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RESEARCH ARTICLE

Activity of intestinal carbohydrases responds to multiple dietary signals in nestling house sparrows

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SUMMARY

The 'adaptive modulation hypothesis' predicts that activity of digestive enzymes should match the amount of their substrates in diet. Interestingly, many passerine birds do not adjust the activity of intestinal carbohydrases to dietary carbohydrate content. It is difficult to assess the generality of this rule, because in some studies passerines fed on low-carbohydrate and high-lipid diet showed reduced activity of intestinal carbohydrases. However, as carbohydrase activity may be inhibited by high dietary lipid content, it is unclear whether observed effects reflected lack of induction by the low carbohydrate levels or suppression by the high lipid levels. Here, we isolated the specific effects of dietary carbohydrate and lipid on carbohydrases. We hand-fed house sparrow nestlings on diets with 25% starch and 8% lipid (diet HS), no starch and 20% lipid (HL), or 25% starch and 20% lipid (HSL). Our results show that activity of intestinal carbohydrases is simultaneously induced by dietary carbohydrates and decreased by dietary lipid, although the latter effect seems stronger. Activities of maltase and sucrase summed over the total intestine decreased in the order HS>HSL>HL. We observed a complex interaction between diet composition and intestinal position for mass-specific activity of these enzymes, suggesting site-specific responses to changes in digesta composition along the intestines caused by digestion and absorption. We re-interpret results of earlier studies and conclude that there is no unequivocal example of adaptive modulation of intestinal carbohydrases by dietary carbohydrate in adult passerine birds, whereas the present experiment confirms that nestlings of at least some species possess such capacity.

Key words: phenotypic flexibility, digestive enzymes, house sparrow, ecological physiology, ontogeny.

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INTRODUCTION

Morphology and physiology of the gastrointestinal tract show several adaptations to different food types, habitats and life strategies (reviewed in Karasov and Hume, 1997; Starck and Wang, 2005; Karasov and Martinez del Rio, 2007; Karasov et al., 2011). Presumably, any unnecessary spare digestive capacity involves costs of maintaining excess organs or enzymes. In contrast, insufficient digestive capacity could allow some energy and nutrients to escape the gastrointestinal tract unutilized. Thus, natural selection is expected to match capacity of the digestive system to animals' needs and conditions. This can be especially true for intestinal digestive enzymes that play a key role in food hydrolysis, but at the same time must share limited space in intestinal epithelium. The 'adaptive modulation hypothesis' predicts that the activity of digestive enzymes should be matched to relative levels of their dietary substrates (Karasov and Diamond, 1988).

The avian digestive system is characterized by high phenotypic flexibility to cope with high energetic costs and physical constraints of flight, rapid growth rate or long migration (reviewed in McWhorter et al., 2009). Many bird species have variable diet or show ontogenetic or seasonal switches in their food habits. Thus, it is reasonable to expect that birds should be able to adjust activity of their intestinal digestive enzymes to diet composition. However, the pattern that emerges from previous studies is that in adult passerine birds, there is an inter-specific correlation between diet composition and activity of intestinal carbohydrases, but not proteases (Kohl et al., 2011; Ramirez-Otarola et al., 2011), whereas on an intra-specific level adult passerines are able to adjust activity of intestinal proteases to diet composition, but not carbohydrases (reviewed in McWhorter et al., 2009). In contrast, adult galliforms and anseriforms seem able to modulate activity of their carbohydrases but usually do not modulate proteases (McWhorter et al., 2009).

Described patterns suggest the presence of fundamental, phylogenetic differences in the strategy for flexibility of intestinal enzyme activities in birds. However, two studies on passerine birds – adult pine warblers (*Dendroica pinus*) (Levey et al., 1999) and nestling house sparrows (*Passer domesticus*) (Brzęk et al., 2009; Brzęk et al., 2011) – found significant, adaptive modulation of maltase activity by dietary carbohydrates. This is particularly puzzling because neither adult yellow-rumped warblers (*Dendroica coronata*, a congener of the pine warbler) (Afik et al., 1995) nor adult house

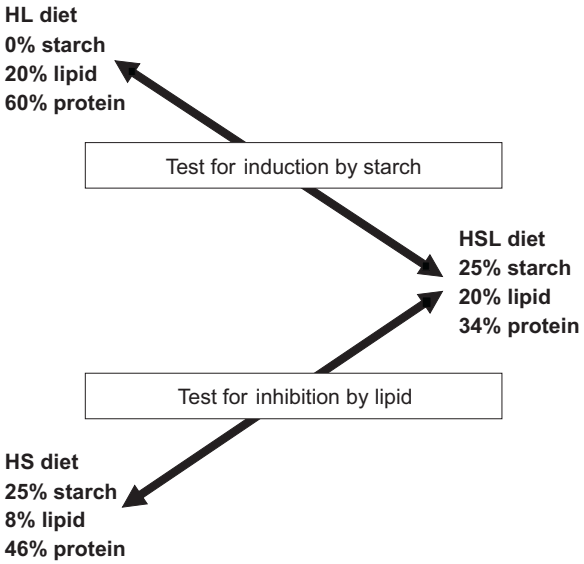

Fig. 1. Different experimental diets and predictions of the 'induction by starch' and 'inhibition by lipid' hypotheses.

sparrows (Caviedes-Vidal et al., 2000) show such flexibility. However, both mentioned studies manipulated the contents of both carbohydrate and lipids in the diet. In laboratory rodents, high levels of dietary lipids decrease activity of carbohydrases (McCarthy et al., 1980; Takase and Goda, 1990; Goda and Takase, 1994), and there is evidence of this in adult house sparrows (Caviedes-Vidal et al., 2000), but this effect has never been carefully tested in birds. Because of different dietary lipid content, the enzyme flexibility observed in adult pine warblers and young house sparrows could be explained by an increase in activity of carbohydrases by carbohydrates in birds fed on high carbohydrate/low lipid diet ('induction by starch'), by a decrease in activity of carbohydrases by lipids in birds fed on low carbohydrate/high lipid diet ('inhibition by lipid'), or by a combination of both mechanisms (compare Goda and Takase, 1994; Goda et al., 1995).

The goal of the present experiment was to isolate the specific effects of dietary carbohydrate and of dietary lipid on carbohydrase activities in nestlings of the house sparrow (*Passer domesticus* L.). Young house sparrows show a gradual increase in the consumption of high-starch dietary items (e.g. seeds) during ontogeny (Anderson, 2006), which is accompanied by a simultaneous increase in the activity of pancreatic and intestinal enzymes involved in the digestion of starch (Caviedes-Vidal and Karasov, 2001; Brzęk et al., 2009; Brzęk et al., 2011; Brzęk et al., 2013). We hand-fed young house sparrows under laboratory conditions on three diets: high-starch and low-lipid (hereafter referred to as HS), starch-free and high-lipid (HL), and a diet that combined the starch content of diet HS with the lipid content of diet HL (hereafter HSL). By comparing enzyme activity in house sparrow nestlings raised on these diets, we could distinguish between both hypotheses explaining the effect of high carbohydrate–low lipid diet on carbohydrases (Fig. 1). If activity of carbohydrases is induced by higher starch content, then we should observe that birds fed on diet HSL should have a higher activity of these enzymes than birds fed on diet HL with the same lipid content but without starch. However, if the activity of carbohydrases is reduced by high lipid content, we predict that birds fed on diet HSL should have lower activity of these enzymes than birds fed on diet HS with the same starch content but lower lipid content.

MATERIALS AND METHODS

Animal maintenance and experimental treatments

Two experimental groups used in the present experiment were part of the study described in our previous paper (Brzęk et al., 2011), and methods used in the present experiment are identical as those described there, which were in turn based on methods used in Brzęk et al. (Brzęk et al., 2009). Briefly, 3-day-old house sparrow nestlings (day 0=day of hatch) were collected in May–July 2008 from natural nests located on the campus of the University of Wisconsin, Madison, and housed individually in our laboratory. Nestlings were hand-fed using three synthetic diets. One group of nestlings was fed a starch-free and high-lipid diet (HL), intended to mimic insects consumed by very young house sparrows, and another group was fed a starch-containing diet (HS), intended to mimic the mixture of insects and seeds typical of older nestlings (see Table 1 for their compositions). These two groups are the same as nestlings dissected in day 12 in our previous study (Brzęk et al., 2011). HL and HS diets were referred to, respectively, as '0' and '+' in our previous papers (Brzęk et al., 2009; Brzęk et al., 2011), but in the present study we assigned them new abbreviations that better explain differences between diets. In the present experiment, we added a third group of nestlings that were raised together with the two others groups but were fed a diet that combined the starch content of the HS diet and lipid content of the HL diet (hereafter referred to as HSL; Table 1). To control for nest effect, when more than one nestling was collected from the same natural nest, individuals were randomly assigned to different experimental diets. The sample size was 9 in all three groups.

In comparison to previously described feeding techniques (Brzęk et al., 2009), hourly meal sizes offered to nestlings on days 3–5 were increased to 0.4, 0.55 and 0.65 g, respectively. Food intake did not differ between the three treatment groups over the entire experimental period (ANOVA, P=0.3).

When nestlings reached day 12, they were killed with CO_2, and dissected to remove and weigh the intestines, gizzard, liver, pancreas and pectoral muscles. Intestines were flushed with ice-cold avian

Table 1. Composition of diets used in the present study

	HL (% of dry mass)	HS (% of dry mass)	HSL (% of dry mass)
Corn starch	0	25.4	25.4
Casein (protein)	59.63	46.23	34.23
Corn oil	20	8	20
Alphacel non-nutritive bulk	4.9	4.9	4.9
Silica sand	5	5	5
Amino acids, vitamins, mineral salt, etc.*	10.47	10.47	10.47

HL, high-lipid and starch-free diet; HS, low-lipid and high-starch diet; HSL, high-lipid and high-starch diet. Diets were mixed with distilled water at a ratio by mass of 1:3 (diet:water).
*Content as described by Lepczyk et al. (Lepczyk et al., 1998).

Ringer's solution to clear them and cut into three sections, corresponding to the proximal, medial and distal regions of intestine, which were weighed again (each section separately) and immediately preserved in liquid nitrogen.

All experimental procedures were approved by the University of Wisconsin, Madison, ethics committee (permit no. RARC A-01269-4-10-06).

Intestinal enzyme assays

To test our predictions we quantified the rate of hydrolysis of maltose, sucrose and L-alanine-*p*-nitroanilide. Maltose hydrolysis (i.e. maltase activity) quantifies the combined activity of three intestinal brush-border membrane (BBM) enzymes against substrates of starch hydrolysis. Two of these enzymes – maltase, which hydrolyzes the disaccharide maltose, and glucoamylase, which hydrolyzes di-, tri- and tetra-saccharides (all with α-1,4 glucose-glucose bonds) – are coded for by the same gene complex *MGAM* (Quezada-Calvillo et al., 2007). The third enzyme, isomaltase, which hydrolyzes α-1,4 and α-1,6 glucose-glucose bonds, is coded for by the gene complex *SI* (sucrase-isomaltase) (Quezada-Calvillo et al., 2007). *SI* also codes for sucrase activity against the α-1,2 glucose-fructose bond of sucrose. Hydrolysis of L-alanine-*p*-nitroanilide quantifies the activity of the BBM enzyme aminopeptidase-N.

We used homogenates of intestinal tissue, which yield a measure with high repeatability of the activity of BBM enzymes. In mice, there is no significant difference between sucrase activity measured in tissue homogenates and in everted sleeves that isolate the measurement to the BBM (Lee et al., 1998). Thus, the homogenate likely gives a good indication of BBM activity, and there does not seem to be an overestimate due to a lot of other possible intracellular sources of activity. Also, another method involving isolation of BBM vesicles may result in an underestimation of enzymatic capacity because of inefficient recovery during the isolation process (Martinez del Rio, 1990).

Assays of intestinal digestive enzymes were performed as described in detail elsewhere (Brzęk et al., 2009), with two modifications: (1) intestinal samples analyzed in a previous study (Brzęk et al., 2009) represented only part of each intestinal section (proximal, medial and distal), but in the present experiment we cut intestines from each section longitudinally and used one half of each section for enzyme assays (the other was preserved for other assays), resulting in better analysis of enzyme activity over the whole intestine length; and (2) in addition to maltase and aminopeptidase-N, we also analyzed sucrase activity. The sucrase assay was identical to the maltase assay described in Brzęk et al. (Brzęk et al., 2009), except sucrose was substituted as the substrate.

We expressed mass-specific activity of enzymes in each intestinal section as micromoles of substrate processed per minute per gram of wet tissue. We then calculated the summed hydrolysis activity of the entire small intestine, which is an index of the total hydrolytic capacity, by multiplying mass-specific activity in the proximal, medial and distal intestinal regions by their respective masses, and summed over the three regions. Finally, we calculated the ratio of values found for maltase to that of aminopeptidase-N (hereafter M/A ratio) for both mass-specific and summed enzymatic activities. This ratio represents relative investment in carbohydrate- and protein-digesting enzymes and is not affected by potential differences in intestinal morphology or villus area between groups.

Data analysis

Data were analyzed by means of one-way ANOVA/ANCOVA, with diet treatment as the main factor. For organ size, initial and/or final body mass were included in the model as covariates when they were significant. Repeated-measures ANOVA was used to examine the effect of diet and intestinal position (proximal, medial, distal) on mass-specific enzyme activities. We inspected the distribution of tested variables and carried out Bartlett's and Levene's tests to check for homogeneity of variance. Mass-specific and summed activities of maltase, as well as values of the M/A ratio, were log-transformed to improve homogeneity of variance (however, non-transformed data are depicted in figures). All tests were carried out using SAS software (SAS Institute, Cary, NC, USA). In all tests, the significance level was set at $P<0.05$. We did not adjust our significance level for multiple comparisons, but relied mostly on the M/A ratio for interpretation of our results, which offers a single estimate of relative investment into carbohydrate and protein digestion intestinal enzymes.

RESULTS

Experimental groups did not differ in their initial body mass on day 3, the final body mass on day 12 or the size of any part of the digestive system (liver, pancreas, gizzard, intestines) or pectoral muscle measured on day 12 (for all variables, effect of diet, $P>0.05$).

Summed mass-specific enzyme activities

Diet treatment exerted a significant effect on the summed activities of maltase and sucrase, as well as on the M/A ratio of summed enzyme activities (Table 2, Fig. 2). For all these variables, the diet effect followed the same pattern: nestlings fed on the HS diet had higher values of studied parameters than those fed on the HL and HSL diets [least significant difference (LSD) test; for M/A ratio in HS *versus* HSL birds, $P=0.023$, for all other comparisons, $P<0.005$], and nestlings fed on the HSL diet had higher values than those fed on the HL diet (LSD test, $P<0.002$ for all comparisons). In contrast, summed activity of aminopeptidase-N was not significantly affected by dietary treatment (Table 2, Fig. 2C).

Mass-specific enzyme activity in different intestine sections

Repeated-measures ANOVA revealed the presence of a highly significant interaction between diet treatment and intestinal position for mass-specific activity of maltase, sucrase and M/A ratio (Table 3). Therefore, we analyzed the effect of diet separately for each section of intestine by means of one-way ANOVA (Table 2), followed by LSD test to identify statistical differences between diets.

Nestlings fed on the HS diet had significantly higher values of mass-specific activities of maltase, sucrase and the M/A ratio in the proximal and medial part of the intestines than nestlings fed on the two other diets ($P<0.015$ for all comparisons; Table 2, Fig. 3). There was no significant difference between the HSL and HL diets for these parameters in the proximal part of the intestines ($P>0.05$ for all comparisons). In the medial part of the intestines, nestlings fed on the HSL diet had higher mass-specific activity of maltase ($P=0.027$) but not sucrase ($P=0.12$), as well as higher values of the M/A ratio ($P=0.0006$) than those fed on the HL diet. Finally, the effect of dietary manipulation in distal section of intestines was very different from that observed in the proximal and medial sections, because nestlings fed on the HSL diet showed higher mass-specific activity of both disaccharidases and values of the M/A ratio than nestlings fed on the HS or HL diets ($P<0.0002$ for all comparisons; Table 2, Fig. 3). Nestlings fed on the HS and HL diets did not differ in mass-specific activities of maltase and sucrase in the distal section of the intestines ($P>0.5$ for both comparisons), but the HS-fed group had higher M/A ratios ($P=0.043$).

Table 2. Summary of results of ANOVA for the effect of dietary treatment on summed enzyme activity and mass-specific enzyme activity in different intestinal sections

	Maltase		Sucrase		aminopeptidase-N		M/A ratio	
	$F_{2,24}$	P	$F_{2,24}$	P	$F_{2,24}$	P	$F_{2,24}$	P
Summed activity	38.59	<0.0001	27.76	<0.0001	0.41	0.67	22.91	<0.0001
Proximal	10.39	0.0006	6.07	0.0074	0.81	0.46	10.05	0.0007
Medial	23.86	<0.0001	24.60	<0.0001	7.38	0.0032	22.82	<0.0001
Distal	17.64	<0.0001	16.63	<0.0001	2.62	0.093	31.99	<0.0001

M/A ratio, maltase/aminopeptidase-N ratio.

The interaction between diet treatment and intestinal position was not significant for mass-specific activity of aminopeptidase-N (Table 3). Nevertheless, we carried out separate one-way ANOVAs independently for each section of the intestines for comparison to trends observed in carbohydrases. Diet had a significant effect only in medial sections (Table 2, Fig. 3C): nestlings fed on the HSL diet had lower mass-specific activity of aminopeptidase-N than HL and HS nestlings (P<0.01 for both comparisons), whereas the latter two groups did not differ (P=0.5).

DISCUSSION

Patterns of the effect of diet components on activities of intestinal enzymes

Diet composition significantly modulated the activity of intestinal carbohydrases in studied house sparrow nestlings. Summed activities of maltase and sucrase, as well as values of the M/A ratio calculated for summed activities, changed in the order HS>HSL>HL (Table 2, Fig. 2). These results confirm predictions of both the 'induction by starch' and 'inhibition by lipid' hypotheses (see Fig. 1). However, a highly significant interaction between dietary treatment and intestinal position for mass-specific activities of disaccharidases and the M/A ratio suggests that the effect of different diet components on studied parameters changed in a proximal-to-distal gradient (Table 3, Fig. 3).

In the proximal section of the intestines, nestlings fed on the HS diet had significantly higher mass-specific activities of maltase and sucrase, and higher values of the M/A ratio than birds fed on the lipid-rich HL and HSL diets, whereas nestlings fed on the HL and HSL diets did not differ (Fig. 3). This pattern confirms the prediction of only the 'inhibition by lipid' hypothesis (see Fig. 1). In the medial section of the intestines, mass-specific activity of maltase and values of the M/A ratio changed in the order HS>HSL>HL (Fig. 3), a pattern that confirms predictions of both tested hypotheses. However, mass-specific activity of sucrase was not elevated significantly by starch in this section (Fig. 3B), and the prohibitive effect of lipid seems stronger than the effect of starch (Fig. 3A; changes in values of the M/A ratio in the group fed on the HSL diet partly reflected changes in activity of aminopeptidase-N, cf. Fig. 3C,D). Finally, in the distal part of the intestines, the mass-specific activities of carbohydrases and values of the M/A ratio were higher in nestlings fed on the HSL diet than in those fed on the HS and HL diets (Fig. 3). As we describe below, this unexpected pattern can also be explained by both hypotheses.

The effect of diet composition on aminopeptidase-N activity was much weaker than in the case of carbohydrases, an expected result because relative changes in protein content of experimental diets were lower than for starch content (Table 1). Nevertheless, the decline in mass-specific activity of aminopeptidase-N in the order

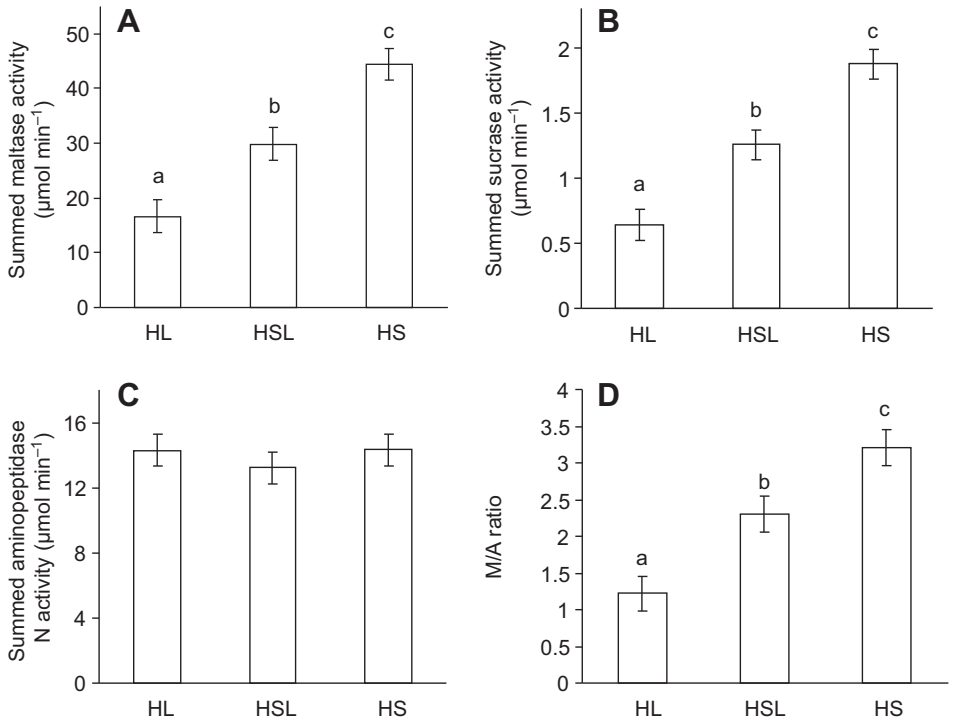

Fig. 2. Summed activity of maltase (A), sucrase (B), aminopeptidase-N (C) and the ratio of summed activity of maltase to that of aminopeptidase-N (D) in the present experiment. Means ± s.e.m. are shown. Different letters indicate significant differences between diets.

Table 3. Summary of results of repeated-measures ANOVA for intestinal enzyme mass-specific activities and maltase/aminopeptidase-N ratio (M/A ratio)

	Diet		Intestinal position		Diet × intestinal position	
	$F_{2,24}$	P	$F_{2,48}$	P	$F_{4,48}$	P
Maltase	14.68	<0.0001	38.71	<0.0001	21.25	<0.0001
Sucrase	18.46	<0.0001	55.92	<0.0001	21.89	<0.0001
aminopeptidase-N	3.57	0.044	21.40	<0.0001	1.69	0.17
M/A ratio	23.01	<0.0001	64.06	<0.0001	14.35	<0.0001

HL>HS>HSL (Fig. 3C) was in agreement with the decrease in dietary protein content of these diets, but we reiterate that the differences in aminopeptidase-N activity usually were non-significant and were not large enough to affect summed enzyme activity (see Fig. 2C). Thus, activity of aminopeptidase-N was probably weakly induced by dietary protein but did not depend on dietary lipid (HL and HSL diets with the same lipid content tended to show, respectively, the highest and lowest activity of aminopeptidase-N). Similarly, composition of dietary lipids inhibited activity of carbohydrases but not leucine aminopeptidase in piglets (Dudley et al., 1994).

Molecular basis for observed dietary modification of intestinal enzymes

Diet can modulate activity of digestive enzymes on intestinal epithelium either by (1) non-specific effects, such as those on morphology of villi, microvilli, fluidity of cell membranes, etc., which might simultaneously affect several enzymes in the same fashion, or (2) specific effects, such as those on the rate of expression or degradation of a particular enzyme at the BBM. Because changes in mass-specific activities of carbohydrases and aminopeptidase-N in our experiment differed in both direction and magnitude (Fig. 3), we hypothesize that observed diet effects were probably mediated by site-specific changes in the rate of synthesis or degradation of carbohydrases. Studies in rodents revealed several genetic and molecular mechanisms that can be responsible for such changes (Takase and Goda, 1990; Shinohara et al., 1993; Kishi et al., 1999; Goda, 2000; Honma et al., 2007; Tanaka et al., 2008; Mochizuki et al., 2010a; Mochizuki et al., 2010b). We cannot pinpoint the mechanisms that were responsible for dietary modulation of intestinal carbohydrase activities observed in our experiment. However, our results suggest that the relative strength of the opposite effects of dietary starch and lipid on the expression of disaccharidases changes in a proximal-to-distal gradient, as shown by significant interaction between diet composition and intestinal section (Fig. 3). Dietary manipulations often result in such regionally specific changes in enzyme activities (Karasov and Hume, 1997).

We hypothesize that the interaction between diet composition and intestinal section reflects changes in concentration of luminal nutrients caused by their gradual hydrolysis and absorption. Digestion of carbohydrates in the proximal and medial parts of intestines might be less efficient in HSL-fed than in HS-fed nestlings because activities of disaccharidases were suppressed here by high lipid content in the HSL diet. Therefore, concentration of carbohydrates in the distal section of the intestines in HSL-fed nestlings might be higher than in those fed on the HS diet. At the same time, as lipid concentration in the intestinal lumen presumably decreased gradually (in all groups) because of its digestion and absorption, its inhibitive effect on expression of disaccharidases might be weaker in the distal than

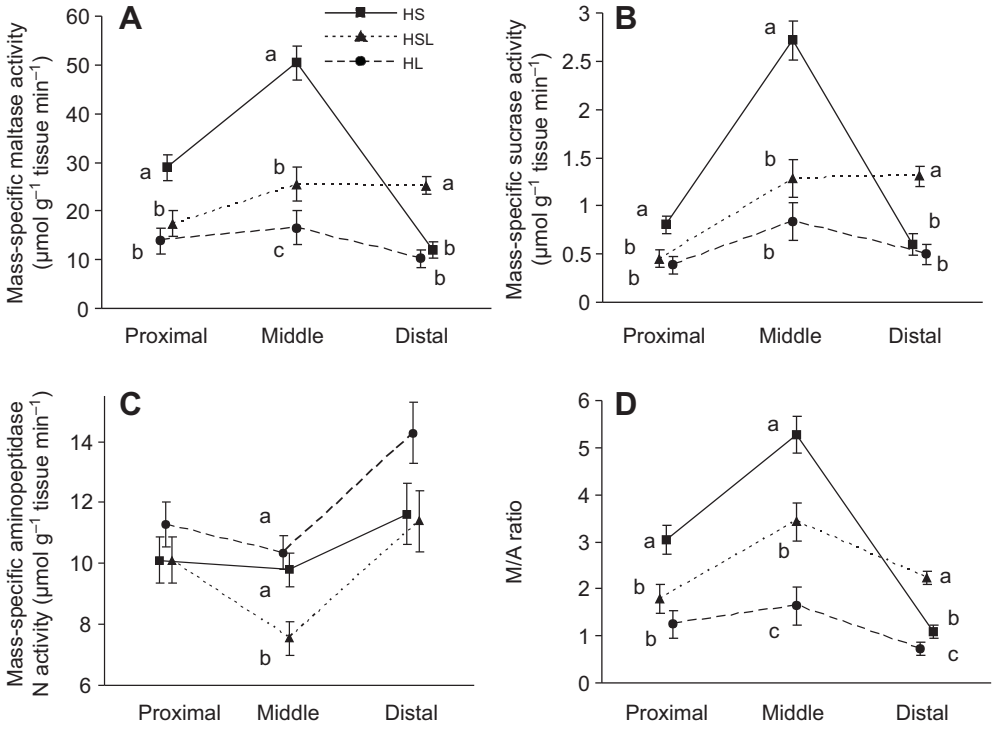

Fig. 3. Mass-specific activity of maltase (A), sucrase (B), aminopeptidase-N (C) and the ratio of mass-specific activity of maltase to that of aminopeptidase-N (D) in the present experiment. Means ± s.e.m. are shown. Squares and solid lines indicate the group fed on the HS diet, triangles and dotted lines indicate the group fed on the HSL diet, and circles and dashed lines indicate the group fed on the HL diet. Different letters indicate significant differences between diets (tested independently within each intestinal position).

in the proximal and medial sections of the intestines. As a result of these two factors, HSL-fed birds showed higher activity of maltase and sucrase in the distal part of the intestines than nestlings fed on the HS diet. However, even though HL-fed nestlings might show similar proximal-to-distal decreases in concentration of luminal lipids, a complete lack of starch in their diet did not upregulate expression of carbohydrases in the distal section of the intestines. Interestingly, a reduction in sucrase activity caused by high content of dietary corn oil may also be weakest in the distal part of rat intestines, as suggested by fig. 1 in Takase and Goda (Takase and Goda, 1990).

We cannot exclude the presence of other mechanisms, such as lower susceptibility of the distal epithelium to the inhibitory effects of lipids. Moreover, we must add three caveats to our interpretation of obtained results. First, our tests assume implicitly that differences in protein content among applied diets (Table 1) did not affect the activity of disaccharidases. However, observed patterns in enzyme activities do not suggest that dietary protein affects activity of disaccharidases or that dietary starch/lipid affect activity of aminopeptidase-N (cf. Figs 2, 3 and Table 1). Second, substrate concentration in the intestinal lumen depends not only on diet composition but also on activity of pancreatic enzymes that carry out the first steps of food hydrolysis. However, we hypothesize that activity of pancreatic enzymes may not be limiting. The human pancreas may possess an enormous, tenfold excess capacity (DiMagno et al., 1973), which represents one of the highest safety factors observed in any biological structure [see fig. 26 in Piersma and van Gils (Piersma and van Gils, 2010)]. Whether the same holds in birds is not known, but one feature of regulation of their digestive enzymes is consistent with this pattern. We found little evidence for the presence of adaptive, diet-related modulation of pancreatic amylase in both passerine and galloanserine species (Brzęk et al., 2013). That study included the same house sparrow nestlings that were the subject of the present experiment, and activity of their pancreatic amylase did not differ significantly across the HS and HL diets. Third, our maltase assay quantified the activity of several enzymes (see Materials and methods), and their relative roles in digestion of carbohydrates is still not fully understood even in mammals (see Quezada-Calvillo et al., 2007). However, we think that our maltase assay offers a reliable estimate of the functional activity of all the BBM enzymes that process products of starch hydrolysis by pancreatic amylase. Moreover, because diet composition modulated both maltase activity and sucrase activity in the same way, it also seems likely that both genes that are responsible for maltase activity (*MGAM* and *SI*) were affected by diet similarly.

Finally, we do not know how different patterns of expression of intestinal disaccharidases affect digestion of starch. House sparrow nestlings fed on the HS diet tended to have better efficiency of starch assimilation than nestlings fed on the HL diet, but this effect was virtually absent in older nestlings [see fig. 6A in Brzęk et al. (Brzęk et al., 2009)]. Moreover, diet composition usually had no detectable effect on growth and development of nestlings (Brzęk et al., 2009; Brzęk et al., 2011; present study). Thus, different summed activity and different proximal-to-distal gradients of mass-specific activity of intestinal carbohydrases have no effect on development of young sparrows, at least under laboratory conditions.

Implications for interpretation of other studies

In some studies that investigated the effect of diet composition on activity of intestinal enzymes in passerine birds, lipid content of experimental diets was kept constant (Martinez del Rio et al., 1995; Sabat et al., 1998). Two studies on *Dendroica* warblers, however, used natural diets that were either rich in carbohydrates but with low lipid content (fruits), or had relatively low carbohydrate and high lipid content (insects and seeds). Diet did not affect mass-specific activity of carbohydrases in yellow-rumped warblers (Afik et al., 1995), but pine warblers fed on a fruit diet had higher activity of maltase and sucrase than birds fed on insects and seeds (Levey et al., 1999). However, results of the present experiment show that identifying the dietary signal for the latter result is equivocal as it matches predictions of both ‘induction by starch’ and ‘inhibition by lipids’ hypotheses. Finally, adult house sparrows fed on a high lipid and low starch diet had lower activity of maltase and sucrase than birds fed on diets with low lipid and either low or high starch content (Caviedes-Vidal et al., 2000), a pattern that supports the ‘inhibition by lipid’ but not the ‘induction by starch’ hypothesis. None of the cited studies found significant interactions between diet composition and intestinal position for activity of carbohydrases. However, we are not aware of any previous experiment that included a diet similar to the HSL diet in our study, i.e. combining high lipid with high carbohydrate content and, as we explained earlier, such a combination is particularly likely to produce complex interactions between intestinal position and mass-specific activity of the studied enzymes.

In conclusion, this is, to the best of our knowledge, the first study that has shown unequivocally that dietary carbohydrate induces activity of intestinal disaccharidases in altricial birds, although this effect is most pronounced when the level of dietary lipid is low (i.e. the effect of lipid is stronger than that of carbohydrates). However, there are some important issues that must be solved before we can generalize our findings. First, the HSL and HL diets in the present experiment were rich in highly unsaturated corn oil, which is not typical of all natural diets. In mammals, the effect of lipid on intestinal carbohydrases may depend on lipid type (Takase and Goda, 1990; Dudley et al., 1994; Yasutake et al., 1995). Second, species may differ in their susceptibility to the effect of lipid [at least in mammals; see Dudley et al. (Dudley et al., 1994) and discussion there]. Finally, our study may explain the single outlier among studies on the effect of diet on intestinal carbohydrases in adult passerines (i.e. Levey et al., 1999), and thus support the hypothesis about the lack of adaptive modulation of these enzymes in adult passerine birds. However, the present experiment simultaneously confirms that dietary starch induces intestinal carbohydrases in house sparrow nestlings. Thus, it is likely that at least some young passerine birds can possess developmental flexibility of intestinal carbohydrases that is lost later in life (Brzęk et al., 2010; cf. Toloza and Diamond, 1990).

LIST OF ABBREVIATIONS

BBM	brush-border membrane
HL	high-lipid and starch-free diet
HS	low-lipid and high-starch diet
HSL	high-lipid and high-starch diet

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AUTHOR CONTRIBUTIONS

P.B., E.C.-V. and W.H.K. designed the research; P.B. conducted the research, carried out statistical analyses of the data and wrote the first draft of the article; K.D.K. carried out biochemical assays; all authors revised and approved the article.

COMPETING INTERESTS

No competing interests declared.

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REFERENCES

Afik, D., Caviedes-Vidal, E., Martinez del Rio, C. and Karasov, W. H. (1995). Dietary modulation of intestinal hydrolytic enzymes in yellow-rumped warblers. *Am. J. Physiol.* **269**, R413-R420.

Anderson, T. R. (2006). *Biology of the Ubiquitous House Sparrow*. Oxford: Oxford University Press.

Brzęk, P., Kohl, K., Caviedes-Vidal, E. and Karasov, W. H. (2009). Developmental adjustments of house sparrow (*Passer domesticus*) nestlings to diet composition. *J. Exp. Biol.* **212**, 1284-1293.

Brzęk, P., Caviedes-Vidal, E. and Karasow, W. H. (2010). House sparrow fledglings leave the nest digestively immature but more flexible than adults. *Integr. Comp. Biol.* **50 Suppl. 1**, E19.

Brzęk, P., Kohl, K. D., Caviedes-Vidal, E. and Karasov, W. H. (2011). Fully reversible phenotypic plasticity of digestive physiology in young house sparrows: lack of long-term effect of early diet composition. *J. Exp. Biol.* **214**, 2755-2760.

Brzęk, P., Ciminari, M. E., Kohl, K. D., Lessner, K., Karasov, W. H. and Caviedes-Vidal, E. (2013). Effect of age and diet composition on activity of pancreatic enzymes in birds. *J. Comp. Physiol. B* **183**, 685-697.

Caviedes-Vidal, E. and Karasov, W. H. (2001). Developmental changes in digestive physiology of nestling house sparrows, *Passer domesticus*. *Physiol. Biochem. Zool.* **74**, 769-782.

Caviedes-Vidal, E., Afik, D., Martinez del Rio, C. and Karasov, W. H. (2000). Dietary modulation of intestinal enzymes of the house sparrow (*Passer domesticus*): testing an adaptive hypothesis. *Comp. Biochem. Physiol.* **125A**, 11-24.

DiMagno, E. P., Go, V. L. and Summerskill, W. H. (1973). Relations between pancreatic enzyme ouputs and malabsorption in severe pancreatic insufficiency. *N. Engl. J. Med.* **288**, 813-815.

Dudley, M. A., Wang, H., Hachey, D. L., Shulman, R. J., Perkinson, J. S., Rosenberger, J. and Mersmann, H. J. (1994). Jejunal brush border hydrolase activity is higher in tallow-fed pigs than in corn oil-fed pigs. *J. Nutr.* **124**, 1996-2005.

Goda, T. (2000). Regulation of the expression of carbohydrate digestion/absorption-related genes. *Br. J. Nutr.* **84 Suppl. 2**, S245-S248.

Goda, T. and Takase, S. (1994). Dietary carbohydrate and fat independently modulate disaccharidase activities in rat jejunum. *J. Nutr.* **124**, 2233-2239.

Goda, T., Yasutake, H., Suzuki, Y., Takase, S. and Koldovský, O. (1995). Diet-induced changes in gene expression of lactase in rat jejunum. *Am. J. Physiol.* **268**, G1066-G1073.

Honma, K., Mochizuki, K. and Goda, T. (2007). Carbohydrate/fat ratio in the diet alters histone acetylation on the sucrase-isomaltase gene and its expression in mouse small intestine. *Biochem. Biophys. Res. Commun.* **357**, 1124-1129.

Karasov, W. H. and Diamond, J. M. (1988). Interplay between physiology and ecology in digestion. *Bioscience* **38**, 602-611.

Karasov, W. H. and Hume, I. D. (1997). The vertebrate gastrointestinal system. In *Handbook of Physiology, Section 13: Comparative Physiology*, Vol. 1 (ed. W. H. Dantzler), pp. 407-480. New York, NY: Oxford University Press.

Karasov, W. H. and Martinez del Rio, C. (2007). *Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins.* Princeton, NJ: Princeton University Press.

Karasov, W. H., Martínez del Rio, C. and Caviedes-Vidal, E. (2011). Ecological physiology of diet and digestive systems. *Annu. Rev. Physiol.* **73**, 69-93.

Kishi, K., Takase, S. and Goda, T. (1999). Enhancement of sucrase-isomaltase gene expression induced by luminally administered fructose in rat jejunum. *J. Nutr. Biochem.* **10**, 8-12.

Kohl, K. D., Brzęk, P., Caviedes-Vidal, E. and Karasov, W. H. (2011). Pancreatic and intestinal carbohydrases are matched to dietary starch level in wild passerine birds. *Physiol. Biochem. Zool.* **84**, 195-203.

Lee, E. A., Weiss, S. L., Lam, M., Torres, R. and Diamond, J. (1998). A method for assaying intestinal brush-border sucrase in an intact intestinal preparation. *Proc. Natl. Acad. Sci. USA* **95**, 2111-2116.

Lepczyk, C. A., Caviedes-Vidal, E. and Karasov, W. H. (1998). Digestive responses during food restriction and realimentation in nestling house sparrows (*Passer domesticus*). *Physiol. Zool.* **71**, 561-573.

Levey, D. J., Place, A. R., Rey, P. J. and Martínez Del Rio, C. (1999). An experimental test of dietary enzyme modulation in pine warblers *Dendroica pinus*. *Physiol. Biochem. Zool.* **72**, 576-587.

Martinez del Rio, C. (1990). Dietary, phylogenetic, and ecological correlates of intestinal sucrase and maltase activity in birds. *Physiol. Zool.* **63**, 987-1011.

Martinez del Rio, C., Brugger, K. E., Rios, J. L., Vergara, M. E. and Witmer, M. (1995). An experimental and comparative study of dietary modulation of intestinal enzymes in European starlings (*Sturnus vulgaris*). *Physiol. Zool.* **68**, 490-511.

McCarthy, D. M., Nicholson, J. A. and Kim, Y. S. (1980). Intestinal enzyme adaptation to normal diets of different composition. *Am. J. Physiol.* **239**, G445-G451.

McWhorter, T. J., Caviedes-Vidal, E. and Karasov, W. H. (2009). The integration of digestion and osmoregulation in the avian gut. *Biol. Rev. Camb. Philos. Soc.* **84**, 533-565.

Mochizuki, K., Honma, K., Shimada, M. and Goda, T. (2010a). The regulation of jejunal induction of the maltase-glucoamylase gene by a high-starch/low-fat diet in mice. *Mol. Nutr. Food Res.* **54**, 1445-1451.

Mochizuki, K., Igawa-Tada, M., Takase, S. and Goda, T. (2010b). Feeding rats a high fat/carbohydrate ratio diet reduces jejunal S/I activity ratio and unsialylated galactose on glycosylated chain of S-I complex. *Life Sci.* **86**, 524-531.

Piersma, T. and van Gils, J. A. (2010). *The Flexible Phenotype: a Body-Centred Integration of Ecology, Physiology, and Behaviour*. Oxford: Oxford University Press.

Quezada-Calvillo, R., Robayo-Torres, C. C., Opekun, A. R., Sen, P., Ao, Z., Hamaker, B. R., Quaroni, A., Brayer, G. D., Wattler, S., Nehls, M. C. et al. (2007). Contribution of mucosal maltase-glucoamylase activities to mouse small intestinal starch α-glucogenesis. *J. Nutr.* **137**, 1725-1733.

Ramirez-Otarola, N., Narváez, C. and Sabat, P. (2011). Membrane-bound intestinal enzymes of passerine birds: dietary and phylogenetic correlates. *J. Comp. Physiol. B* **181**, 817-827.

Sabat, P., Novoa, F., Bozinovic, F. and Martínez del Rio, C. (1998). Dietary flexibility and intestinal plasticity in birds: a field and laboratory study. *Physiol. Zool.* **71**, 226-236.

Shinohara, H., Goda, T., Takase, S. and Sugawa-Katayama, Y. (1993). Feeding medium-chain triglycerides to rats decreases degradation of sucrase-isomaltase complex in the jejunum. *J. Nutr.* **123**, 1161-1167.

Starck, J. M. and Wang, T. (2005). *Physiological And Ecological Adaptations To Feeding In Vertebrates*. Enfield, NH: Science Publishers, Inc.

Takase, S. and Goda, T. (1990). Effects of medium-chain triglycerides on brush border membrane-bound enzyme activity in rat small intestine. *J. Nutr.* **120**, 969-976.

Tanaka, T., Suzuki, A., Kuranuki, S., Mochizuki, K., Suruga, K., Takase, S. and Goda, T. (2008). Higher expression of jejunal LPH gene in rats fed the high-carbohydrate/low-fat diet compared with those fed the low-carbohydrate/high-fat diet is associated with in vitro binding of Cdx-2 in nuclear proteins to its promoter regions. *Life Sci.* **83**, 122-127.

Toloza, E. M. and Diamond, J. M. (1990). Ontogenetic development of transporter regulation in bullfrog intestine. *Am. J. Physiol.* **258**, G770-G773.

Yasutake, H., Goda, T. and Takase, S. (1995). Dietary regulation of sucrase-isomaltase gene expression in rat jejunum. *Biochim. Biophys. Acta* **1243**, 270-276.