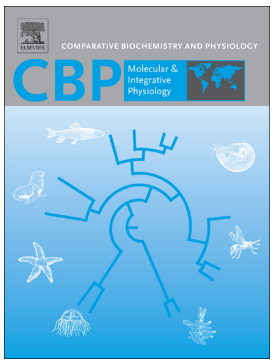

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PII: S1095-6433(20)30213-0

DOI: https://doi.org/10.1016/j.cbpa.2020.110860

Reference: CBA 110860

To appear in: *Comparative Biochemistry and Physiology, Part A*

Received date: 1 November 2020

Revised date: 27 November 2020

Accepted date: 28 November 2020

Please cite this article as: W.H. Karasov and E. Caviedes-Vidal, Adaptation of intestinal epithelial hydrolysis and absorption of dietary carbohydrate and protein in mammals and birds, *Comparative Biochemistry and Physiology, Part A* (2019), https://doi.org/10.1016/j.cbpa.2020.110860

Adaptation of intestinal epithelial hydrolysis and absorption of dietary carbohydrate and protein in mammals and birds

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Running title: Intestinal adaptation in mammals and birds

Abstract

The small intestine of mammals and birds exhibits fascinating variation across taxa, body size, and life history features such as locomotion and diet. In the intestine's brush border membrane (BBM), hydrolases are more abundant than transporters in both mammals and birds, but there are differences among the groups in abundance of certain hydrolases and possibly in transporters. For example, mammals express two α-glucosidases, sucrase-isomaltase (*SI*) and maltase glucoamylase (*MGAM*), whereas songbirds we studied have only *SI*, and the chicken expresses *SI* plus another α-glucosidase that functions similarly to *MGAM* but is not a true ortholog. For intestinal absorption of sugars and amino acids, small fliers rely on a paracellular pathway to a greater extent than do nonflying mammals, which rely more on transporters. Possibly having evolved in fliers as compensation for lower intestinal nominal surface area (NSA), the fliers' reliance on paracellular absorption is supported by their greater villous surface enlargement that leads to more (per cm^2 NSA) tight junctions and greater clearance of passively absorbed compounds. To match digestive capacity to nutrient load, a positive relationship is often observed between dietary intake of macronutrients and intestinal activity of the enzymes and transporters of their respective constituents. In enterocytes, rapid, fine-tuned adjustment to high dietary carbohydrate and protein involves rapid, specific correlated increase in activity and abundance of hydrolases and transporters in the BBM and increases in their mRNA.

Key words: aminopeptidase-N, evolution, proteomics, rodent, SGLT1

Introduction

Mammals and birds adapt to increases in nutrient load. We refer to adaptation broadly, within individuals and among populations and taxa, to matches achieved by changes in digestive structure and function that compensate, for example, for increases in food intake

during cold acclimation, during reproduction or migration, or when diet changes to one of higher macronutrient content (Karasov and Douglas, 2013)[1]. The exposition and explication of this topic demands incorporation of the diversity of animals, which makes it so appropriate as part of an homage to August Krogh, who promoted incorporating animal diversity as part of making progress in understanding physiology (Krogh, 1929).

Inspired by theatre, we present our review in three parts: Part I The Stage, which will mainly be the epithelium of the small intestine and most specifically its apical membrane; Part II The Players, which will be processes mediated by enzymes, transporters and other proteins that populate the stage; and Part III The Action: Achieving the Match.

Part I - The Stage

The small intestine's nominal surface area (NSA) increases allometrically with a body mass exponent similar to that of metabolism ($\propto M^{-3/4}$) (Fig. 1A). In small (<600 g) mammals and birds, NSA is lower in fliers (Fig. 1A) which translates to lower intestine volume and carried mass of digesta, thus saving on flight costs.

In both birds and mammals the small intestine's surface area is magnified by villi, cell-lined finger-like projections inward into the lumen, which are separated at their base by villus crypts that contain undifferentiated stem cells (Starck, 2003). The crypt stem cells give rise to a columnar absorptive cell lineage, from hereon called enterocytes, which account for >90% of all villous cells in rats (for other cells see (Kurtz et al., 2020)) The enterocytes change their structure and function during migration toward the villus tip, where they slough off. The fastest cell migrations measured so far in mammals are 24 h in laboratory rats and in birds 48-72 h in hatchlings (Starck, 2003). The continuous flow of cells from villus crypt to tip allows phenotypically flexible responses but also creates high turnover and likely high maintenance costs for the intestine, which have been estimated to be 20-30% of basal metabolism and 28-46% of whole body protein synthesis (Starck, 2003).

The villi enlarge surface area, and the surface enlargement factor (SEF) for villi is greater in bats than in nonflying mammals (Fig. 1B) because bats have taller villi that are thinner and are spaced more closely together (crypts between them are narrower) (Brun et al., 2019b). Birds appear also to exceed mammals in villous SEF (Fig. 1B). Larger SEF in bats and birds may largely compensate for their smaller NSA, so that villous surface area of bats and birds becomes similar to that in nonflying mammals (Fig. 1C). Additionally, because the diameter of enterocytes lining the villi do not seem to differ between the taxa or with body mass (Fig. 1D),

[1] Transport of essential nutrients may also be upmodulated in nutrient deficiency Karasov, W.H., Douglas, A.E., 2013. Comparative digestive physiology. Comprehensive Physiology 3, 741-783.

bats also exceed rodents in number of enterocytes/cm^2 NSA and thus more cell-cell junctions per cm^2 NSA (Brun et al., 2019b), a factor of significance when considering the capacity for paracellular absorption.

Microvilli, cylindrical membrane projections from the apical membrane of enterocytes, often referred to as the brush border membrane (BBM), further increase surface area 20-80X above that of the villous surface area. This microvillous SEF does not seem to vary among the taxa or with body size (Fig. 1D).

Part II – The Players

The BBM of enterocytes is populated with a diversity of enzymes, transporters and channels that perform the final stages of intestinal digestion and absorption of carbohydrates and proteins (Fig. 2A). Semi-quantitative proteomic analysis of the key players can be achieved using shotgun mass spectrometry (McConnell et al., 2011). Proteins are categorized into functional groups by gene ontology annotation using data bases such as the Human Protein Atlas (http://www.protein-atlas.org/;(McConnell et al., 2011)). Relative abundances of all BBM peptidases are consistently greater than all the BBM amino acid and peptide transporters, by as much as 10X (Fig. 2B). Likewise, the relative abundances of all BBM α-glucosidases are at least 4-8 times more abundant than the BBM sugar transporters (Fig. 2C). As more species are studied this might be an interesting pattern to interpret in terms of ideas about capacity matching in series systems (Diamond, 2002). The most abundant hydrolases in the BBM are the enzymes historically measured by intestinal physiologists – SI and or MGAM (34 - 49% of all BBM hydrolases) and Aminopeptidase-N (APN; 12 - 18 %) (Brun et al., 2019a; McConnell et al., 2011). The most abundant of the BBM transporters is the Na^+/D-glucose cotransporter (SGLT1) in mice (McConnell et al., 2011) and in all species we studied (Brun et al., 2019a).

Based on phylogenetic analysis of 129 vertebrates, it appears that all vertebrates have a single gene encoding the α-glucosidase that is orthologous to mammalian sucrase-isomaltase (*SI*), but there have been multiple independent duplications of this, of which maltase-glucoamylase (*MGAM*) is one example that is present in all mammalian genomes surveyed. Other duplications occurred in some fish lineages, and chickens have an avian *SI* duplicate referred to as *ADAG* (avian derived α-glucosidase) (Brun et al., 2020b). The songbirds we surveyed lacked *ADAG*, but some appear to have it (Brun et al., 2020b). Consequently, in the proteomic survey there are two α-glucosidases in rodents and chickens, but only SI in the songbird (Fig. 2D). The relative abundance of SI is greater in the songbird than the rodents and chicken (Fig. 2D). Is this because the capacity for hydrolyzing maltose during starch digestion is based entirely on this single enzyme in the songbird rather than the sum of two enzymes with

perhaps complementary kinetic characteristics in the other three species? Overall, comparative genomic and proteomic analysis of the BBM has only just begun, but studies like this foretell how these tools can incorporate evolutionary thinking to help solve outstanding questions and also raise and inform new studies of mechanism.

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Part III – The Action: Achieving the Match

The hypothesis of evolutionary economic design assumes that there is natural selection for a match between both digestive and absorptive capacity of the vertebrate small intestine and nutrient load (Diamond, 2002) so that ingested nutrients are not wasted in excreta due to insufficient digestive capacity, and so that membrane space and/or expenditures building and maintaining the intestinal machinery to hydrolyze and absorb substrates are not wasted when substrate levels are low. Hence, digestive capacities should cover their corresponding nutrient loads plus a safety margin, neatly captured in the motto "enough but not too much" (Diamond, 1991). A wide variety of studies indicate that the majority of species studied have quite modest immediate spare capacities (range 9– 50%) (Karasov and Douglas, 2013).

A positive relationship is often observed between dietary intake of macronutrients and intestinal activity of the enzymes and transporters of their respective constituents (Fig. 3). In documenting the relationship for sugar and amino acid transport, it was useful for several reasons to analyze the patterns using the ratio of glucose:proline, defined as the intestine's summed mediated uptake capacity for glucose divided by that for the amino acid proline (Karasov and Diamond, 1988). Analogously, ratios of intestinal α-glucosidase activity:peptidase activity correlate positively with dietary carbohydrate content (Fig. 3).

Our understanding of genetic and molecular mechanisms accounting for the phenotypic variation in activity is sparse. Variation in gene copy number may be one mechanism. For example, in a phylogenetically informed analysis of more than 40 mammalian species in multiple branches of mammalian phylogeny the copy number of pancreatic amylase (*AMY2*) and salivary amylase (*AMY1*) was positively correlated with relative starch consumption (Pajic et al., 2019). The extent to which copy number variation explains variation in phenotypic amylase activity is not clear because correlations may be low (Carpenter et al., 2017) and the tissue of expression and perhaps the exact function of additional copies in many species is yet to be determined (Pajic et al., 2019). Perhaps the gene duplications of intestinal α-glucosidases (Brun et al., 2020b) might prove to provide somewhat of an analog to that mode of molecular adaptation. A second mode is changes in nucleotides within genes coding for digestive proteins that alter their functionality. Emerging examples of this include the functional loss of intestinal

trehalase activity, *i.e.,* pseudogenization of the *Treh* gene, in noninsectivorous species among bats and some mammalian lineages (Jiao et al., 2019), and functional loss of intestinal sucrase activity in the *SI* gene of some birds (Brun et al., 2020b). A third mode is differences in expression of the gene that result in differences in functional copies of its coded protein. In humans, differences or changes in lactase expression occur in the context of stable lactase (*LCT*) gene sequence and copy number, and so changes in LCT abundance and lactase activity are thought to be regulated at the transcriptional level (i.e., regulation of mRNA level) and/or in post-translational processing (Leseva et al., 2018). Linear regression analysis showed that DNA methylation at both the *LCT* promoter and enhancer correlates inversely with LCT mRNA and lactase activity (Leseva et al., 2018).

Digestive enzyme flexibility in response to changes in diet macronutrient level is widespread in birds and mammals (Karasov and Douglas, 2013) and the underlying mechanism may be transcriptional control of enzyme abundance in the BBM. When rodents are switched to higher carbohydrate diet, the activities of intestinal SI and MGAM increase within 6-12 h, preceded by rapid increase in enzyme transcription followed by translation and translocation to the BBM (Mochizuki et al., 2010). We performed similar dietary switching experiments in nestling house sparrows (*Passer domesticus*) which switch naturally from low starch insect diet to higher starch seed diet and in whom SI is responsible for all maltase and sucrase intestinal activities. Within 24 hours of a switch to higher starch diet, SI mRNA, as well as abundance and activity in whole tissues and in BBM, were specifically increased (Fig. 4A,C,E,F). Twenty-four hours after a reverse switch back to the lower starch diet, both SI mRNA and SI activity were decreased (not shown). Analogous studies with the nestlings indicates the same for flexible matching of intestinal APN activity to dietary protein level (Fig. 4B,D). But, enzymatic flexibility is certainly not universal as, for example, flexibility in SI may be absent in species or life-stages in which the birds are dietary specialists (Gatica-Sosa et al., 2018). In rats, increased MGAM mRNA transcription on high starch diet seems regulated through histone acetylation and binding of several cofactors in the promoter/enhancer and transcriptional regions of *MGAM* (Mochizuki et al., 2010). The extent to which underlying mechanisms are similar in birds will be exciting to explore.

For nutrient absorption, flexible phenotypic matching of absorption rate to dietary substrate level is afforded by multiple mechanisms (Ferraris, 2001; Ferraris et al., 2018). In rodents, uptake of glucose and amino acids increases within 24 h of a switch to a high carbohydrate, or high protein diet, respectively (Karasov and Douglas, 2013), mediated predominantly by increase in density of transporters in the BBM (Ferraris, 2001). In diet switch

experiments with rats, piglets and horses, elevated sugar and peptide transport was associated with elevated expression of the respective mRNA (reviewed in (Karasov and Douglas, 2013)). According to Ferraris (Ferraris, 2001), regulation of glucose transport by diet does not occur in mature villus cells but involves increased transcription of SGLT1 mainly in crypt cells. In contrast, modulation of fructose transport by its transporter GLUT5 (Ferraris et al., 2018), and peptide transport by Pept-1 (Karasov and Douglas, 2013), involves mRNA transcription and protein synthesis in mature cells lining the villus. Another cellular mechanism that may mediate rapid change in sugar transport is recruitment of preexisting GLUT2 and GLUT5 and their insertion into the BBM in response to increased luminal substrate, although the nutritional significance of this is debated for GLUT2 (Ferraris et al., 2018; Karasov, 2017).

In birds, induction of mediated glucose transport by higher dietary carbohydrate is rarely observed (Karasov and Douglas, 2013), and birds have lower mediated D-glucose absorption capacities than nonflying mammals (*c.f.* Fig. 5 in (Price et al., 2015)). But another way to achieve the match might be greater reliance on passive absorption, whose rate is linearly correlated (i.e., matched) with glucose concentration at the BBM. Birds and bats have less intestinal nominal NSA than nonflying mammals (Fig. 1), and reliance on a passive paracellular absorptive path between adjacent enterocytes (Fig. 2A) can help compensate for lower mediated absorption (Fig. 5). The major barrier to solute flux by this paracellular pathway is the tight junction (TJ), composed of membrane spanning proteins including occludin and claudins that form charge- and size-selective TJ pores for molecules (Karasov, 2017). In experiments with intact animals fed paracellular permeability probes (Fig. 5A), fractional absorption, *f*, declines with increasing molecule size (Fig. 5B), as expected for sieving through effective pores. For probe molecules in the size range of sugars and amino acids, *f* is about 3 times higher in flying birds and bats than in nonflying mammals (Fig. 5B).

The differences in *f* among intact animals can be demonstrated at the tissue level using *in situ* recirculating intestinal perfusions (Fig; 5C). Both the whole-animal and *in situ* measurements indicate that bats and birds have higher small intestinal permeability to hydrophilic organic solutes than do nonflying mammals, and the simultaneous measurements of absorption of both the paracellular probes and glucose indicate that the majority of glucose is absorbed passively in bats (Fig. 5C) and in small birds (5A and (Garro et al., 2018)).

We think that the aforementioned higher villous area per cm^2 NSA in bats and birds compared to rodents (Fig. 1B), which likely corresponds to more cell-cell junctions in fliers, partly explains the higher clearance of passive probe molecules in the fliers (Fig 5D). Could differences in the claudin protein makeup of the TJ be a causative factor for differences in

intestinal paracellular permeability between flying and non-flying vertebrates (Brun et al., 2019b; Karasov, 2017)? Could the study of the cellular and subcellular details of paracellular absorption in general be advanced by the study of species such as bats and birds with relatively high paracellular absorption, in accordance with August Krogh's dictum (Krogh, 1929)?

Epilogue

Animals' structural, functional, and molecular modes of matching digestive capacity to energy and nutrient flow, as focused on here, are but a part of the adaptation of the entire gastrointestinal tract and its resident microbes (see also (Kurtz et al., 2020)). These topics will remain key components in the future, broader study of nutritional ecology and physiology.

Acknowledgments

We salute our many collaborators as well as colleagues whose work we were unable to cite due to space limitations. Financial support over three decades came most notably from the U.S. National Science Foundation to both authors, the Argentinian Agencia Nacional de Promoción Científica y Tecnológica and National Scientific and Technical Research Council, and our respective universities (Department of Forest and Wildlife Ecology at the University of Wisconsin-Madison and Department of Biology of Universidad Nacional de San Luis). Funding agencies had no role in study design, analysis or interpretation of the data, or writing and submitting the article.

Figure 1. In mammals and birds over a range of body sizes, the main differences in intestinal morphometry are lower nominal surface area and higher villous surface area enlargement in fliers. (A) Small intestine nominal (smooth bore tube) surface area (NSA), the product of its length and circumference (inset figure), increases with body mass in mammals and birds with similar allometric slope on a log-log plot (common slope = 0.73). The body size range considered here is up to ~10 kg, considering the size range of most flying birds and bats. NSA is about 35-40% lower in the flyers (Brun et al., 2019b; Lavin et al., 2008). Regression plot adapted from (Price et al., 2015). **(B)** Villi, finger-like projections into the intestinal lumen that are covered with enterocytes, further increase surface area (SA). Stereological methods are used to determine how, and to what extent, villi increase surface area, through the calculation of villous surface enlargement factor (SEF), defined as villous SA/NSA. For illustration purposes the outline of villi relative to that of intestine circumference is shown, but SEF more typically factors in measures of villi heights, widths, and density (or indices thereof) (Brun et al., 2019b). Phylogenetically informed comparative analyses found that SEF was independent of body mass in mammals and birds and higher in flyers than nonflying mammals (Brun et al., 2019b)(Lavin et al., 2008) (higher by 59% and 44% for bats and birds, respectively, in data shown here). **(C)**

Taking into account the larger villous SEF in the flyers, the difference between flyers and nonfliers that was apparent for NSA disappears for villous SA (Brun et al., 2019b). **(D)** The majority of cells covering the villi are enterocytes (shown as an inset in D), and their diameter (clustered near or below 10 μ) does not seem to vary much with body size or taxonomic group (data from (Brun et al., 2019b; Lavin, 2007; Mitjans et al., 1997)). Microvilli, cylindrical membrane projections at the top of enterocytes, further increase SA 20-80X. Microvillous SEF, defined as microvillous SA/villous SA, also does not seem to vary much with body size or taxonomic group based on limited data available (points clustered near or above 20X). Figures 1B,C,D rely on data in (Brun et al., 2019b; Lavin, 2007; Lavin et al., 2008; Mitjans et al., 1997).

Figure 2. In the intestine's BBM, hydrolases are more abundant than transporters in mammals and birds, and there are differences among the groups in abundance of certain hydrolases and possibly in transporters. (A) Cartoon illustrating two adjacent enterocytes and some mechanisms by which carbohydrates and proteins are digested and absorbed, adapted from (Price et al., 2015). Substrates are hydrolyzed by a variety of brush-border enzymes (e.g., SI, MGAM, APN). Water-soluble monomers can move across the epithelium via transporters in the brush border [e.g., SGLT1, fructose transporter (GLUT5), peptide transporter (PEPT1) & amino acid transporters] but also through the tight junction (composed of a number of interacting protein strands such as claudins, occludin) to the lateral intracellular space (LIS) via diffusion or solvent drag (bulk movement along with absorbed water). Na^+ ions, which enter enterocytes down their gradient, are expelled from within the cell by Na^+-K^+-ATPase in the basolateral membrane (the exact stoichiometries of solute fluxes are not depicted). GLUT2 is a pathway for glucose to leave the enterocyte at the basolateral membrane. Lipophilic compounds, such as fatty acids and lipophilic xenobiotic chemicals, can diffuse into and across the cell phospholipid bilayer membranes. The numbers of symbols is not meant to reflect relative abundances. **(B)** In mammal and bird species we studied (n=4 individuals/species), 15 μg of BBM protein yielded, through shotgun mass spectrometry, 13,666±204 mass spectra (P>0.3 for difference among species) that corresponded, via comparison to species-specific databases (e.g., UniProt [https://www.uniprot.org/proteomes/] and RefSeq [https://www.ncbi.nlm.nih.gov/refseq/]), to ~640 discrete proteins (Brun et al., 2019a; Brun et al., 2020b). Semi-quantitative analysis of proteomic data is achieved through comparisons of protein specific spectra normalized to total protein spectra within and among specific protein categories (McConnell et al., 2011). In this comparison across 2 mammalian and 2 avian species, relative abundances of all BBM peptidases are consistently greater than the relative abundances of all the BBM amino acid and peptide transporters (& channels), by as much as

10X (Brun et al., 2019a). (**C)** In this comparison in the same animals as in B, relative abundances of all BBM carbohydrases are consistently greater than the relative abundances of all the BBM sugar transporters (& channels), by as much as 10X (Brun et al., 2019a). Spectra for GLUT transporters (e.g., GLUT5 and GLUT2) were below the necessary threshold or not found, perhaps because detectability follows their insertion into the BBM that may occur immediately following a meal ((Ferraris et al., 2018). (**D**) The α-glucosidases accounted for ~0.4 (i.e., 39.4 $\pm$ 3.4%, n=4 species) of the hydrolases in whole BBMs (Brun et al., 2020b). As predicted by genomic analysis, SI and MGAM were present in *M. musculus* and *R. norvegicus*, and SI and ADAG in *G. gallus,* but only SI and, not ADAG, was identified in the songbird *T. guttata*. The relative abundance of SI in the songbird *T. guttata* (0.40 $\pm$ 0.02) respectively) was roughly twice as high as that measured in *M. musculus* (0.20$\pm$ 0.02), *R. norvegicus* (0.23$\pm$ 0.01), and *G. gallus* (0.26$\pm$0.02); (n=4 individuals/ species) (Brun et al., 2020b). Within C and D, different lower case letters above the bars indicate significant differences among species by 1-way ANOVA.

Figure 3. Relative hydrolase activity is matched to diet macronutrient levels across multiple species of birds and mammals. (**A**) Relative rates of hydrolysis of maltose and peptide in wild-caught birds that eat different diets. The ordinate is the ratio of the intestine's hydrolytic capacity for maltose to that for l-alanine-p-nitroanilide, which is a substrate for amino-peptidase-N. This index of relative investment in starch vs. protein hydrolysis is convenient because (i) it bypasses the question of how to normalize tissue-specific hydrolysis rate (i.e., to intestine mass, area, protein content), and (ii) it permits comparisons across species of different mass because the ratio is relatively independent of body mass (e.g., Fig. 2 in (Karasov and Diamond, 1988)). Remarkably, the positive Model II regressions for each data set do not differ significantly (P>0.35), despite the fact that the data sets are from different research groups on birds in different continents (Kohl et al., 2011; Ramirez-Otarola et al., 2011), or from a data set ("Several studies") composed of many single studies performed by many investigators (McWhorter et al., 2009). The abscissa values for diet macronutrient composition were calculated by us based on the diet food descriptor for each species (e.g., insectivore, granivore, omnivore) and average nutritional values for each food type (Karasov and Martínez del Rio, 2007). (**B**) Relative rates of hydrolysis of sucrose and peptide in wild-caught bats, from (Schondube et al., 2001). Because the bats were mainly frugivores, nectarivores and insectivores we used the intestine's hydrolytic capacity for sucrose to calculate the value on the ordinate. For a different approach for characterizing dietary macronutrient levels on the abscissa, we used the reported data on tissue δ^{15}N, which indexes trophic level (Karasov and Martínez

del Rio, 2007) and was also used successfully by (Ramirez-Otarola et al., 2011). The resulting significant correlation is negative because $\delta^{15}N$ is highest in insectivores and lowest in nectarivores (Karasov and Martínez del Rio, 2007). The correlation was also significant and negative when we used maltase activity and calculated diet composition using the same methods as for the birds in A. (**C**, **inset**) Most of the variation in the ratio for bats (and the birds; data not shown) arises from species differences in carbohydrate rather than protein processing, as shown by the lack of correlation for APN activity in the bats. It has been suggested that this is because carbohydrate is a nonessential macronutrient whereas protein is required by all animals and all maintain a substantial capacity to assimilate it (Karasov and Diamond, 1988). Three of the four comparative analyses in both A and B were also phylogenetically informed and the patterns were not considered to be simply legacies of common descent within each feeding type (Kohl et al., 2011; Ramirez-Otarola et al., 2011; Schondube et al., 2001).

Figure 4. Rapid adjustment by juvenile house sparrows to high dietary carbohydrate and protein involves specific increase in hydrolase activity and abundance in whole tissues and in isolated BBM and increased mRNA in isolated enterocytes. (**A**) Within 24-hours of a diet switch from the low starch-high protein P diet (59.5% w/w/ casein, 5% corn starch, 8% corn oil, 10.5% other essential nutrients) to the high starch-lower protein S diet (respectively, 26.5%, 38%,8%, 10.5%), house sparrow nestlings nearly doubled maltase activity, and it remained high for days as they continued to feed on the higher starch S diet, in comparison to nestlings that remained on the low starch P diet. Filled orange circles and orange line indicates nestlings that were switched to S diet, and filled brown circles and brown line indicates nestlings that remained on the P diet for the number of days indicated on the abscissa. The maltase activity was calculated as activity of the entire small intestine/mass of entire small intestine. Values are means±s.e. (*n*=10–13 nestlings in each treatment group). Different letters denote significant differences in enzyme activity between treatment groups (one-way ANOVA, followed by Tukey HSD, across the 5 treatment groups. Adapted from (Rott et al., 2017). (**B**) In an analogous experiment with other nestlings that used the same diets, within 24-hours of a diet switch from the lower protein S diet to the higher protein P diet, house sparrow nestlings increased APN activity, and it remained high for days as they continued to feed on the higher protein P diet, in comparison to nestlings that remained on the lower protein S diet (*n*=10-13 nestlings per diet group). The dietary-induced upward modulation of both maltase and APN was reversible. Adapted from (Rott et al., 2017). (**C & D**) In other experiments on nestlings using the same diets, maltase and APN activity were measured in isolated BBM, and the respective hydrolase mRNA was measured in isolated enterocytes. Both SI mRNA and BBM maltase activity, which

is entirely accounted for by SI (Brun et al., 2020b), were elevated significantly (P<0.05, indicated by asterisk) in nestlings 24 hours following the switch to higher starch diet S in comparison to same-age nestlings that remained on the lower starch P diet (C). Samples sizes were 7-10 nestlings per group. Both APN mRNA and BBM APN activity were elevated in nestlings 24 hours following the switch to higher protein P diet in comparison to same-age nestlings that remained on the lower protein S diet (D). Adapted from (Brun et al., 2020a). (**E** & **F**) Proteomic analysis of BBM of nestlings eating diet S or diet P for one day showed that the former group had significantly higher relative abundance of SI (calculated as SI spectra relative to total hydrolase spectra in isolated BBM) (0.56 ± 0.19, n=7 nestlings) than the latter group (0.23 ± 0.13, n=6) (P=0.002), and that SI abundance and maltase activity in BBM of the 13 nestlings were correlated (Model II regression P<0.001) (F). Proteomic analysis of BBM also showed that the starch-induced increase in SI abundance was specific for SI, because comparisons by diet of relative abundances of 10 other BBM hydrolases were nonsignificant (E) Adapted from (Brun et al., 2020a); the acronyms of the BBM hydrolases are defined in (Brun et al., 2020b).

Figure 5. For intestinal absorption of sugars and amino acids, small fliers rely on a paracellular pathway to a greater extent than do nonflying mammals. (**A**) Paracellular absorption is studied using passive permeability probe molecules - hydrophilic, metabolically inert, organic molecules in the MW size range of amino acids and sugars (approximately 75–180 Da; effective molecular radius 3–4 Å) that in validation experiments with birds and mammals do not interact with intestinal transporters. Examples here include L-glucose, L-arabinose, and L-rhamnose, from (Karasov, 2017). They are administered simultaneously with hydrophilic nutrients, or their nonmetabolizable analogues (e.g., 3-O-methyl-D-glucose (3OMD-glucose) here), that are absorbed via intestinal transporters as well as passively. In these experiments with intact animals, the amounts absorbed into blood were measured as a function of time since oral administration, and simple pharmacokinetic models were used to calculate the fractional absorption, f, values of which range between 0 (no absorption) and 1 (complete absorption). The fractions absorbed into blood typically rise rapidly to a plateau in 60–100 min for both probes; there is no particular long lag for the paracellular probes. For paracellular probes, both f and apparent rates at early time points were higher in the fliers than in nonfliers. Adapted from (Karasov, 2017). (**B**) In the experiments with intact animals, fractional absorption of the passively absorbed probes declined with increasing molecule size, as expected for sieving through effective pores in the TJs (Karasov and Douglas, 2013). For the passive probe molecules in the size range of sugars and amino acids, f is about 3 times higher in flying birds

and bats (0.74±0.05, s.e.m) than in nonflying mammals (0.25±0.05) (Price et al., 2015). Shown are box plots of data from (Price et al., 2015) based on samples sizes indicated in the inset legend. (**C**) In experiments by Brun et al. (Brun et al., 2014) using *in vivo* recirculating intestinal perfusions, clearance of L-arabinose at 10mM was about 5X higher in bat species (13.0±2.1 μL min^{-1} cm^{-2} NSA; *n*=3 species, each with measurements in 5-6 individuals) than in rodent species (2.4±0.9, *n*=3 species, each with measurements in 6-7 individuals) (*P*<0.001). Simultaneously in these experiments, D-glucose absorption rate at 10 mM did not differ between bats (85.3±12.7 nmol min^{-1} cm^{-2} NSA) and rodents (109.7±9.3) (*P*=0.07), but the calculated percentages of glucose absorbed passively was about 4 times higher in the bats (113±6%, not different from unity) than in the rodents (31±2%) (*P*<0.001). Species codes are: 1Cp=*Carollia perspicillata*, 2Sl-*Sturnira lilium*, 3Al=*Artibeus lituratus*, 4Mm=*Mus musculus*, 5Ah=*Akodon montensis*, 6Rv-*Rattus norvegicus*. All six species normally consume high carbohydrate diets. (**D**) In all species we studied using uniform *in vivo* recirculating intestinal perfusions, which includes 5 of the 6 species shown in 5C, clearance of L-arabinose correlated significantly with villous area per cm^2 NSA (i.e., villous SEF). Each point is the mean value for a species, and the solid line is the significant fit by Model II regression (*P*=0.0002), which was chosen because both *x* and *y* values were measured with experimental error. This figure is adapted from (Brun et al., 2019b) with data from two studies by A. Brun and colleagues using uniform methodology (Brun et al., 2014; Garro et al., 2018). The data are coded for the main dietary macronutrient (C = carbohydrate, P = protein) because both arabinose clearance and SEF tend to be lower in animals that eat mainly proteinaceous foods (insects, blood) (Price et al., 2015).

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

Graphical Review

Title: Adaptation of intestinal epithelial hydrolysis and absorption of dietary carbohydrate and protein in mammals and birds

William H. Karasov and Enrique Caviedes-Vidal

Fliers have lower intestinal nominal surface area but higher villous surface area enlargement than nonfliers
Consequently, fliers have more tight junctions which allow more paracellular absorption.
Mammals have 2 α-glucosidases, SI and MGAM, but many birds have only SI
Commonly, intestinal hydrolases & transporters correlate with their respective dietary macronutrient levels
Phenotypically, it's mediated by specific increase in activity and abundance of hydrolases in the BBM and increases in their mRNA

Surface area at the site of intestinal digestion and absorption

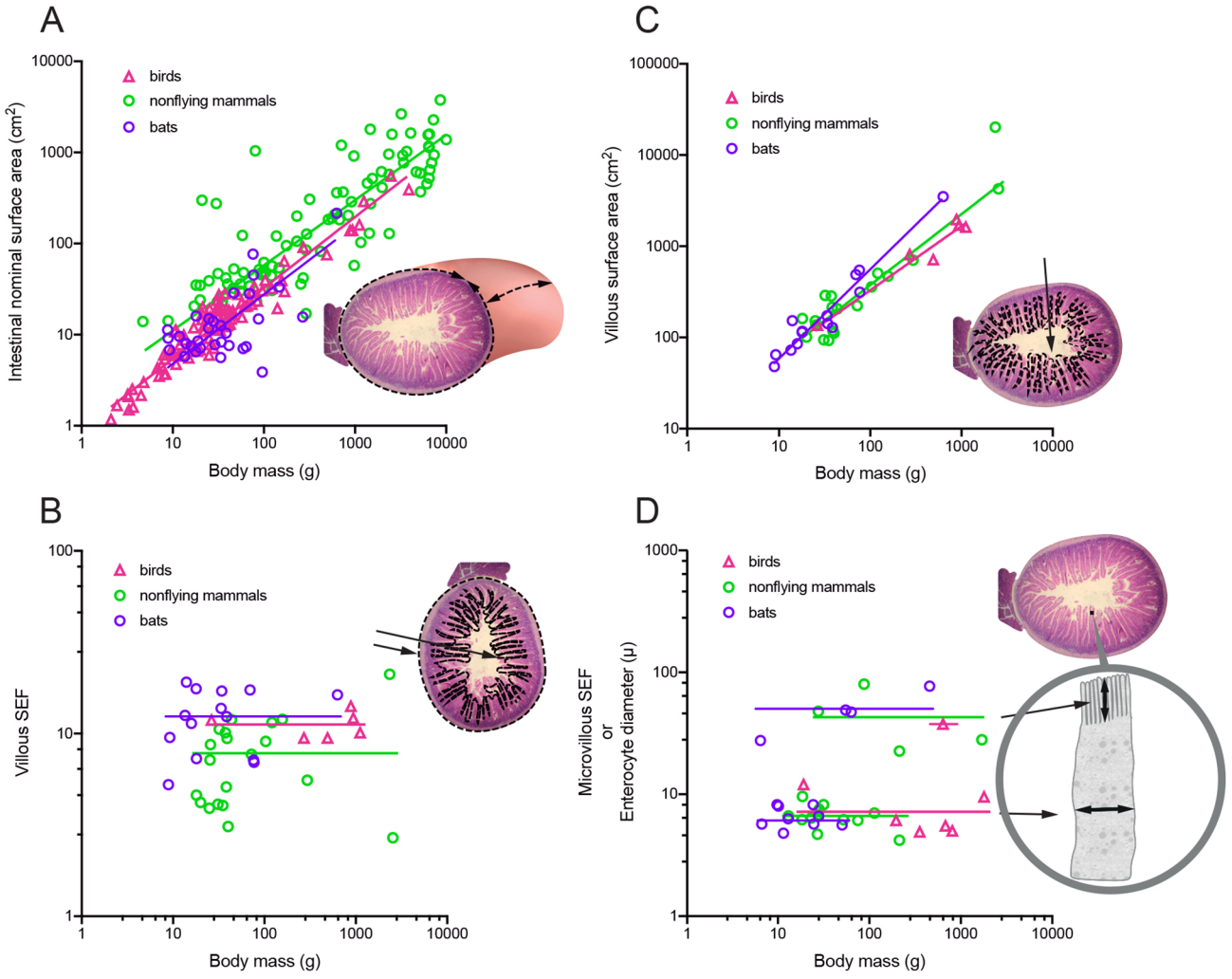
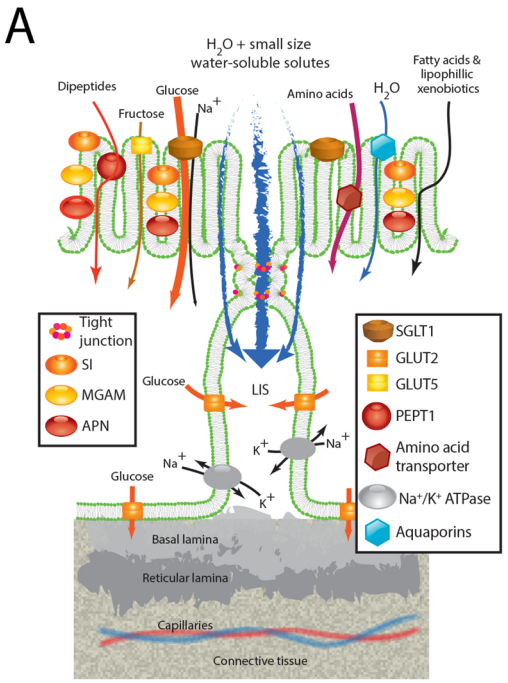
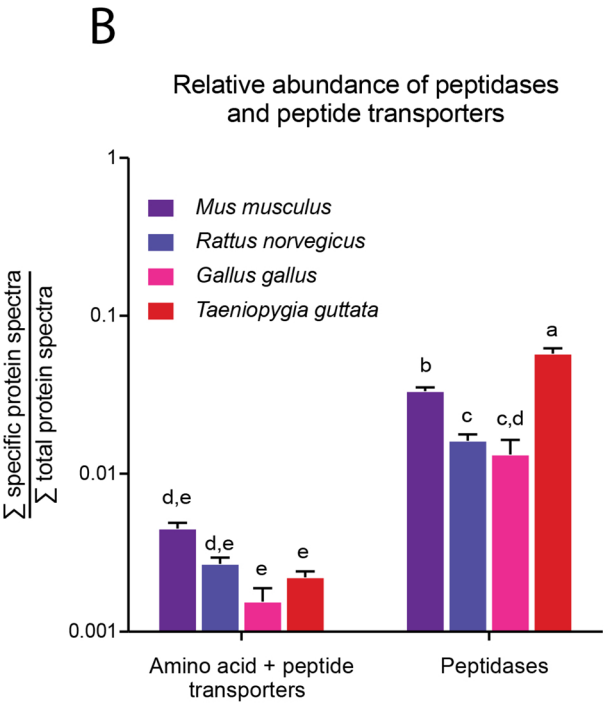
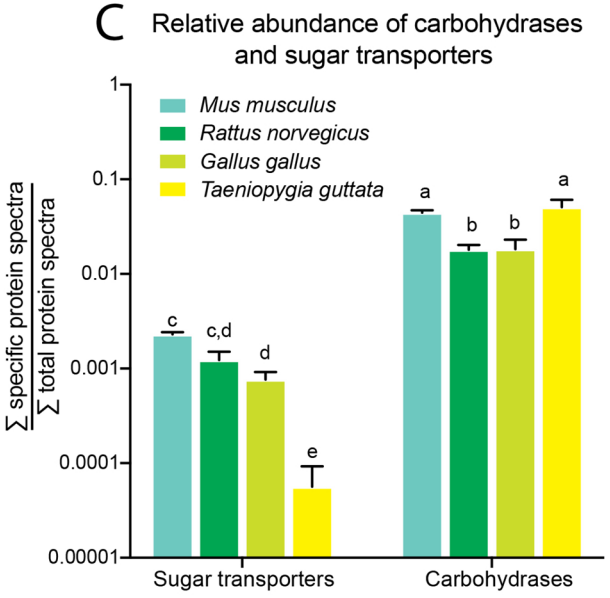


Figure 1


A
H2O + small size water-soluble solutes
Dipeptides
Glucose
Fructose
Na+
Amino acids
H2O
Fatty acids & lipophillic xenobiotics
Tight junction
SI
MGAM
APN
SGLT1
GLUT2
GLUT5
PEPT1
Amino acid transporter
Na+/K+ ATPase
Aquaporins
Glucose
LIS
Na+
K+
Glucose
Basal lamina
Reticular lamina
Capillaries
Connective tissue


B
Relative abundance of peptidases and peptide transporters
Mus musculus
Rattus norvegicus
Gallus gallus
Taeniopygia guttata
∑ specific protein spectra / ∑ total protein spectra
1
0.1
0.01
0.001
d,e
d,e
e
e
b
c
c,d
a
Amino acid + peptide transporters
Peptidases


C
Relative abundance of carbohydrases and sugar transporters
Mus musculus
Rattus norvegicus
Gallus gallus
Taeniopygia guttata
∑ specific protein spectra / ∑ total protein spectra
1
0.1
0.01
0.001
0.0001
0.00001
c
c,d
d
e
a
b
b
a
Sugar transporters
Carbohydrases

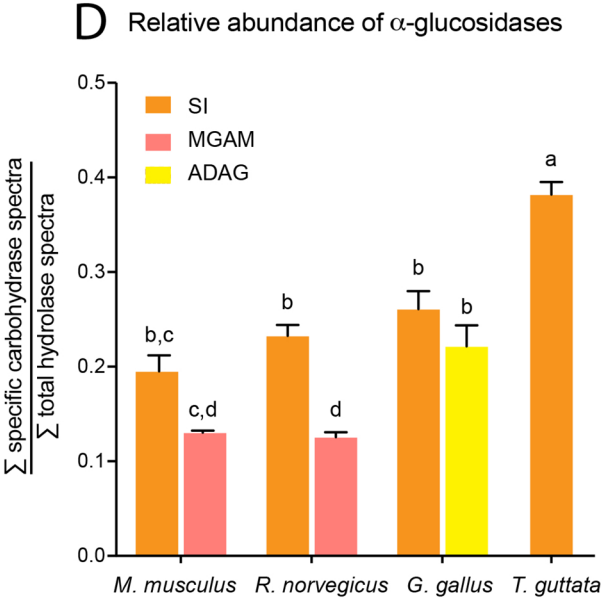

D
Relative abundance of α-glucosidases
SI
MGAM
ADAG
∑ specific carbohydrase spectra / ∑ total hydrolase spectra
0.5
0.4
0.3
0.2
0.1
0.0
b,c
c,d
b
d
b
b
a
M. musculus
R. norvegicus
G. gallus
T. guttata

Figure 2

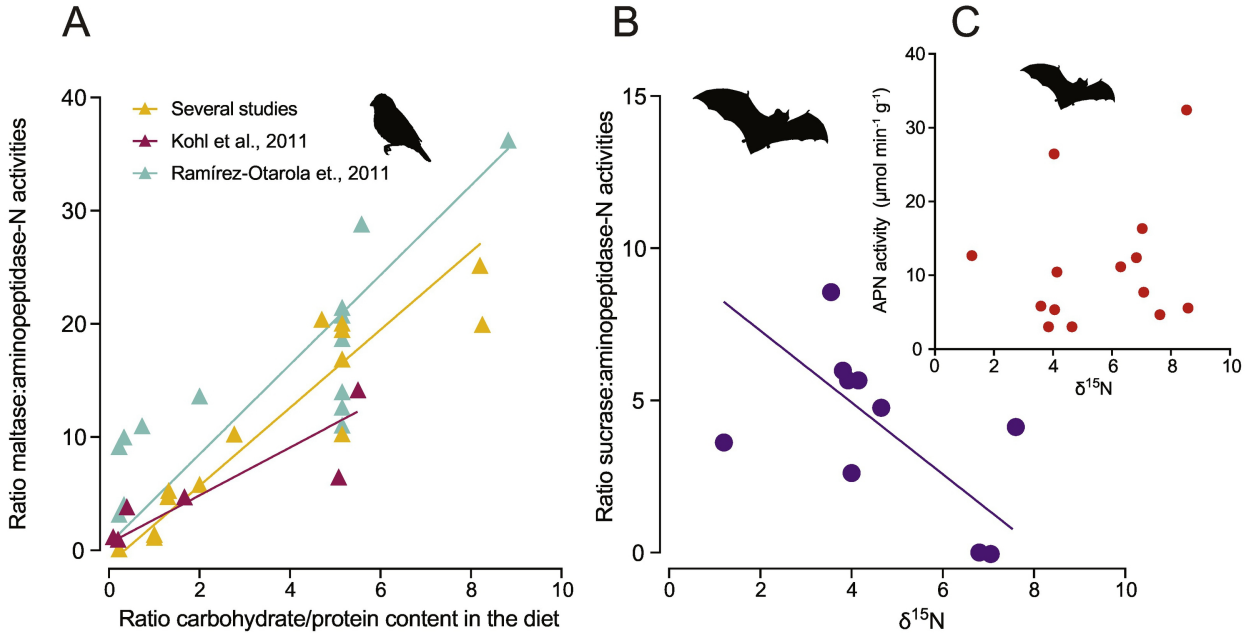

Relative hydrolase activity is matched to diet macronutrient level
A
B
C
Several studies
Kohl et al., 2011
Ramírez-Otarola et., 2011
Ratio maltase:aminopeptidase-N activities
Ratio carbohydrate/protein content in the diet
Ratio sucrase:aminopeptidase-N activities
δ15N
APN activity (μmol min-1 g-1)
δ15N

Figure 3

Cellular basis for the match of hydrolysis to diet macronutrient level

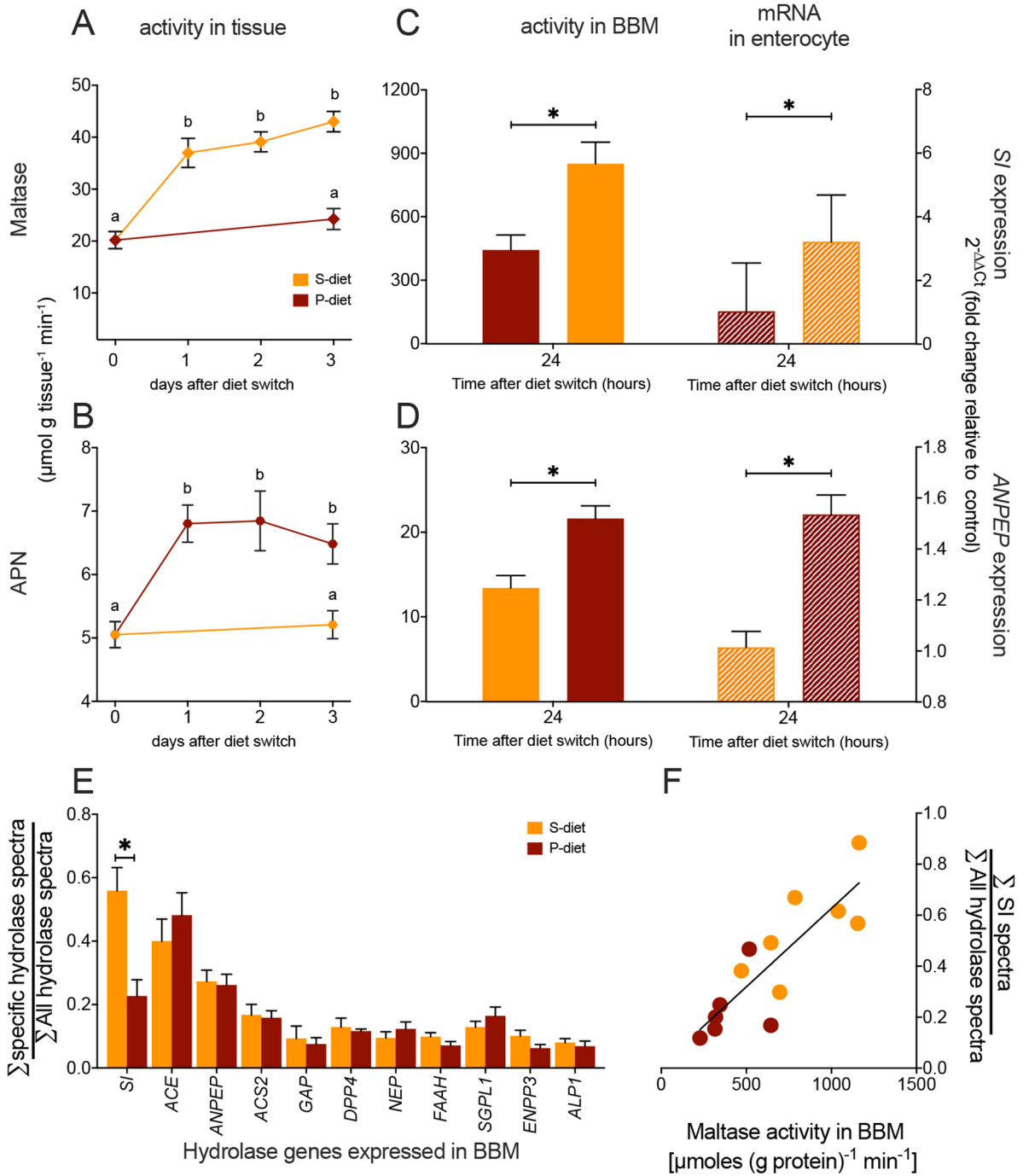


Figure 4

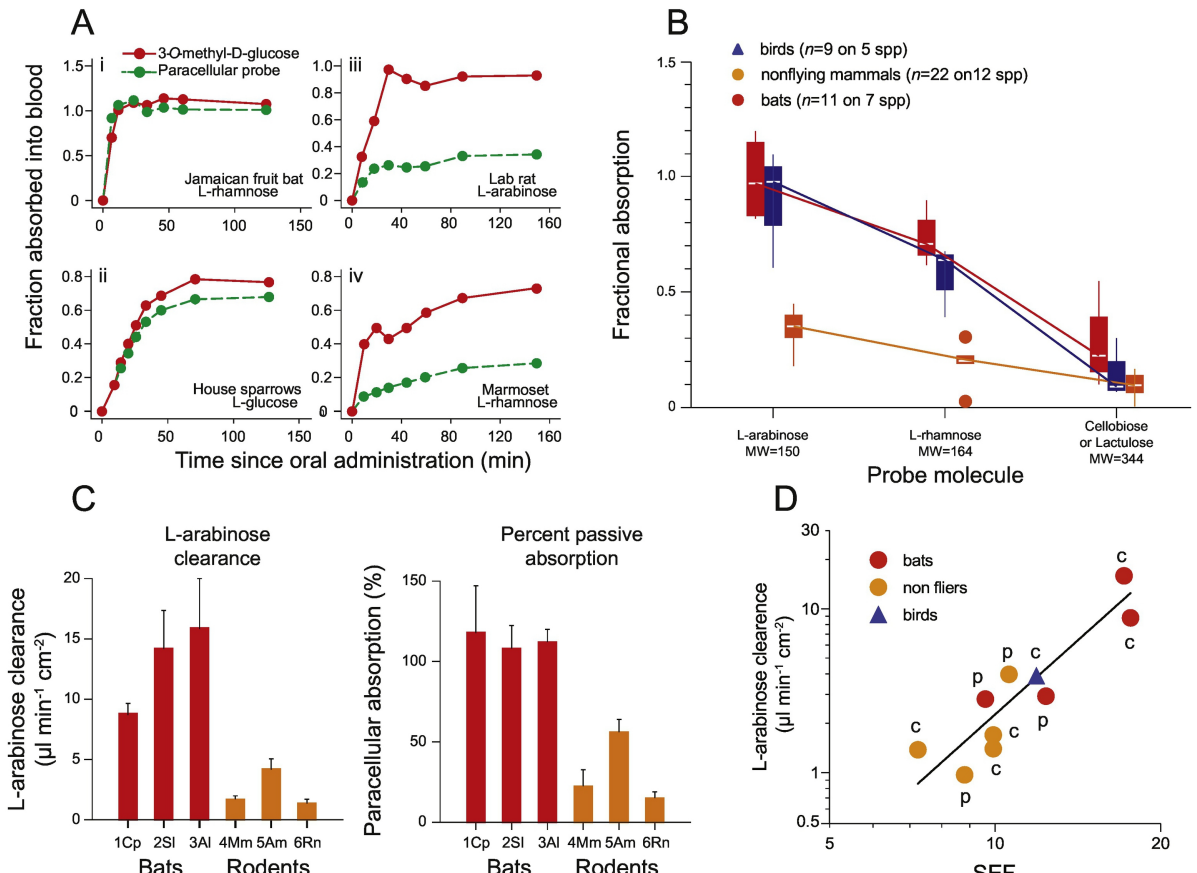

A
3-O-methyl-D-glucose
Paracellular probe
Fraction absorbed into blood
i
Jamaican fruit bat
L-rhamnose
iii
Lab rat
L-arabinose
ii
House sparrows
L-glucose
iv
Marmoset
L-rhamnose
Time since oral administration (min)
B
birds (n=9 on 5 spp)
nonflying mammals (n=22 on12 spp)
bats (n=11 on 7 spp)
Fractional absorption
L-arabinose MW=150
L-rhamnose MW=164
Cellobiose or Lactulose MW=344
Probe molecule
C
L-arabinose clearance
L-arabinose clearance (µl min-1 cm-2)
1Cp 2Sl 3Al 4Mm 5Am 6Rn
Bats
Rodents
Percent passive absorption
Paracellular absorption (%)
D
bats
non fliers
birds
L-arabinose clearence (µl min-1 cm-2)
SEF

Figure 5