Digestive modulation in a seasonal frugivore, the American robin (Turdus migratorius)

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Levey, Douglas J., and William H. Karasov. Digestive modulation in a seasonal frugivore, the American robin (Turdus migratorius). Am. J. Physiol. 262 (Gastrointest. Liver Physiol. 25): G711–G718, 1992.—American robins (Turdus migratorius) switch from eating fruits in the fall to insects in the spring. Our objective was to determine the physiological and morphological changes associated with such a switch. Three nonexclusive hypotheses addressed possible mechanisms operating on different levels. First, we hypothesized that birds on the two diets would differ in intestinal absorption rates of sugars and amino acids. We predicted that individuals on a high-protein low-carbohydrate insect diet would exhibit higher proline and lower glucose absorption than individuals on a low-protein high-carbohydrate fruit-based diet. Uptake rates of glucose and proline were measured in vitro, using an everted sleeve technique. We found no significant differences in uptake between the two groups and so rejected the hypothesis. The second hypothesis, that retention time of digesta in the gut changes with diet, was tested using an inert marker (polyethylene glycol) injected into insects or fruit and fed to birds. We predicted and found that the marker was excreted significantly faster in birds eating fruit rather than insects. Thus this hypothesis is supported. The third hypothesis focused on changes in gut morphology as a basis for shifts in digestive processing. We found no differences in gut length, nominal surface area, or volume and so rejected this hypothesis. Note that hypotheses two and three are closely related, because retention time is proportional to gut volume/digesta flow. Because gut volume did not differ between the two groups, the difference in retention time must have been due to a change in flow. Taken together, these results suggest that short retention times are likely an important adaptation to frugivory.

Digestive efficiency, the proportion of ingested food that is digested and absorbed, reflects how effectively an animal exploits food resources; it is one of the most basic parameters determining energetic gain. In birds, digestive efficiency is similar across different taxonomic groups if type of diet is held constant (13). Nevertheless, digestive efficiency on a given diet is not fixed (e.g., 22). An important consequence of its variation is that the nutritional content of a food item is not an accurate predictor of its nutritional value. Nutritional value, the accessibility of nutritional contents to an animal, is determined by digestive efficiency, which is a function of many factors, including enzyme activity, nutrient absorption rates and digesta retention time (time a meal spends in the gut; 29), which can be a function of gut volume and digesta flow rate (23, 27). Changes in these parameters are likely to play an important role in determining changes in digestive efficiency when a bird shifts diet. Yet, we know little about the plasticity of avian digestive traits, even though they are key to understanding which species can switch diets and how complete the switch can be.

Among temperate birds, perhaps the most dramatic dietary switch is from insects to fruit. In an earlier experiment (22), we examined changes in digestive efficiency when American robins (Turdus migratorius) were fed fruit and then insect diets. We found a significant increase in efficiency when the birds were switched from fruit to insects and another increase in efficiency from the beginning to the end of the insect-feeding trial. Two factors, food composition (i.e., proportion of material refractory to digestion) and food processing (i.e., physiological and morphological traits), likely explain our results. In this study we focus on the latter.

Changes in digestive efficiency within a trial must have been due to changes in food processing, because food composition was kept constant. Delineating processing’s contribution to between-trial differences is difficult, because both food composition and food processing are involved. But several lines of evidence suggest that differences in food processing were indeed important, that food composition alone could not account for differences in digestive efficiency between diets. First, taking the refractory portion of fruit into account (13), robins had surprisingly low efficiency on the fruit diet (22), implying that they did not absorb all of the sugars, amino acids, or lipids. Second, this was confirmed for sugar in a trial in which D-[14C]glucose in agar cubes was fed to robins; their digestive efficiency was 73% (17). Third, the cricket diet had the lowest proportion of easily digestible components, such as simple carbohydrates, protein, and lipid, yet had the highest efficiency (22). Thus it appeared that the robins’ digestive processing of the two diets was somehow different.

We pose and test three nonexclusive hypotheses relating to mechanisms of changes in digestive efficiency that accompany changes in diet. On the cellular level, we hypothesized that changes in absorption rates of sugars and amino acids may underlie differences in digestive processing among animals on disparate diets (hypothesis 1). A wide variety of transporters are phenotypically modulated by dietary nutrient concentration and changes in transporter activity can occur rapidly after a diet shift (6, 19). Because crickets are relatively rich in proteins and low in carbohydrates whereas fruits generally show the opposite pattern (22, 25), we predicted that proline uptake rates would be higher and glucose uptake rates lower in robins eating crickets than in those eating fruit.

On the organ level of gut function, we hypothesized that changes in retention time would explain variation in digestive efficiency (hypothesis 2). This idea is based
on the assumption that short gut retention times decrease digestive efficiency and high retention times increase it (28). Because robins had apparently low digestive efficiency on fruit (22), we predicted shorter retention times for fruits than for insects. This prediction also derives from a recently proposed model (23).

Finally, on the highest level of gut processing, we hypothesized that gross changes in gastrointestinal morphology could underlie differences in gut function (hypothesis 3). Changes in gut morphology are well-known to be associated with shifts in diet (1, 2). The functional significance of these changes, however, remains unclear (25). Nevertheless, changes in gut morphology are important to examine, because models (see below) indicate that gut volume could influence retention time and absorption (the latter via changes in surface area). Thus, measurements for this hypothesis will likely provide insights into the first two hypotheses.

Our hypotheses can be integrated by a simple model (after Ref. 27), which makes clear the relationship among our response variables

\[
\text{digestive efficiency} = \frac{\text{retention time} \times \text{absorption rate}}{\text{concentration} \times \text{volume of digesta}}
\]

Digestive efficiency in Eq. 1 can theoretically relate to the entire diet, but also to specific components of the diet such as the simple-to-digest sugars. Thus for example, the digestive efficiency for simple sugars in fruits could be lower as a result of high digesta volume (which relates to hypothesis 3) or low absorption rate (hypothesis 1) or retention time (hypothesis 2). Note that retention time, in turn, is related to gut volume and flow, the volume of digesta passing through the gut per unit time

\[
\text{retention time (min)} = \frac{\text{gut volume (ml)}}{\text{flow (ml digesta/min)}}
\]

This means that hypothesis 3 can be viewed as a subset of hypothesis 2; gut volume is an important determinant of retention time. Thus if we detect a change in retention time, our measurements of gut volume will allow us to determine if it was due to a change in gut volume or flow.

METHODS

Ten robins (captured near Madison, WI, in the fall of 1986) were kept in separate indoor cages at constant temperature (23°C) and photoperiod (12 h dark:12 h light). They were maintained on a synthetic fruit-based diet (5) and regained capture mass. We ran two feeding trials. We fed all birds a mixed diet of three fruit species (gray dogwood, Cornus racemosa; toothed viburnum, Viburnum dentatum; and wild grape, Vitis sp.) in the first trial and then a diet of 4-wk-old house crickets (Acheta domestica) in the second trial. Food and water were presented in petri plates ad libitum. We maintained the birds on the cricket diet for 10 days and planned to do likewise for the fruit diet but after 3 days most birds had lost considerable body mass. To prevent their death, we offered them banana mash for 4 days before completing the final 3 days of the trial with the three fruit species.

Retention Times

At the end of each 10-day period, we measured gut retention times of the diet each bird had been consuming. Grapes and crickets were injected with 5–10 μl of [H]+polyethylene glycol (PEG; a water-soluble marker; mol wt = 4,000) and partially painted with orange enamel paint (a solid marker). Birds were deprived of food for no longer than 30 min and were then offered several labeled food items of the same type. Usually they consumed one or two within 5 min. If not, they were force fed. Total ingested activity was 5–10 μCi and, on average, meal size (g wet mass ingested) was similar for the two diets at the start of the trials. All labeled food was removed after 5 min and replaced with unlabeled food of the same type. Birds were then watched continuously from behind one-way mirrors, and the time and location of each defecation recorded. After 60 min, the plastic-backed paper towel on the cage floor was replaced, and defecations were recorded every 15 min for at least another 20 min. During the entire time, birds ate normally.

Each defecation was sprayed lightly with distilled water, scraped from the plastic backing, placed in a sealed vial, and covered with distilled water. The samples were stored in a refrigerator for at least 12 h and periodically shaken. (A pilot study established that 12 h was sufficient for PEG concentrations to equilibrate in the water.) Aliquots of 1.5 ml were then taken and dissolved in scintillation cocktail (Aquasol) for counting.

We calculated several estimates of how long digesta is retained in the gut. Mean retention time is defined as the sum of the products of the proportion of PEG excreted during each 10-min interval (29). The mode is the time of the defecation that contained the highest disintegrations per minute (dpm). The time for 50% is the time of the defecation at which cumulative percentage of dpm exceeded 50. The first appearance or "transit time" is the time of the first defecation to contain enamel paint or >2% of the total dpm counted in a trial. Finally, the median was determined by first noting all defecations that contained paint or >2% of total dpm and then identifying the time of the middle defecation in this distribution.

We started the second trial immediately after the completion of the first trial. Five birds were randomly selected and returned to the fruit-based diet; the others remained on crickets. On days 7, 8, 10, 14, and 16 after this switch, one bird was randomly chosen from each group and killed for measurement of gut morphology and nutrient uptakes. In all statistical comparisons between these groups, birds were paired by day to control for length of time on diet.

In Vitro Uptakes of Glucose and Proline

Uptake of D-[1-3H]glucose, L- [5-3H]proline, and L-[1-14C]-glucose across the brush-border membrane (not transmural flux) was measured as described by Karas and Diamond (15). In brief, 1-cm sleeves of everted tissue were preincubated in 37°C Ringer solution and suspended for 1 min above a stir bar (1,200 rpm) in a solution containing labeled glucose or proline. Tissues were then rinsed or blotted, removed from the rod, weighed, incubated in a tissue solubilizer, and counted for dpm. To correct for passive uptake and nonabsorbed nutrients in adherent mucosal fluid, we used tracer concentrations of nonactively absorbed (L- [1-14C]glucose) or membrane-impermeable (isobutyryl-1-C]inulin) markers (15). Na+ dependency of L-proline uptake was estimated by incubating adjacent tissues in identical solutions of 5 mM proline, except that in one, Na+ was replaced with choline. Uptake measurements of D-glucose (50 mM) and L-proline (5 mM) were made in the proximal, middle, and distal thirds of the intestine. Measurements of L-
glucose uptake (the passive component of glucose absorption) were made only in the middle third. Values were normalized to centimeter of intestinal length. Summed uptakes over the entire intestine were calculated by interpolating linearly between adjacent sections and integrating over the entire intestinal length.

Concentration of 50 mM for glucose was chosen for measurements at all intestinal levels (foregut, midgut, and hindgut) to permit comparison with other studies and because uptake was nearly saturated (see below). Concentration dependency of glucose uptake was measured in midgut at 0.5, 1, 5, 25, and 50 mM. Because we measured mediated uptake of D-glucose by using L-glucose as a marker (15), we fit the data to the equation 

\[ u = (J_{\text{max}} \times \text{conc})/(K_u + \text{conc}) \]

where \( J_{\text{max}} \) is maximal flow and \( K_u \) is the concentration at which uptake was 0.5 \( J_{\text{max}} \) and is uncorrected for effects of unstirred layers. For comparing kinetics of uptake rates in different diet groups, we used the procedures recommended by Motulsky and Ransnas (26).

For measuring proline uptake, we used 50 mM in the midgut to permit comparison with other studies, but we also used 5 mM in the hopes that the majority of uptake would be \( \text{Na}^+ \) dependent. (The selection of a lower solute concentration stems from the fact that the proportion of solute uptake that is \( \text{Na}^+ \) dependent is inversely related to solute concentration when uptake is the sum of mediated \( \text{Na}^+ \)-dependent and -independent components and a nonmediated \( \text{Na}^+ \)-independent pathway (i.e., diffusion).

An incubation time of 1 min was used for both glucose and proline solutions. This time was selected for two reasons. 1) It was sufficient to allow adherent fluid to equilibrate with labeled markers in the bathing solution, and 2) uptake rates were detectable and still linear with time (to ensure measurement of unidirectional flux). Documentation is provided in APPENDIX.

Gut Morphology

The intestine (pylorus to cloaca) was carefully excised and everted over a small-diameter glass or stainless steel rod. One end was tied to the rod, and the other was gently pulled until the entire piece was taut. On release, the length was recorded. The intestine was then cut into 1-cm segments, each of which was mounted on a separate rod of diameter great enough to spread but not rip the tissue. Nominal surface area was calculated by multiplying the circumference of each rod size by the length of tissue mounted on it and summing these products over all rod sizes. Gut volume was calculated in a similar way, substituting area for circumference. Intestine mass was recorded for each 1-cm piece of tissue after it had been removed from an isotonic solution and gently blotted.

RESULTS

Retention Times

Measurements of how long digesta remain in the gut, no matter how calculated (see METHODS) were significantly shorter for fruits than for crickets (\( P \) values < 0.01, paired t tests; Table 1). Based on mean retention times, PEG in crickets required ~1.5 times longer to pass than in fruit.

Because of a methodological bias, the difference between fruit and crickets is even more pronounced than our data suggest. When we changed the toning on the cage floor after 60 min, the disturbance usually caused the birds to defecate. In the fruit trials, most of the marker had been defecated by this time and so the disturbance had a negligible effect on retention time. In the cricket trials, however, relatively little PEG was defecated before 60 min and the defecation caused by our disturbance often contained the highest concentration of PEG. This explains why the estimates of retention time for crickets clustered at 60 min (Table 1). It also suggests that "normal" retention times would have been longer.

Nutrient Transport

We found significant day-to-day variation in measurements of both glucose and proline uptakes (\( F_{4,33} = 3.4, P < 0.02; F_{4,25} = 10.9, P < 0.001 \), respectively). This variation showed no detectable pattern. To control for it, we included day of measurement as an independent variable in two-way analysis of variances (ANOVA) that tested the effects of diet and intestine level (i.e., position along the intestine) on uptake rates. No interaction terms were significant in any of these analyses.

The intestinal level at which uptake was measured (foregut, midgut, hindgut) had no significant effect on glucose uptake rate per centimeter (\( F_{3,18} = 0.54, P = 0.66 \); Fig. 1B). Glucose uptake at 50 mM did not differ between birds on the cricket and fruit diets at any intestinal level or summed over the entire intestine length (\( F_{1,28} = 0.81, P = 0.38 \), Fig. 1B; Table 2). The midgut apparent passive permeability coefficient for glucose (\( K_3 \); apparent because it is uncorrected for unstirred layers), also did not differ between the birds on the two diets (Table 2). Assuming that passive permeabilities of L- and D-glucose are equal, the \( K_3 \) values can be used to calculate total glucose uptake (i.e., the sum of active and passive components). Using the average \( K_3 \) and carrier-mediated D-glucose uptakes of all individuals (1.4 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-1} \) and 64.7 nmol.min\(^{-1} \cdot \text{cm}^{-1} \), respectively), we estimate total glucose uptake at 50 mM is 133.2 nmol.min\(^{-1} \cdot \text{cm}^{-1} \), 51% of it is passive.

Glucose uptake increased sublinearly with increasing glucose concentration, as expected for a carrier-mediated process (Fig. 2). There was no significant effect of diet (\( F_{2,26} = 0.03; P > 0.5 \)). The estimated kinetic parameters were \( J_{\text{max}} = 1.16 \pm 0.12 \) (corresponding to a maximal rate of 75 nmol.min\(^{-1} \cdot \text{cm}^{-1} \)) and \( K_m = 10.2 \pm 4.0 \) mM (\( n = 10 \) birds).

Proline uptake rate per centimeter was independent of intestinal level (\( F_{2,13} = 0.31, P = 0.74 \); Fig. 1C). As with glucose, proline uptake did not differ between birds on the two diets (5 mM: \( F_{1,20} = 0.52, P = 0.48 \), Fig. 1C; 50 mM: \( F_{1,7} = 3.4, P = 0.10 \), Table 2). Summed proline uptake at 5 mM also did not differ (Table 2). Finally, robins on fruit and cricket diets showed no difference in either total or \( \text{Na}^+ \)-independent uptake of proline (Table 2). Note that \( \text{Na}^+ \)-independent proline uptake is partly due to diffusion and that \( \text{Na}^+ \) dependency suggests carrier-mediated transport (although there may be \( \text{Na}^+ \)-independent carriers, too). \( \text{Na}^+ \)-independent uptake accounted for ~64% of total proline uptake at 5 mM, and probably much more at 50 mM.

The ratio of glucose uptake (50 mM) to proline uptake (5 mM) did not vary significantly along the intestine or between diets (\( F_{2,15} = 0.83, P = 0.45; F_{1,20} = 1.0; P = 0.32 \); Fig. 1D). In short, all uptake rates were remarkably
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DIGESTIVE RESPONSES TO DIET SHIFTS IN ROBINS

Table 1. Residence times of a liquid ($^{14}$H/PEG) and solid (enamel paint) marker placed in or on fruits and crickets and fed to robins

<table>
<thead>
<tr>
<th>Diet</th>
<th>Mean, min</th>
<th>Mode, min</th>
<th>Time for 50%, min</th>
<th>First Appearance, min</th>
<th>Median, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid marker</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit</td>
<td>47.7±1.7*</td>
<td>32.8±2.8b</td>
<td>37.2±1.9c</td>
<td>20±2.2c</td>
<td>53.3±2.7*</td>
</tr>
<tr>
<td>Cricket</td>
<td>65.2±1.8a</td>
<td>65.5±4.9b</td>
<td>63.8±3.3c</td>
<td>50±4.7c</td>
<td>63.0±3.3*</td>
</tr>
<tr>
<td>Solid marker</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit</td>
<td>28.9±2.0f</td>
<td>47.4±4.1f</td>
<td></td>
<td></td>
<td>74.6±6.6*</td>
</tr>
<tr>
<td>Cricket</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

Times are ± SE; n = 10 robins. *c* Significant (P < 0.01) difference between retention times with the same letter (paired t tests). PEG, polyethylene glycol. For solid marker, data are insufficient or of wrong type (discrete rather than continuous) to calculate statistics for mean, mode, and time for 50%.

constant; we found no significant source of variation due to diet or intestinal level.

Gut Morphology

Gut mass varied significantly with position along the intestine ($F_{2,24} = 33.0$, $P < 0.001$, Fig. 1A). We detected no significant differences between the five birds on the cricket diet and those on the fruit diet in any of the morphological attributes we measured (Table 2, Fig. 1A).

DISCUSSION

We will discuss our results at two levels. First, we review the three hypotheses on a proximate level, focusing on mechanistic determinants of digestive efficiency: uptake, retention time, and gut morphology. Note that one could pursue this mechanistic approach at a different level of biological organization by asking: “what hormonal signals or cellular events underlie changes in uptake, retention time, and morphology?” Our study was not designed to address such questions. In the second part of the DISCUSSION, we switch from an examination of how digestion works in robins to why it works the way it does. We assume that the design and function of the gut have been determined by natural selection; the gut does not teleologically “know” how to respond to changes in diet.

Determinants of Digestive Efficiency

**Hypothesis 1: nutrient uptake.** Because we found no differences in glucose or proline uptake between robins on the two diets, we reject this hypothesis. This lack of difference cannot be explained by the failure of everted sleeves to maintain full metabolic activity and transport capabilities. Our uptake estimates are within the range found in other passerine birds (17). Neither do we believe that our negative results were due to insufficient time for acclimation to the different diets. On average, birds were on the cricket diet for 21 days and the banana mash diet for 11 days. We know of no comparable studies on dietary modulation of nutrient transport in birds fed foods similar to their natural diet. In white mice, changes in glucose and proline transport rates are evident within 1 day after a switch in diet (19). More generally, in trials lasting 0.5–14 days, Diamond and Karasov (6) found that dietary substrate levels regulated transporter activity for a wide diversity of amino acids, monosaccharides, vitamins, and minerals in rodents fed diets varying in carbohydrate or protein levels. Similar results have been reported for glucose modulation in several other vertebrates (Karasov, unpublished observations).

Given the numerous studies that have shown dietary modulation in nutrient uptake, we were surprised to find that robins displayed no modulation when they switched diets. A possible explanation is that their modulation is controlled by an endogenous rhythm, not directly by diet. Because robins feed heavily on insects in the spring and fruit in the fall, perhaps uptake rates of glucose rise and proline fall from spring to fall. These changes could be induced by abiotic cues (e.g., photoperiod). If such an endogenous rhythm occurs, it would not be surprising that we found no modulation because we did all our experiments in the fall. A small data set, however, suggests that this is not the case. Three robins captured in April and June, 1989, had ratios of glucose-to-proline uptake (50 mM:5 mM) of 3.9, 1.3, and 2.7, not lower than our fall values, as predicted by the endogenous rhythm explanation.

Robins' apparent inability to modulate nutrient uptake demonstrates that well-established interspecific patterns of absorption do not necessarily hold intraspecifically. For example, species that naturally consume carbohydrate-rich foods tend to have higher levels of glucose uptake than those that subsist on carbohydrate-poor foods (14). Proline uptake, on the other hand, stays relatively constant across species that consume different diets (14). This diet-related pattern of uptake has been demonstrated across all major vertebrate classes (16) and even within classes across species that show relatively minor differences in diet (17). Yet, within a single species, the American robin, a dramatic change in diet has no effect on glucose or proline uptakes.

To the best of our knowledge, this is the first test of dietary modulation of nutrient transport in birds fed natural foods. It helps resolve a central issue in comparative studies of nutrient uptake: the relative contributions of phenotypic and genotypic factors to the rate of nutrient transport. The role of each has been demonstrated in several species (e.g., Ref. 4), but in robins, glucose and proline uptake rates seem genetically fixed, at least in adults.
Table 2. Gut morphology and uptake parameters for glucose and proline in robins on a fruit-based diet and a cricket diet

<table>
<thead>
<tr>
<th></th>
<th>Fruit Diet</th>
<th>Insect Diet</th>
<th>P value</th>
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<tbody>
<tr>
<td>Uptake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed (50 mM), μmol/min</td>
<td>1,466±231</td>
<td>1,464±460</td>
<td>0.99</td>
</tr>
<tr>
<td>Passive permeability coefficient, μmol·min⁻¹·cm⁻¹</td>
<td>1.5±0.2</td>
<td>1.3±0.2</td>
<td>0.61</td>
</tr>
<tr>
<td>Proline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed (5 mM), μmol/min</td>
<td>1,509±100</td>
<td>1,554±206</td>
<td>0.80</td>
</tr>
<tr>
<td>With Na⁺ in midgut (50 mM), nmol·min⁻¹·cm⁻¹</td>
<td>353±21</td>
<td>598±107</td>
<td>0.10</td>
</tr>
<tr>
<td>With Na⁺ in foregut (5 mM), nmol·min⁻¹·cm⁻¹</td>
<td>57.7±7.5</td>
<td>56.2±7.1</td>
<td>0.30</td>
</tr>
<tr>
<td>Without Na⁺ in foregut (5 mM), nmol·min⁻¹·cm⁻¹</td>
<td>35.1±4.4</td>
<td>37.7±4.4</td>
<td>0.51</td>
</tr>
<tr>
<td>Gut morphology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal length, cm</td>
<td>23.5±2.4</td>
<td>23.9±2.4*</td>
<td>0.83</td>
</tr>
<tr>
<td>Nominal area, cm²</td>
<td>24.3±2.0</td>
<td>24.0±2.8</td>
<td>0.81</td>
</tr>
<tr>
<td>Volume, cm³</td>
<td>1.96±0.22</td>
<td>1.93±0.26</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 10 robins. Passive permeability coefficients are uncorrected for unstirred layers. P values are from paired t tests (see text).

Fig. 2. D-Glucose uptake as a function of glucose concentration in American robins fed fruit mash (n = 5) or crickets (n = 5). Uptakes were first normalized to each individual’s mean uptake at 50 mM. Vertical bars give SE. Curve is based on equation: relative uptake = (1.16 × concn)/(10.2 + concn).

Thus robins may provide a good model for studying the genetic or ontogenetic basis of nutrient uptake.

The $K_m$ for glucose uptake (10 ± 4 mM) was higher than reported previously for chickens (5 mM; 14) and Anna’s Hummingbirds (4 mM; 18). But the variance of our estimate was large and for the subset of the data on robins that could be compared with the data on hummingbirds, there was no significant difference in relative uptake as a function of concentration ($F_{2,24} = 2.46; P > 0.1$). We note, however, that apparent differences in $K_m$ between species might also relate to misapplication of a single carrier model of transport to a multiple carrier system. While evidence exists for multiple aldohexose carriers in mammals (9), extensive studies in chick intestinal cells (20) have not provided evidence for more than one type of transporter. We note that none of the studies using the everted sleeve technique on birds used...
enough concentrations to discriminate multiple carriers.

**Hypothesis 2: retention times.** Robins had shorter retention times for fruits than for insects, thereby supporting this hypothesis. Our results are consistent with numerous comparative studies that have found the shortest retention times in the most frugivorous species (8, 10, 17). Our results extend this pattern by demonstrating for the first time that short retention times are characteristic of fruit processing not only among bird species but within species (see also Ref. 11).

Because retention time is the quotient of gut volume (ml) and flow rate (ml/min), and gut volume did not change on the two diets (see below), we can deduce that the flow rate was higher on the fruit diet. Indeed, robins ate more frequent and larger meals when feeding on fruits than on crickets (22). A consequence of increased intake is that it can compensate for low digestive efficiency.

A shorter retention time may have been the mechanism underlying the apparently lower digestive efficiency of nonreferred material by robins feeding on fruits versus insects (see introduction). Also, changes in retention time could account for the temporal shifts in digestive efficiency that we observed immediately after switching a bird onto a new diet (22). For example, when we took robins that had been eating a fruit diet and fed them crickets, their digestive efficiencies increased significantly over a 10-day period. Most of the adjustment occurred within the first 3 days after the switch. The initially low digestibility could have resulted from the birds initially processing crickets at the same rate they had been processing the fruit diet. Then, as retention time acclimated to the new diet, the digestive efficiency increased.

The validity of this explanation depends largely on how quickly retention time adjusts to a change in diet. Little information is available. Herrera (8) estimated gut transit times in frugivores by intubating them with a barium sulfate solution, placing them in cloth bags, and noting the time of first appearance of marker. He did this on birds caught in spring eating insects (when fruits were unavailable) and compared the results with different individuals of the same species caught in autumn eating fruits (when fruits were abundant). He found that frugivorous species had short retention times that changed little, if any, during a switch from insects in the spring to fruits in the fall. He also noted that “hard insects, especially beetles, are often recovered intact from feces of seed dispersers (i.e., frugivores), but this never occurs among nondispersers” (Ref. 8, p. 615). Presumably, fast processing by frugivores precludes their complete digestion of insects. Worthington (32) reached a similar conclusion after feeding mixed fruit and insect diets to several avian frugivores. Herrera’s and Worthington’s results suggest that retention time is a key variable determining digestive efficiency in frugivores and that it is an inherent feature of the bird, independent of diet. An invariant retention time might also explain why nonfrugivorous birds with longer gut retention times would find fruit unprofitable. It would take them too long to process such a nutritionally poor meal.

Note that our results disagree with Herrera’s: our birds clearly changed retention times. Furthermore, the response we observed was obvious after 3 days, whereas Herrera failed to detect a response after several months. This disagreement could be resolved if it were found that the gut has a baseline level of motility, which Herrera was measuring each season with his intubation suspension. The time course of our experiment was shorter, and we compared retention times of different food types, each of which could elicit a different motility pattern.

In general, gut motility (and hence retention time) is negatively correlated with luminal solute concentration (7). For example, high concentrations of amino acids and fats in the duodenum inhibit gastric motility in poultry through hormonal [possibly CCK-8 and avian pancreatic polypeptide (aPP)] and neural reflexes (7). Crickets are much higher in amino acids and fats than is fruit pulp, the dilute sugar solutions of which would be expected to elicit short retention times. Solute-level control over retention allows retention time to vary from meal to meal, presumably in a way that optimizes nutrient uptake across a wide variety of meal types. This would certainly be an advantage for most fruit-eating birds, because they consume a diverse diet that includes many insects (31).

**Hypothesis 3: gut morphology.** Because we found no difference in intestine length, mass, or nominal area between the robins on the two diets, we reject this hypothesis. The lack of difference may have been due to insufficient time for acclimation. Note, however, that several studies have documented substantial changes in gut dimensions over approximately the same time period as our study when birds switched from one diet to another (e.g., Ref. 2). Given that the gut measurements of our birds were strikingly similar and that acclimation to the diets was apparently complete by 10 days (22), we doubt that the morphological characters we measured play an important role in determining digestive efficiency of robins acclimating to new diets.

Note that our results run against a common assumption: short guts facilitate short retention times. We found that gut length and retention times are not necessarily correlated; robins with equally long guts passed fruits and crickets at different rates (see also Refs. 3, 10).

**Evolutionary Implications**

Short retention time is thought to be an “indispensable” adaptation to frugivory (8). By processing fruits quickly, birds can overcome the digestive bottleneck that results from eating a bulky food item high in calories but low in protein and other nutrients (10, 21). Because uptake rates stay relatively constant across diets, a consequence of short retention times in these birds is low digestive efficiency (Ref. 17, this study). Nonetheless, nutrient uptake is maintained at a relatively high level, because the birds keep their guts full and continually provided with new ingesta, thereby providing carriers with a relatively constant high concentration of nutrients (23). This is a digestive “strategy” akin to that seen in horses and aphids (28). Note that processing fruits in this way would likely preclude optimal processing of insects if both were ingested at the same time. We
hypothesize that this is why robins more often eat insect or fruit meals than meals that combine the two types of food (30; see also Ref. 31).

Does Digestive Strategy Determine Diet or Vice Versa?

Food digestion is often viewed as a tradeoff between the rate of processing and thoroughness of digestion (28). According to this framework, the digestive strategy of an animal is determined by where it lies along an “efficiency-velocity” gradient; some animals process food quickly and incompletely, whereas others process it slowly and thoroughly (24). A key issue is how an animal’s position along the efficiency-velocity gradient affects its food choice. Milton (24) argued that in monkeys the position is essentially fixed and thus that diet is largely determined by digestive processes. Bjornsdal (3), on the other hand, demonstrated that tortoises modulated aspects of digestive function and thus their diet determined digestive strategy. Our results agree with those of Bjornsdal. Robins are dietary generalists that dramatically change the relative proportions of fruits and insects in their diet (30). Their retention times and digestive efficiencies are dependent on diet. However, their inability to rapidly acclimate these features to a new food type suggests an initial cost to switching diets. This cost may influence their willingness to sample new foods. In short, a robin’s digestive strategy may affect day-to-day food choices. But, when considering the large seasonal changes in diet caused by shifts in food availability and/or endogenous rhythms, the type of food ingested clearly affects digestive processing. Thus the argument about whether diet affects digestive strategy or vice versa seems misdirected. Both processes can operate on different time scales.

Conclusion

Our results are only a first step in uncovering which digestive traits respond to shifts in diet. Villus growth and production of hydrolitic enzymes and bile are examples of traits we did not examine but that may be associated with the changes we observed in digestive efficiency. Still, our results emphasize the importance of a single variable, retention time. Note that robins lack many digestive features that would presumably increase their ability to process fruit. In particular, they cannot digest sucrose (because they lack sucrase; 17) and their uptake rates are generally low and appear fixed (17, this study). Despite these “handicaps,” they still are able to subsist on a diet of mostly fruit (30). Apparently, their ability to modulate retention times is a key factor that allows them to do so.

APPENDIX

Determination of Incubation Time

An incubation time of 1 min was used for both glucose and proline solutions. This time was sufficient to allow adherent fluid to equilibrate with labeled markers in the bathing solution, as indicated by nonsignificant variation in inulin space ([dpm of inulin per cm]/[dpm of inulin per μl incubation solution]) among incubation times of 1, 2, and 4 min ($F_{2,14} = 1.1$; $P = 0.37$; two-way ANOVA with incubation time and individual bird as independent variables). Incubation time was also chosen so that uptake rates were detectable and still linear with time (to ensure measurement of unidirectional flux). We tested whether a 1-min incubation time satisfied these conditions by standardizing all uptakes to 1 min (e.g., by multiplying 0.5-min uptakes by 2), running a two-way ANOVA (same independent variables as above), and examining differences between incubation times with Scheffé’s post hoc tests. A significant decline in standardized uptake between two successive time intervals would indicate a fall-off of unidirectional flux. For glucose, uptake at 0.5 and 1 min were similar (99 ± 25 and 76 ± 32 nmol·min⁻¹·cm⁻², respectively) and significantly greater than uptake at 2 min (46 ± 23 nmol·min⁻¹·cm⁻²; $F_{2,18} = 10.8, P < 0.001$; Scheffé’s $S = 52$ and 29 for 0.5 and 1 min, respectively, $P$ values < 0.05). For proline, uptakes fell significantly between our two shortest incubation periods (1- and 2-min uptakes $= 1,055 ± 153$ and $802 ± 93$ nmol·min⁻¹·cm⁻², respectively; $F_{2,14} = 15.6; P < 0.001$; Scheffé’s $S = 185, P < 0.01$). We therefore chose the shortest incubation time that would ensure equilibration of adherent fluid (1 min) and assumed that uptake rate was still linear (or almost so) up to this time.

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