otherwise diminished absorptive capacity that should be imposed by their smaller small intestines. Bats nonetheless have high absorption of paracellular probes, which could be an important mechanism for compensating for any diminished capacity for mediated uptake.

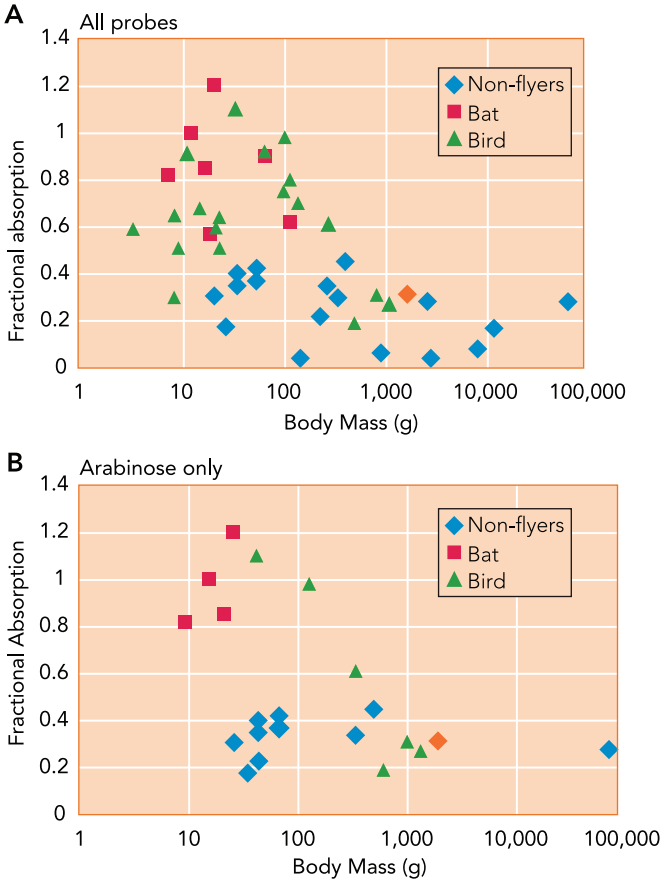

FIGURE 6. **Fractional absorption of paracellular probes in various species**
A: absorption of L-arabinose, L-rhamnose, L-glucose, or mannitol. When more than one probe was measured for a species, we chose L-arabinose or the probe closest in size to arabinose. Fractional absorption may vary depending on diet sugar concentration (75, 78); when more than one measurement was available for a probe in a species, we averaged the measurements. *B*: absorption of L-arabinose by various species. The orange-filled diamond denotes the sole lizard in the dataset, which was grouped with nonflying mammals for statistical analysis.

Mechanistic Basis of High Paracellular Nutrient Absorption in Flying Vertebrates

High paracellular nutrient absorption might result from *1*) small intestines that have more tight junctions across which nutrients/probes can be absorbed or *2*) tight junctions that are more permeable to nutrient-sized molecules. This is an area of active investigation, but there is some evidence that both may be occurring.

Birds and bats sometimes have more villous amplification than nonflyers (57, 87, 123) and sometimes have smaller enterocytes (87). Both properties would lead to more tight junctions per nominal surface area. However, due to their smaller small intestine, birds and bats still often have fewer total enterocytes, and presumably tight junctions, summed over the entire small intestine in studies that demonstrate higher paracellular nutrient absorption in the flying species (57, 87, 123). Furthermore, the greater villous amplification in flying species is often not enough to explain their greater paracellular probe absorption in in situ perfusion

studies (57, 87). That is to say, paracellular probe absorption per villous surface area is still higher in flying species than in nonflyers.

Greater permeability of the tight junctions has been suggested previously for flying species (57, 87), although rarely has it been directly assessed, excepting the previously mentioned in situ perfusion studies. Differences in tight-junction permeability could derive from several mechanistic origins. These could include differences in tight-junction protein sequence/structure, types, and concentrations of proteins that localize to the tight junctions, differences in the frequency of temporary breaks in the tight-junction barrier (5, 112, 116), and differences in the shuttling of tight-junction proteins between the tight junction and the intracellular pools (101, 102). Tight junctions are complex structures composed of a number of interacting proteins (e.g., claudins, occludin), and one simple hypothesis is that the composition of the tight junction, in terms of proteins expressed, could determine the permeability to macronutrients (33). Modifications to the expression of certain proteins (e.g., CLDN1, CLDN2, OCLN) in cell monolayers has sometimes been associated with differences in permeability to water-soluble solutes (4, 60, 99, 106, 111, 113, 114). Our data indicate that there are differences in expression patterns of claudins and occludin that vary among species (87) (Price E, Fernández-Marinone G, unpublished observations), although these differences between bats and nonflyers may be more related to diet or taxonomic affinity. The mechanistic underpinning of variation in permeability remains a frontier in this research.

Implications of High Paracellular Nutrient Absorption

At an ecological level, the high intestinal paracellular permeability of flying species can both increase and decrease the effects of natural and synthetic toxins, depending on the action of the toxin. Birds and bats should have greater exposure to water-soluble toxins that can be absorbed across their more permeable intestinal epithelia (23, 40). On the other hand, the functional effects of certain plant secondary compounds that target glucose transporters should be minimized in flying species. For example, glucose absorption by the robin (*Turdus migratorius*) was unhindered by several naturally occurring flavonoids, including the well known SGLT1-inhibitor phloridzin, whereas glucose absorption was diminished in rats by the same compounds (104). The difference in response is probably because robins rely mainly on the paracellular pathway for glucose absorption (64). Such physiological differences could, in turn, mediate certain plant-animal interactions. For example, plants that favor long-distance seed dispersers could attract flying fruit consumers while using flavonoids to repel nonflying consumers that presumably move smaller distances before dropping or egesting seeds.

From a biomedical point of view, altered intestinal permeability is evident in several gastrointestinal diseases (100), and the paracellular route of absorption is important in pharmaceutical delivery. Birds and bats have high epithelial permeability in their natural states and might be useful models for understanding how high paracellular permeability is regulated and how its detrimental effects can be mitigated.

Water Absorption

Aside from the need to process nutrients rapidly, flying vertebrates must rapidly absorb and excrete water to reduce the mass carried. Some birds can pass water through and out the digestive tract

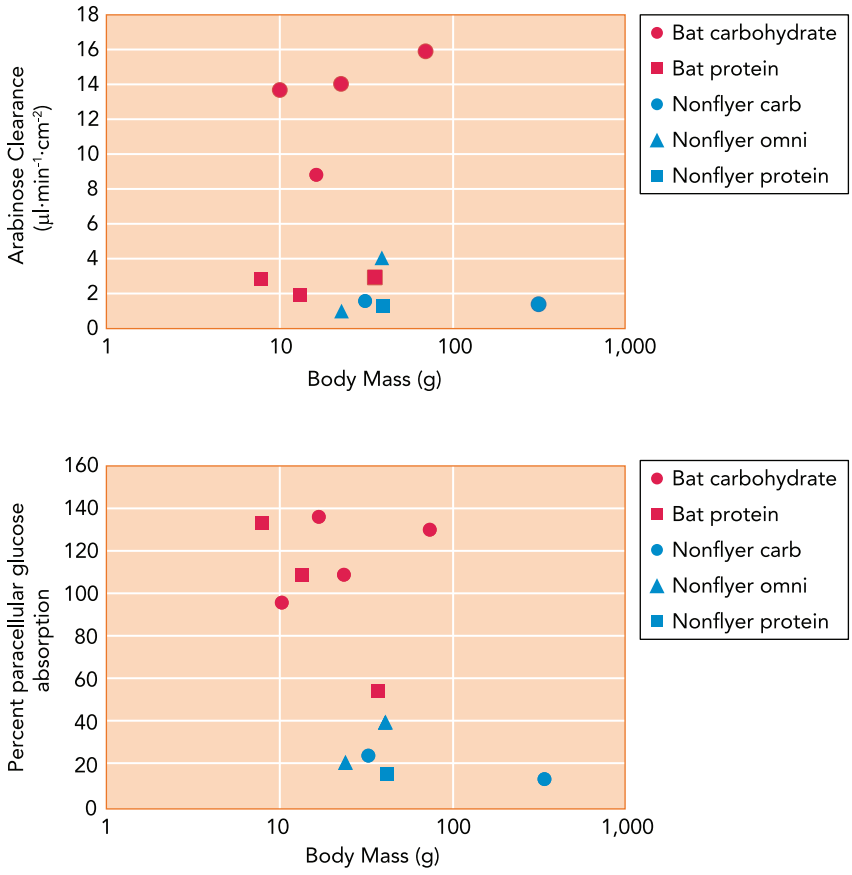

FIGURE 7. **Absorption of nutrient-sized probes in in situ intestinal luminal perfusions**
Top: clearance (µl·min^{-1}·cm^{-2}) of L-arabinose (M_r 150). *Bottom*: proportion of glucose absorption that was paracellular, estimated using L-arabinose absorption. The luminal concentration of glucose was 10 mM in these experiments. In some cases, radiolabeled 3-*O*-methyl-D-glucose was used as a tracer to estimate glucose absorption. Bats (in red) are designated as those that typically eat carboydrate-rich diets (filled circles) or protein-rich diets (filled squares). Nonflyers (in blue) are designated as either those that eat those diets or those that are omnivores (blue triangles), eating diets mixed with carbohydrate- or protein-rich foods.

without absorbing it, although the mechanism for avoiding absorption is unknown (65). Other birds and bats absorb most dietary water after feeding on watery diets (36, 66, 70). Bats with the most watery diets (i.e., vampire bats, nectarivores, frugivores) have high water absorption rates in isolated small intestinal perfusion experiments (Price E, Caviedes-Vidal E, unpublished observations).

Conclusions and Future Directions

Birds and bats have smaller small intestines relative to similarly sized nonflying mammals, a trait presumably favored by evolution to reduce mass, but one that should constrain the capacity for digestion and absorption. Both birds and bats may partially or completely compensate with higher villous amplification. However, birds at least apparently still have a deficit of transporter-mediated glucose absorption relative to nonflyers. Accumulating evidence demonstrates that small birds and bats have high rates of paracellular absorption of glucose-sized molecules, which could compensate for any reduction in transporter-mediated absorption associated with their small guts and rapid transit times. Such differences in absorption among taxa are apparent not only in intact animals but in isolated intestinal loops.

We suggest research in the following areas would be fruitful: *1*) directly testing for differences between flyers and nonflyers in summed enzymatic capacities of the small intestine, while accounting for diet and body size; *2*) comparing the summed mediated transport rates of bats vs. nonflying mammals; *3*) exploring the paracellular permeability of the intestines of large bats; *4*) incorporating retention time into analyses, particularly for bats; *5*) determining the genetic bases for the differences between flyers and nonflyers, including exploration of the molecular architecture of tight junctions as a potential mechanistic driver of rates of paracellular absorption; and *6*) evaluating the importance of the gut microbial community in flying vertebrates. These research directions should shed light not only on the mechanisms that explain differences in absorption among taxa but also increase our understanding of the ultimate selection pressures that drive these differences. ■

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