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RESEARCH ARTICLE

*Obesity, Diabetes and Energy Homeostasis*

# Dietary adaptation to high starch involves increased relative abundance of sucrase-isomaltase and its mRNA in nestling house sparrows

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## Abstract

Dietary flexibility in digestive enzyme activity is widespread in vertebrates but mechanisms are poorly understood. When laboratory rats are switched to a higher carbohydrate diet, the activities of the apical intestinal α-glucosidases (AGs) increase within 6–12 h, mainly by rapid increase in enzyme transcription, followed by rapid translation and translocation to the intestine's apical, brush-border membrane (BBM). We performed the first unified study of the overall process in birds, relying on activity, proteomic, and transcriptomic data from the same animals. Our avian model was nestling house sparrows (*Passer domesticus*), which switch naturally from a low-starch insect diet to a higher starch seed diet and in whom the protein sucrase-isomaltase (SI) is responsible for all maltase and sucrase intestinal activities. Twenty-four hours after the switch to a high-starch diet, SI activity was increased but not at 12 h post diet switch. SI was the only hydrolase increased in the BBM, and its relative abundance and activity were positively correlated. Twenty-four hours after a reverse switch back to the lower starch diet, SI activity was decreased but not at 12 h post diet switch. Parallel changes in SI mRNA relative abundance were associated with the changes in SI activity in both diet-switch experiments, but our data also revealed an apparent diurnal rhythm in SI mRNA. This is the first demonstration that birds may rely on rapid increase in abundance of SI and its mRNA when adjusting to high-starch diet. Although the mechanisms underlying dietary induction of intestinal enzymes seem similar in nestling house sparrows and laboratory rodents, the time course for modulation in nestlings seemed half as fast compared with laboratory rodents. Before undertaking modulation, an opportunistic forager facing limited resources might rely on more extensive or prolonged environmental sampling, because the redesign of the intestine's hydrolytic capacity shortly after just one or a few meals of a new substrate might be a costly mistake.

*intestinal carbohydrases;* Passer domesticus*; phenotypic flexibility; protein; transcriptional regulation*

## INTRODUCTION

Many omnivorous species exhibit dietary flexibility to seasonal or unexpected changes in food availability and types (1). Varying diets may be composed of different levels of substrates that require different particular complements of enzymes for their breakdown. The intestinal α-glucosidases (AGs) mediate the assimilation of carbohydrates by hydrolyzing the bonds in *1*) dietary disaccharides, enabling the uptake of glucose and fructose, and *2*) the products of the digestion by amylase(s) of starch and glycogen (2). Based on arguments of economic design, one might predict that for an omnivore switching among diets with differing amounts of hydrolysable carbohydrates, such as sucrose and starch, there will be a positive relationship between dietary substrate levels and the abundance of AGs necessary for their breakdown (1). Although the existence of dietary flexibility in digestive enzyme activity has been described in scores of terrestrial and aquatic species (1), much less is known about the underlying mechanisms.

In laboratory rats, transition to higher starch diets induces mRNA, protein abundance, and activities of the intestinal AGs sucrase-isomaltase (SI) and maltase-glucoamylase (MGAM) (3–6). Dietary induction of SI in rats can be very

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rapid, with significant increases in SI mRNA within 3 h of a diet switch (3) and significant increases in activity and enzyme abundance within 3–6 h post diet switch (4). These changes occur in enterocytes along the villus (7). Dietary modulation of enzyme activity in rodents does not rely on replacement of villus cells by differentiation of new enterocytes from stem cells in the intestinal crypts, which is a slower process (4, 8).

In most passerine bird species, which include house sparrows (*Passer domesticus*)—the avian model in this study—SI alone appears to account for all sucrase and maltase activity in the small intestinal brush-border membrane (BBM), based on genomic, proteomic, and functional data (9). In house sparrow nestlings, but not adults (10), intestinal maltase and sucrase activity in intestinal tissue homogenates are induced by high dietary carbohydrate (11–13) and the increased activity is associated with increased SI mRNA (10). What is unknown for the nestlings, or any other bird including poultry as far as we know (2), is whether dietary induction of SI and its mRNA occurs as fast as in laboratory rodents and whether or not increased SI activity in tissue homogenates corresponds with increased SI activity and abundance in the BBM.

In two experiments that mirror each other, we tested the hypothesis that adaptive modulation of intestinal SI activity relies on transcriptional control (i.e., regulation of mRNA level) of enzyme abundance in the BBM. House sparrow nestlings, which naturally experience large changes in dietary carbohydrate and protein content as their parents transition them from consuming insects to seeds (14), are a very tractable system for these tests because they can be raised in captivity and fed artificial diets whose composition can be rapidly altered and mimic the different diet chemistries in nature (13). There is evidence, in a prior study of house sparrow nestlings by Rott et al. (13), that sucrase and maltase activity in intestinal tissue homogenates are increased 24 h after switch to a higher carbohydrate diet and decreased 24 h after the reverse switch to a lower carbohydrate diet, but the evidence is not conclusive because the study lacked a direct comparison of nestlings switched with nestlings not switched (i.e., simultaneous controls). Therefore, for *experiment 1*, which focused on dietary induction of SI, our *prediction 1* was that SI activity in both intestinal tissue homogenates and BBM of nestling house sparrows would be induced within 24 h by sudden dietary increase in carbohydrate (starch). *Prediction 2* was that increase in SI activity would be associated with, or preceded by, increased abundance of its mRNA. *Prediction 3* was that increased SI activity in the BBM would be correlated with increased SI abundance. *Experiment 2* focused on downward modulation of SI and tested *prediction 4* and *5* that were the reverse of *prediction 1* and *2*, respectively. We did not test for the correlation between BBM SI activity and abundance a second time but we assumed that the results would be similar.

Overall, our study was designed to advance the understanding of fundamental aspects of intestinal modulation in birds. However, the study is also important for understanding the ability of wild birds to adjust to changes in their nutritional resources that occur due to human activities such as habitat alterations and climate change.

## METHODS

### Study Site, Bird Collection, Experimental Diets, and Feeding

Our study site, bird collection and manipulation, experimental diets, and daily feeding routine were exactly that of Rott et al. (13). During summers 2016 and 2017, house sparrow nestlings 3 days posthatch were collected from their nests in the University of Wisconsin (UW)-Madison campus vicinities and were immediately housed in environmental chambers under controlled conditions (ambient temperature 35°C, relative humidity 50–55%, 15 h:9 h light: darkness regime) and manually fed semisynthetic diets hourly for 16 h/day beginning at 0600 h exactly, following earlier protocols (13). The UW-Madison ethics committee approved experimental procedures (permit IACUC-A005514).

*Experiment 1* in 2016 was designed to study induction of SI. Upon arrival in environmental chambers all nestlings ($n$ = 55) were fed a high-protein, low-starch "P" diet [59.5% wt/wt casein, 5% corn starch, 8% corn oil, 10.5% other essential nutrients, 17% nonnutritive bulk; (13)]. After 3 days on this diet, birds were randomly assigned to one of two diet groups, one ($n$ = 23) switched at 0600 h to a high-starch, lower-protein "S" diet (38% corn starch, 26.5% casein, all other components identical to "P" diet (13)), and the rest ($n$ = 17) maintained on "P" diet. The protein content of the lower protein "S" diet still contained sufficient levels of essential amino acids and total protein to support growth in birds (15).

*Experiment 2* was performed a year later in 2017 and was the reverse of *experiment 1*; designed to study downward modulation of SI. Upon arrival all nestlings ($n$ = 49) were initially fed the high-starch "S" diet. After 3 days on this diet, birds were randomly assigned to one of two diet groups, one switched at 0600 h to the low-starch, higher protein "P" diet ($n$ = 20) and the rest maintained on "S" diet ($n$ = 19).

### Tissue Harvest and Assays for Enzyme Activity, mRNA, and Abundance

In both *experiment 1* and *2*, after 12 h on dietary treatments, nestlings ($n$ = 17 and 19, respectively) were weighed, euthanized with $CO_2$, and dissected to remove the intestines, pancreas and liver, and the remainder of nestlings were similarly processed after 24 h on dietary treatments ($n$ = 23 and 20, respectively). At this age, the nestlings were too sexually immature to assign gender by inspection of gonads, and we did not perform a molecular test for sex. The whole intestine was perfused with ice-cold avian Ringer solution, quickly blotted dry for weighing, and then placed on an iced small steel plate and longitudinally divided into three pieces. One was immediately stored at −80°C for later measurement of intestinal sucrase and aminopeptidase-N (APN) activity in tissue homogenates (13). Enterocytes were isolated from the remaining two-thirds of tissue (16) and half the isolate was stored in RNAlater at −80°C for later measurement of SI and APN mRNAs relative to reference genes (*EEF1A1* and *GAPDH*) by RT-qPCR (10), with only a slight change because for reverse transcription we used the SuperScript III First-Strand Synthesis System (Invitrogen). To design APN primers for house sparrows, we aligned using MUSCLE (17) all relevant sequences of bird peptidases obtained from the


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NCBI database by BLASTn (http://www.ncbi.nlm.nih.gov/BLAST/) using as query the zebra finch's (*Taeniopygia guttata*) APN sequence (XM_030281964.2). Based on this alignment, we picked a specific target region for APN that excludes the rest of the peptidases and chose the sequence of the phylogenetically closest species available (*T. guttata*) to design our primers with the PrimerQuest Tool. The parameters were analyzed using OligoAnalyzer Tool (PrimerQuest program, IDT, Coralville, IA; accessed from https://www.idtdna.com/SciTools) and Tm calculator (New England Biolabs, Ipswich, MA; https://tmcalculator.neb.com/). The designed primers were tested for homology with unrelated sequences by BLAST and were ordered from Invitrogen (Thermo Fisher Scientific, Waltham, MA); primer sequences 5′-3′ [forward-reverse] TACCTCCACACCTTCTCCTAC - CGGTCCATGATGC/TTGCTGAT.

The remainder of the enterocytes were used to isolate BBMs (9), which were then stored at −80°C for later measurement. We measured maltase and aminopeptidase-N activities in isolated BBMs. We used maltose for the BBM measurement because SI hydrolysis rate on maltose (i.e., rate of release of glucose) is much higher than on sucrose, which makes measurements more accurate when using very small amounts of isolated BBM. Parallel changes in both maltase and sucrase activity are expected because SI alone accounts for all maltase and sucrase activity in house sparrows (9, 18). Also in BBM, we measured protein relative abundance by nanoscale liquid chromatography coupled to tandem mass spectrometry (nLC/MS/MS) as done previously in Brun et al. (9, 18). The output of the nLC/MS/MS analysis was subjected to spectral counting quantification. The spectral data were searched against the *Mus musculus* Uniprot amino acid sequence database (87,156 entries) and protein identifications were accepted if they could be established at >99% probability within 1% false discovery rate and contained at least five identified peptides. This yields, in essence, the number of spectra that are assigned to discrete identified proteins. The procedure does not use protein standards to calibrate absolute abundances, but the number of SI spectra relative to the number of total spectra in isolated BBM was determined within each sample and normalized to total spectral counts within that sample. When comparing samples with highly similar total protein backgrounds, as in these experiments, this spectral counting approach allows for identification of shifts in the relative abundance of SI protein. In 2016, nLCMS was performed on BBM from individual birds, but because of low recovery in a large proportion of birds the following year, we performed it on multiple pools created from BBM of 2–3 birds from within each treatment group. All the analyses on stored tissue and tissue isolates were completed within ≤2 yr.

### Data Analysis

Data were analyzed with the statistical package JMP, Version Pro 15.2.0 (SAS Institute Inc., Cary, NC, 1989–2019). Results are given as means ± 1 SE [$n$ = number of birds (or pools of birds) per treatment; each treatment defined according to diet and hours post diet switch]. Siblings were not included within any single treatment group, with the exception of an error in forming one group in *experiment 2*; this pair of siblings was <1% of all birds in this study. Data on mean rates of hydrolysis, abundance, or mRNA were plotted according to their respective diet group (P or S) and as a function of time for the 24 h following the diet switch (along the $x$-axis; time 0 h corresponds to 0600 h). The nestlings in the diet group that was not switched are referred to as controls. We used two-way ANOVA with diet and time (12 h vs. 24 h post diet switch) as factors to test for whether diet and/or time influenced enzyme activities and mRNA. Data at time = 0 were excluded from this two-way ANOVA because only the control group was represented. The contrasts function was used to test for significant ($P < 0.05$) diet effects at 12 and 24 h, and those $P$ values are shown in Figs. 1 and 4. In every two-way ANOVA that we could perform, time and/or its interaction with diet was a significant factor. We were able to test most effectively for a possible time effect by focusing just on control groups in one-way ANOVAs that included data at time = 0 (corresponding to 0600 h), time =12 (1800 h), and time = 24 (0600 h the following day). This added ~33% more data and excluded the confounding effect of a diet switch. Tukey post hoc comparisons were used to test for significant ($P < 0.05$) time differences at 0 h, 12 h, and 24 h, and those differences are shown in Figs. 1 and 4. Preceding these ANOVAs, we confirmed that data were normally distributed and homoscedastic, but in a few cases we first $\log_{10}$-transformed the data to meet test's assumptions. In BBM, we tested for the correlation of SI relative abundance as a function of its activity and whether its intercept was zero, using Model II regression because both $x$ and $y$ values were measured with error. We used the $F$ test recommended by Motulsky and Ransnass (32) to test for difference between the diet groups in this correlation. Studentized residuals from regression were inspected to check for normal distribution and homoscedasticity. Significance level was set at $P < 0.05$.

## RESULTS

### Experiment 1

In *experiment 1*, here and elsewhere, data for time = 0 are shown for visual comparison, but that time point was excluded from the two-way ANOVA because only controls were tested at time = 0. Consistent with our first prediction, nestlings initially raised 3 days on low-starch diet P and then switched to higher starch diet S had higher SI activity in whole tissue homogenates 24 h post diet switch than in similar-aged controls (i.e., nestlings maintained on low-starch diet P throughout; post hoc contrast $F_{1,36} = 44.2$, $P < 0.001$). At an earlier time point, 12 h post diet switch, tissue sucrase activity of nestlings eating higher starch diet was not significantly elevated above that in controls ($F_{1,36} = 1.21$, $P = 0.28$). Controls on the same P diet throughout did not differ in SI activity between times 0 h, 12 h, and 24 h post diet switch (one-way ANOVA: $F_{2,25} = 0.37$, $P = 0.69$). Corresponding to the increased SI activity in tissue homogenates, nestlings that fed 24 h on higher starch diet had higher maltase activity in isolated BBM compared with the controls (lower starch diet group) ($F_{1,19} = 8.90$, $P = 0.0076$; Fig. 1*B*). But in nestlings tested earlier (i.e., at 12 h), there was no difference in BBM maltase activity between diet groups ($F_{1,19} = 0.99$, $P = 0.33$; Fig. 1*B*),


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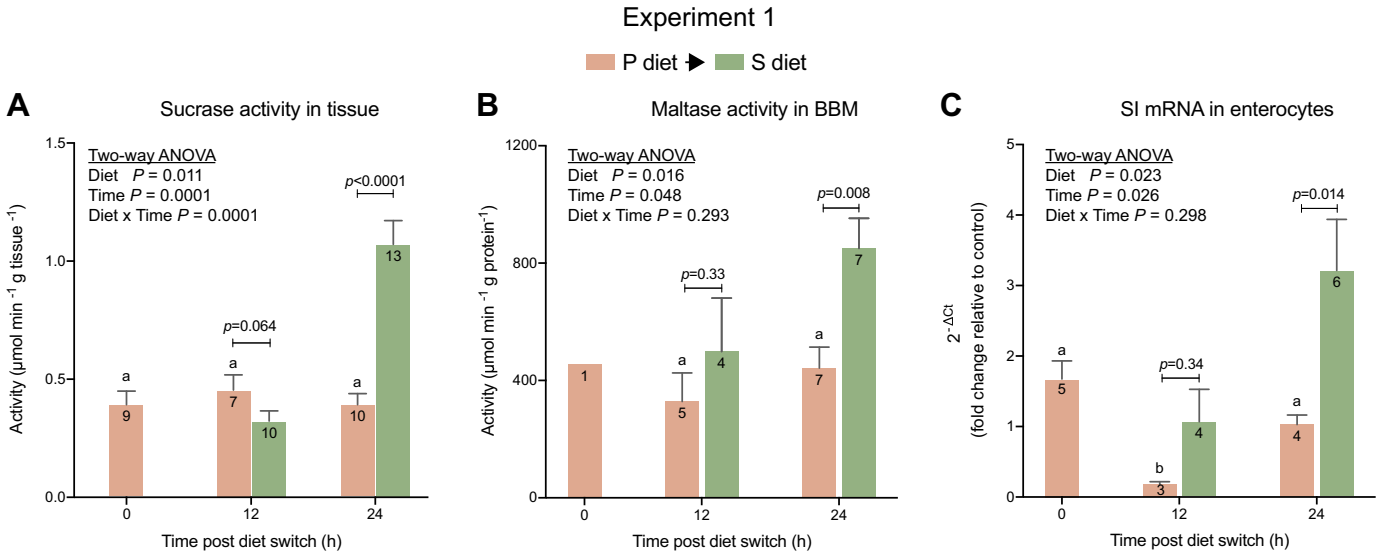


**Figure 1.** α-Glucosidase in homogenates of intestinal tissue (*A*; sucrase activity), in brush-border membrane (BBM) (*B*; maltase activity), and in isolated enterocytes [*C*; sucrase-isomaltase (SI) mRNA in enterocytes] in nestling house sparrows in the hours following a diet switch from the low-starch P diet to the high-starch S diet (*experiment 1*). At the beginning of the experiment, time is 0 h for nestlings on P diet. Values are means ± SE, and numbers within bars are number of nestlings in each treatment group. *P* values for differences between the two diets at specific time are shown above the pairs of bars, based on two-way ANOVA post hoc diet contrasts at 12 h and 24 h post diet switch. Among controls (i.e., unswitched nestlings that remained on P diet throughout), bars with different lower case letters represent mean values that differed significantly among the three time points in one-way ANOVA. Increases in both maltase and sucrase activity are expected because SI alone accounts for all maltase and sucrase activity in house sparrows (9). Maltase was used for the most accurate measurements in the very small amounts of isolated BBM because SI hydrolysis rate on maltose (i.e., rate of release of glucose) is much higher than on sucrose.

and in controls, the BBM activity did not differ between times 0 h, 12 h and 24 h post diet switch ($F_{1,10} = 0.93$, $P = 0.36$).

Consistent with our second prediction, nestlings that were switched to higher starch S diet had higher SI mRNA 24 h post diet switch compared with controls (nestlings that remained eating lower starch P diet; $F_{1,13} = 8.06$, $P = 0.014$; Fig. 1*C*). At an earlier time point, 12 h post diet switch, tissue sucrase activity of nestlings eating higher starch diet was not significantly elevated above that in controls ($F_{1,13} = 0.96$, $P = 0.34$). Controls consuming the P diet throughout differed in SI mRNA ($F_{2,9} = 6.45$, $P = 0.018$), with the value at 12 h being lower than values at both 0 h and 24 h, with no difference between 0 h and 24 h (post hoc Tukey comparisons).

The diet-induced increase in hydrolase activity and mRNA in the S diet group was specific for SI, based on other simultaneous measurements in the same birds. For example, aminopeptidase-N activity and mRNA from nestlings in the high-starch diet S group 24 h after the diet switch was not higher but instead tended to be lower than in same-aged individuals in the diet P group (Table 1). Thus, the upward modulation of SI activity and its mRNA was an enzyme-specific effect because there were not parallel changes for the enzyme APN.

Consistent with our third prediction, SI spectra relative to total spectra in isolated BBM, which corresponds to relative SI abundance (mg protein)$^{-1}$, was also higher in the S diet group (0.559 ± 0.193, $n = 7$ nestlings) compared with the lower starch P diet group (0.226 ± 0.127, $n = 6$) ($t_{10.4} = 3.7$, $P = 0.004$). The diet-induced increase in SI abundance in the S diet group was specific for SI, because comparisons by diet for abundances of 10 other BBM hydrolases were all non-significant (Fig. 2). Also consistent with our third prediction, SI abundance and maltase activity in BBM were correlated (Pearson $r = 0.839$, $P = 0.0003$; Fig. 3). The linear relationship extrapolated back to the origin (95% confidence interval for Model II reduced major axis regression = −0.141 to 0.164), and both diet groups were fit by the same linear model ($F_{2,22} = 0.353$, $P = 0.71$). These proteomic data are consistent with the idea that SI abundance accounts for all the maltase activity in the nestlings, whether they were uninduced or induced for higher starch-digesting capacity.

**Table 1.** *Tests*[a] *for effect of diet on aminopeptidase-N activity its mRNA in nestlings 24 h following switch from low-starch/higher protein P diet to higher starch/lower protein S diet*

| | APN Activity in Tissue Homogenates, mmol min$^{-1}$ g$^{-1}$ tissue | | | APN Activity in BBM, mmol min$^{-1}$ g$^{-1}$ tissue | | | mRNA in Enterocytes, fold change relativeto control | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | *n* | Mean | SE | *n* | Mean | SE | *n* |
| Diet S | 4.89 | 0.29 | 13 | 20.0 | 3.18 | 5 | 0.279 | 0.13 | 7 |
| Diet P | 5.72 | 0.40 | 10 | 27.52 | 6.54 | 5 | 1.295 | 0.36 | 8 |
| *t* test | | $t_{16.9} = 1.71$ $P = 0.11$ | | | $t_{5.2} = 1.07$ $P = 0.33$ | | | $t_{13} = 10.55$[b] $P = 0.0001$ | |

[a]All *t* tests were two-tailed. [b]Log2-transformed values.


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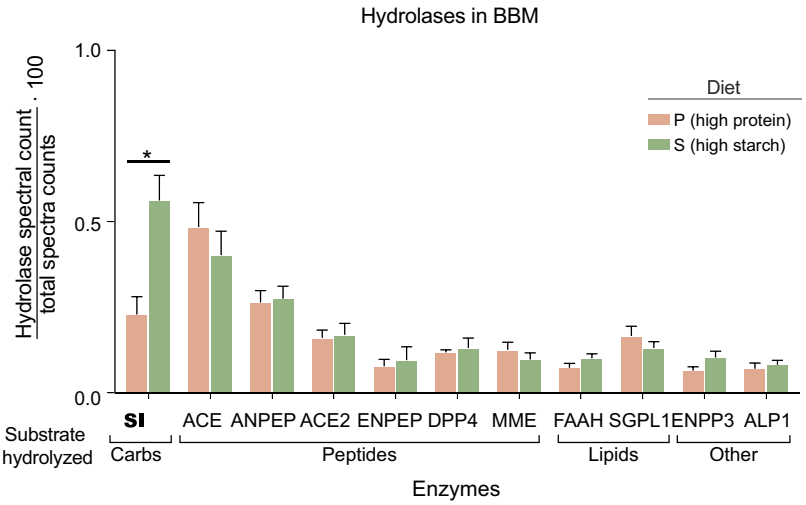


**Figure 2.** Abundance of sucrase-isomaltase (SI) and other hydrolases in brush-border membrane (BBM) isolated from nestlings on the two diets 24 h after the diet switch in *experiment 1*. The asterisk above SI represents a significant difference by diet ($P < 0.004$), whereas comparisons by diet for the other 10 hydrolases were all nonsignificant (all $P$'s $> 0.1$).

Overall, the picture that emerges from analyses in *experiment 1* is that the switch to higher starch diet was associated with higher SI activity at 24 h post diet switch but not at 12 h post diet switch. The higher activity was associated with higher SI mRNA and enzyme abundance.

### *Experiment 2*

In *experiment 2*, consistent with *prediction 4*, house sparrow nestlings initially raised 3 days on high-starch diet S and then switched to low-starch diet P had lower SI activity in whole tissue homogenates 24 h post diet switch than in similar-aged controls (post hoc contrast $F_{1,35} = 6.11$, $P = 0.018$), but not at 12 h post diet switch ($F_{1,35} = 0.50$, $P = 0.48$; Fig. 4*A*). SI activity in whole tissue did not vary with time post diet switch in the controls (one-way ANOVA: $F_{2,26} = 0.68$, $P = 0.06$). For SI activity in BBM (Fig. 4*B*), we show mean values (± SE) for illustrative purposes, but we judged the low sample sizes to be poorly suited to ANOVA analyses, though there was a trend for lower maltase activity in BBM of nestlings 24 h after switch to low-starch P diet, compared with controls ($t_4 = 1.63$, $P = 0.089$, one-tailed $t$ test for a priori prediction).

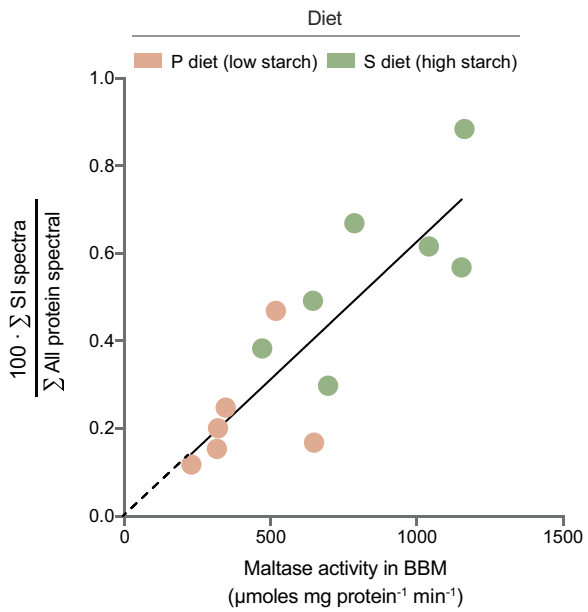


**Figure. 3.** Abundance of sucrase-isomaltase (SI) was correlated with maltase activity in brush-border membrane (BBM) isolates from nestlings 24 h after the diet switch in *experiment 1* ($P < 0.001$; see text). The sum of SI spectra relative to the sum of total spectra in isolated BBM corresponds to relative SI abundance (mg protein)$^{-1}$.

In *experiment 2*, consistent with our fifth prediction, nestlings that were switched to low-starch P diet had lower SI mRNA 24 h post diet switch compared with controls (post hoc contrast $F_{1,17} = 9.06$, $P = 0.008$; Fig. 4*C*), but not at 12 h post diet switch (post hoc contrast $F_{1,17} = 2.87$, $P = 0.11$). In controls consuming the S diet throughout, SI mRNA varied significantly with time ($F_{2,12} = 25.5$, $P < 0.0001$) with value at 12 h being lower than values at both 0 h and 24 h and with no difference between 0 h and 24 h (post hoc Tukey comparisons).

Simultaneous measurements in the same birds of aminopeptidase-N activity and its mRNA help to illustrate that the diet-induced decrease in SI activity and mRNA in the P diet group was specific for SI. Whereas at 24 h after the diet switch, SI activity and mRNA were lower in the P diet group and APN activity and its mRNA were higher in the P diet group (Table 2). Thus, the downward modulation of SI activity and mRNA was an enzyme-specific effect.

The picture that emerges from analyses in *experiment 2* is that the switch from high-starch diet S to low-starch diet P was associated with specific decrease in SI activity at 24 h post diet switch but not at 12 h post diet switch. The decrease in activity was associated with a parallel change in SI relative mRNA. Also, as in *experiment 1*, there was an effect of time on SI relative mRNA in control nestlings, which seems to reflect a rhythm in which mRNA abundance is lower at 1800 h than either 0600 h or 2400 h.

## DISCUSSION

### Time Course for Modulation of Intestinal α-Glucosidases

Studying the time course of change in an intestinal process following a change in dietary substrate level is potentially confounded if there are diet-independent changes over


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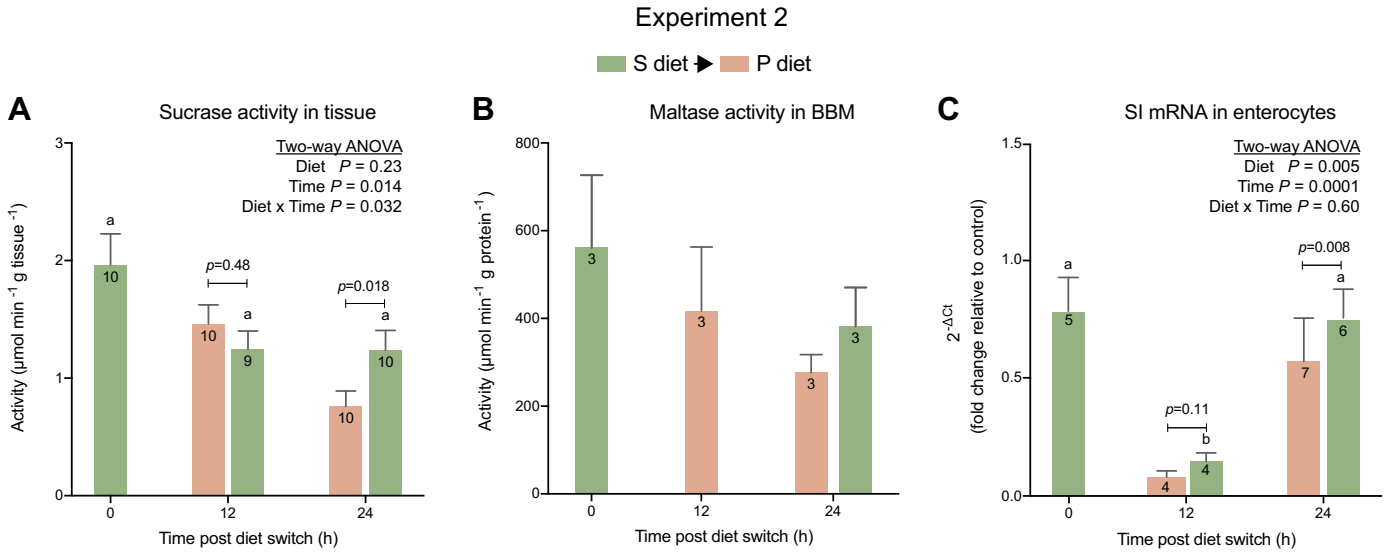


**Figure. 4.** α-Glucosidase in homogenates of intestinal tissue (*A*; sucrase activity), in brush-border membrane (BBM) (*B*; maltase activity), and in isolated enterocytes [*C*; sucrase-isomaltase (SI) mRNA] in nestling house sparrows in the hours following a diet switch from the high-starch S diet to the lower starch P diet (*experiment 2*). Values are means ± SE, and numbers within bars are number of birds (or pools of birds in the case of BBM) in each treatment group. *P* values for differences between the two diets at specific time are shown above the pairs of bars, based on two-way ANOVA post hoc diet contrasts at 12 h and 24 h post diet switch. Among controls (i.e., unswitched nestlings that remained on S diet throughout), bars with different lower case letters represent mean values that differed significantly among the three time points in one-way ANOVA. Decreases in both maltase and sucrase activity are expected because SI alone accounts for all maltase and sucrase activity in house sparrows (9). Maltase was used for the most accurate measurements in the very small amounts of isolated BBM because SI hydrolysis rate on maltose (i.e., rate of release of glucose) is much higher than on sucrose.

time, for example, in a diurnal rhythm in intestinal function even in animals on constant diet (19, 20). For this reason, we routinely *1*) checked for a time as well as diet effect in two-way ANOVA and isolated the diet effect at specified times by using contrasts according to diet, and *2*) tested more extensively, after finding time effects or interactions in two-way ANOVA, for an effect of time by one-way ANOVA in our controls (nestlings not diet-switched) over an entire 24-h cycle. This statistical scheme seemed satisfactory and effective for demonstrating the diet effects that we predicted as well as an unanticipated time effect on one of our measurements, SI mRNA (Figs. 1*C* and 4*C*). Both of these effects are discussed subsequently.

### Mechanisms of Modulation of Intestinal α-Glucosidases

Overall, switch of nestling house sparrows to high-starch diet was associated with increases in SI activity in tissue homogenates, its mRNA in isolated enterocytes, and in SI activity and abundance in BBM. Our findings seem similar to those in young (3 wk post-weaning) rats, where transition to high starch has been linked to increased mRNA of both SI (3, 21) and maltase-glucoamylase (MGAM) (6), to increased sucrase activity in the BBM (4) and to increased SI abundance in jejunal homogenates [measured as sucrase immunoreactivity (5)]. Our study is not only the first on birds but also the only study on dietary modulation of SI (or MGAM), to our knowledge, that combines measures on all these processes simultaneously and also demonstrates that enzyme abundance is increased in the BBM. The similar constellation of findings in rat and sparrow could be interpreted to reflect a general and major role for transcriptional control (i.e., regulation at the mRNA level) of enzyme abundance in dietary adaptation of intestinal α-glucosidases in mammals and birds. However, we cannot entirely rule out activation of nascent enzyme (5) that might be present in enterocyte cytosol but incapable of substrate hydrolysis, which we then would not measure in our tissue homogenates. But, in laboratory rats, the glycosylated form of SI made in the Golgi is fully active (7). Also, nLC/MS/MS would likely detect an inactive form of the protein, so a future study could use that method to interrogate the non-BBM cell fractionation fraction(s) that contain(s) the cytosolic contents.

**Table 2.** *Tests for effect of diet on aminopeptidase-N activity and mRNA in nestlings 24 h following switch from higher starch/lower protein S diet to lower starch/higher protein P diet*

| | APN Activity in Tissue Homogenates, mmol $min^{-1}$ $g^{-1}$ tissue | | | APN Activity in BBM, mmol $min^{-1}$ $g^{-1}$ tissue | | | mRNA in Enterocytes, fold change relativeto control | | |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | *n* | Mean | SE | *n* | Mean | SE | *n* |
| Diet S | 4.714 | 0.453 | 10 | 13.41 | 0.838 | 3 | 1.01 | 0.07 | 10 |
| Diet P | 6.935 | 0.459 | 10 | 21.60 | 0.58 | 3 | 1.54 | 0.07 | 9 |
| *t* test | | $t_{18} = 3.44$ $P = 0.003$[a] | | | $t_4 = 3.87$ $P = 0.009$[b] | | | $t_{17} = 5.17$ $P = 0.0001$[a] | |

[a]Two-tailed *t* test. [b]One-tailed *t* test following demonstrated difference in activity in tissues.


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The changes in SI mRNA relative abundance could be due to changes in gene transcription and/or alterations in mRNA stability, but other methods, such as nuclear run-on transcription assays, favor the former interpretation for other intestinal hydrolases of rats (22, 23). Mechanisms controlling activation of transcription are generally unknown, but there is evidence for dietary adaptation of MGAM activity in rats that mRNA transcription is regulated through histone acetylation and binding of several cofactors in the promoter/enhancer and transcriptional regions of MGAM (6). The extent to which underlying mechanisms are similar in birds will be exciting to explore.

Although increases and decreases in SI mRNA relative abundance are associated with, and presumably precede respectively, increases and decreases in SI protein abundance and activity, why would mRNA vary markedly over a 24-h cycle but not protein abundance and activity (c.f. controls as a function of time in *A* and *B* vs. *C* in both Fig. 1 and Fig. 4)? Gene expression rhythms in small intestine have been observed (20), but if mRNA degrades more rapidly than synthesized enzymes, then protein pool sizes might not fluctuate to the same extent as those of their mRNA (7). Also, there can be lags due, for example, to time for translocation of enzyme from intracellular sites of synthesis to the BBM (3), and lags could make changes in BBM protein abundance and activity out of phase with those of their mRNA. Future studies of this may need to be systematically designed with frequent sampling over time to reveal possible asynchronous rhythms in both mRNA and protein.

### Modulation of Intestinal Aminopeptidase-N

The switch from diet S to diet P in *experiment 2* simultaneously decreased dietary starch content by 33 percentage points and increased dietary protein content by 33 percentage points. Prior study on other nestlings using these same diets has shown that nestlings raised on higher protein diet P had elevated APN activity compared with nestlings raised on lower protein diet S (13). Considering this, it is not surprising that coincident with the decreases in SI illustrated in Fig. 4, aminopeptidase-N activity and its mRNA in the same tissues responded in opposite direction, that is, APN was induced on higher protein diet (Table 2). For APN, the results of the earlier study and this study are consistent with the idea that upward modulation of APN occurs within a day of a switch to higher protein diet, but our new data on APN mRNA suggest a major role for transcriptional control of APN similar to that for SI. In laboratory rats also, intestinal APN activity is increased on higher protein diet (24, 25), though perhaps not with parallel change in mRNA (25).

Some other studies with nestling house sparrows failed to find significant dietary modulation of intestinal APN activity (11, 12, 26) but those studies involved smaller increases in dietary protein content (range 12–26 percentage points) than in the more recent studies with diets S and P. One explanation might be that changes in enzyme activity are in some proportion to changes in dietary substrate levels, and experiments relying on diets with smaller changes in substrate level, i.e., a smaller effect size, have less statistical power to detect differences in enzyme activity. Alternatively, animals typically seem to have ~50% spare digestive capacity (27), and so, they perhaps simply do not respond to changes in dietary substrate that are relatively smaller.

### Perspectives and Significance

Although the mechanisms underlying dietary induction of intestinal AGs seem similar in nestling house sparrows and young laboratory rodents, the time course for modulation of SI activity in nestlings seemed slower compared with in laboratory rodents, in whom intestinal SI activity is induced within 6–12 h by dietary substrate (3–5, 21). We failed to find in nestlings compelling evidence for induction of SI mRNA or activity 12 h post diet shift (Fig. 1). We also failed to find evidence for downward modulation 12 h post diet shift (Fig. 4), but we are not aware of other comparison studies that focused similarly on downward modulation following reduction in the dietary signal. Our results suggest that the most reliable conclusion right now is that nestlings seemed half as fast as young laboratory rats in increasing SI activity following an increase in dietary carbohydrate level.

We are not surprised by this unique finding. Food is potentially more limiting for a wild animal than for ad libitum-fed domesticated laboratory animals, and there are plausible costs associated with dietary modulation of intestinal enzymes, such as allocations of limited biosynthetic energy and/or membrane space (28). Before undertaking modulation, an opportunistic forager might rely on more extensive or prolonged environmental sampling, because the redesign of the intestine's hydrolytic capacity shortly after just one or a few meals of a new substrate might be a costly mistake. There might be selection against an omnivorous bird, like young house sparrow nestlings, that routinely consumes insects, to immediately alter enzyme activity away from insects toward seeds and carbohydrates just because of a short-term lull in insect availability. But, a more sustained decline in insects and protein intake might merit a biochemical restructuring for future reliance on a carbohydrate-rich diet. Thus, nestling house sparrows can track daily changes in resource availability with fine-tuned changes in digestive performance. Their response time for change is fast but not too fast, which might be a temporal analogue to a similar adage about intestinal digestive capacity (29). Wild omnivorous small mammals, such as laboratory rodents and nestling house sparrows, also exhibit dietary modulation of intestinal enzyme activity (30), and future studies might test in them for similar mechanisms to laboratory rodents but somewhat slower response times. Also, future studies comparing response times might consider carefully controlling for differences in age and body mass, the latter of which influences some other features measured in what has been called "biological time" (31). Besides the importance for advancing understanding of fundamental aspects of intestinal modulation, additional studies of this sort are also important for understanding the ability of wild animals to adjust to changes in their nutritional resources that occur naturally or due to human activities, such as habitat alterations and climate change.

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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

A.B., M.E.M., W.H.K., and E.C.-V. conceived and designed research; A.B., M.E.M., and G.A.B.-W. performed experiments; A.B., M.E.M., W.H.K., and E.C.-V. analyzed data; A.B., M.E.M., G.A.B.-W., W.H.K., and E.C.-V. interpreted results of experiments; W.H.K. and E.C.-V. prepared figures; W.H.K. and E.C. drafted manuscript; A.B., M.E.M., G.A.B.-W., W.H.K. and E.C.-V. edited and revised manuscript; A.B., M.E.M., G.A.B.-W., W.H.K., and E.C.-V. approved final version of manuscript.

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