Do Northern Bobwhite Quail Modulate Intestinal Nutrient Absorption in Response to Dietary Change? A Test of an Adaptational Hypothesis

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ABSTRACT. We acclimated northern bobwhite quail (Colinus virginianus) to either chow (high carbohydrate/low protein) or cricket (low carbohydrate/high protein) and tested predictions of hypotheses based on the premise of the economical design of animals. The adaptive modulation hypothesis predicts that d-glucose uptake would be higher and L-proline uptake lower in bobwhites acclimated to chow. The spare capacity hypothesis predicts that the capacity to absorb d-glucose actively will exceed the estimated nutrient load from daily food intake. There was no significant dietary effect on intestinal d-glucose (P = 0.8) and L-proline (P = 0.7) uptake rates measured in vitro using the evverted sleeve technique. In chow eaters maximal mediated d-glucose uptake summed along the entire length of intestine (53 cm) was far too low (7.2 mmol/d) to explain observed rates of glucose absorption in vivo (>35 mmol/d). Hence, both predictions were falsified. In vitro uptake may not be an appropriate measure of the intestine's absorptive capacity because it does not measure possibly important pathways of passive absorption. There is increasing evidence that substantial passive glucose absorption occurs in some birds. If passive absorption predominates the adaptive modulation hypothesis might not apply. Comp Biochem Physiol 113A:3:233--238, 1996.

KEY WORDS. Glucose, proline, active transport, absorption, intestinal adaptation, passive absorption

INTRODUCTION

It has been proposed that, through the action of natural selection, the absorptive capacity of the vertebrate small intestine is matched to nutrient intake (2). If there were not such a match, then valuable food energy might be wasted in excreta when feeding on diets with high substrate loads that are not absorbed, and/or the metabolic expenses of synthesizing and maintaining the molecular machinery to absorb substrate would be wasted when feeding on diets with very low levels of substrate. This premise of economical design has led to two hypotheses: the adaptive modulation hypothesis (5,11); and the spare capacity hypothesis (3).

According to the adaptive modulation hypothesis, the transporters for monosaccharide or amino acids should tend to be upmodulated by their substrates, or by dietary carbohydrate or protein, respectively. Levey and Karasov (16) did not find this to be the case in American robins (Turdus migratorius) fed fruit or crickets, even though this species exhibits a fairly large seasonal switch from insects in spring/summer to fruits in fall/winter. According to the spare capacity hypothesis, the transport capacity of apical sugar and amino acid transporters are matched to meet metabolic demands with some provision for a safety margin (3). According to Diamond (2), typically capacity proves to exceed intake by a factor of ca. 2, although in chickens the match seemed closer to one-to-one and at some ages less than one (20). Note that both hypotheses are based on the premise that transcellular carrier-mediated transport is the primary mechanism of absorption.

In this article, we test two predictions of these hypotheses using northern bobwhite quail (Colinus virginianus). Bobwhites were chosen because they are omnivorous in the wild, relying primarily on plant materials (seeds, fruits, leaves) but also taking arthropods especially in summer and when young (18). Digestive adaptability might be expected in them. However, their form of omnivory differs in scale somewhat from that of American robins in that bobwhites may be more omnivorous within a meal or day whereas the robins' omnivory results more from a seasonal switch in diet.

To test these hypotheses in bobwhites, we raised subadults on diets differing in carbohydrate and protein content, and then measured glucose and proline absorption by intestinal sleeves in vitro. We tested the prediction that d-glucose uptake would be higher and L-proline uptake lower in bobwhites acclimated to high carbohydrate/low
protein diet compared with those acclimated to a low carbohydrate/high protein diet. We also tested the prediction that the capacity to absorb α-glucose actively, measured as maximal mediated uptake rate of α-glucose, would exceed the estimated nutrient load from daily food intake.

MATERIALS AND METHODS

Seventeen 30-day-old quail (91.3 ± 3.0 [SE] g) obtained on July 9, 1991 from the Kidder Game Farm near Milton, WI, were divided into two groups. The birds were housed in separate cages (ca. 0.03 m³) indoors at constant temperature (24 ± 1°C) and day length (12 h) with ad libitum water. One group (chow group; 4 males, 5 females) received pheasant starter pellets (Hamme Feed Service, DeForest, WI) ad libitum for 6 weeks. The manufacturer's guaranteed analysis of this feed was minimum 26% crude protein, minimum 2.5% crude fat and maximum 7.5% crude fiber; we assumed that it contained at least 60% nonfibre carbohydrate (calculated by difference). The feed also contained the antibiotic Amprolium. The other bobwhite group (chicken group; 5 males, 3 females) received crickets (Acheta domestica; Flukers Cricket Farm, Baton Rouge, LA) ad libitum for 4 weeks, then both crickets and the chow ad libitum for 1 week because they gained less mass than birds fed on chow, and then only crickets for the final 2 weeks. Cricket dry matter contains ca. 65% crude protein (15), 26% crude fat (unpublished data) and thus <10% carbohydrate primarily as glycerol and chitin. Feeding rates were determined by disappearance of food, after correction for food water content (chow 7%, crickets 74%).

On each of 4 days during week 11 of life two birds from each diet group (usually one male and female) were studied; on day 5 a single chow bird was studied. This effectively blocks for time in the event of significant day-to-day variation in methodology (16).

We measured mediated (i.e., active) uptake of α-[1-14C]glucose (ICN, Irvine, CA), and total (i.e., mediated plus passive) uptake of α-[2,3-3H]proline (American Radiochemicals Inc., St. Louis, MO) and α-[1-3H]glucose (Dupont, Boston, MA) into the tissue across the brush-border membrane as described in Karasov and Levey (13) and Levey and Karasov (16). In brief, 1-cm sleeves of everted tissue were preincubated in 40°C Ringer solution and suspended, respectively, for 1, 2 or 4 min above a stir bar (1200 rpm) in a solution containing labeled glucose or proline. These incubation times were chosen because in other avian species they are sufficient to allow adherent fluid to equilibrate with labeled markers in the bathing solution, and uptake rates were detectable and still linear with time (to ensure measurement of unidirectional flux; (13,16). 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 α-[1-14C]glucose (Amersham, Arlington Heights, IL) or membrane-impermeable marker (sarcosine-3-[14C]C)ulin; ICN).

Uptakes of 50 mM α-glucose and 50 mM l-proline were measured in three intestinal regions: proximal (first 10% of length), middle (45–55%) and distal (last 10%) small intestine; uptake of α-glucose was measured in midgut only. Uptakes of α-glucose and l-proline were measured at 50 mM because this concentration nearly saturates the transporters and the mediated rates are thus near maximal (16). Summed uptake capacities over the entire intestine were calculated by interpolating uptakes per centimeter linearly between adjacent sections and integrating over the entire intestinal length. Tracer l-glucose uptake in midgut was expressed as an apparent permeability coefficient, i.e., tissue dpm corrected for adherent fluid (solution dpm/mL), expressed as microliters per unit tissue per minute.

Ceca were also removed, perfused clean and blotted, and their length and mass measured. The circumference, and hence nominal surface area (excluding area of villi and microvilli), of ceca and small intestinal sleeves was measured by opening segments and spreading them flat prior to measurement to the nearest millimeter.

Numerical results are given as means ± SEM (n = number of animals). For comparisons of single measures, two-factor analysis of variance (ANOVA) was performed (factors = diet, sex), and body mass was included as a covariate (ANCOVA) for some of the morphometric analyses. Simultaneous tests for effects of intestinal position on intestinal morphology or uptake within quail, and diet effects on these measures between quail, were made by repeated measures ANOVA with two factors (diet, sex). In these analyses we first included measurement day as a factor, but it was not significant for either glucose uptake (P = 0.078) or proline uptake (P = 0.23) and so was excluded from the recalculated ANOVA presented here. If the repeated measures ANOVA indicated a significant (P < 0.05) diet × position interaction, the diet effects were isolated using individual t-tests at each intestinal position. Other statistical tests are described subsequently. The P < 0.05 level was considered significant. Unless reported, interactions between factors in ANOVAs and ANCOVAs were not significant.

RESULTS

Food Intake and Body Mass

At week 11 of age, body mass of chow feeders was similar to that of cricket feeders (F1,14 = 1.8; Table 1), but males were heavier than females (162.6 ± 3.5, [n = 9] vs. 135.6 ± 6.6 [n = 8]; F1,14 = 11.79, P = 0.004). Ad libitum dry matter feeding rate of chow feeders (11.9 ± 0.3 g/d) was higher than that of cricket feeders (7.8 ± 0.3 g/d), probably in part because the metabolizable energy content of crickets is higher and thus less need be eaten to satisfy the same energy requirement.
TABLE 1. Intestinal and cecal measures in bobwhites fed chow or crickets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body mass (g)</th>
<th>Length (cm)</th>
<th>d-Glucose (µmol/min)</th>
<th>L-Proline (µmol/min)</th>
<th>Length (cm)</th>
<th>Area (cm²/cm)</th>
<th>Mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow</td>
<td>154.2 ± 5.1</td>
<td>53.2 ± 2.0</td>
<td>5.28 ± 0.83</td>
<td>28.87 ± 2.19</td>
<td>8.8 ± 0.5</td>
<td>1.4 ± 0.1</td>
<td>1.04 ± 0.11</td>
</tr>
<tr>
<td>Cricket</td>
<td>149.0 ± 8.0</td>
<td>47.4 ± 2.1</td>
<td>4.31 ± 0.32</td>
<td>25.50 ± 1.52</td>
<td>6.6 ± 5.0</td>
<td>0.9 ± 0.1</td>
<td>0.69 ± 0.09</td>
</tr>
<tr>
<td>P-value*</td>
<td>0.6</td>
<td>0.16</td>
<td>0.89</td>
<td>0.62</td>
<td>0.15</td>
<td>0.014</td>
<td>0.096</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = number of individuals).
*P-value for diet effect from ANOVA (see text).

Intestinal and Cecal Morphology

Small intestine length varied significantly with body mass (ANCOVA F₁,₁₃ = 12.1; P = 0.004) but not with diet (F₁,₁ = 2.2; P = 0.16) or sex (F₁,₁ = 1.3; P = 0.27) (Table 1). Wet mass per centimeter of intestine was higher in cricket feeders (ANCOVA F₁,₁ = 12.5, P = 0.004; Fig. 1) but did not vary significantly with sex (F₁,₁ = 1.8; P = 0.21) or body mass (F₁,₁ = 0.9; P = 0.37). The diet effect depended upon position (diet × position interaction F₂,₁₈ = 3.87, P = 0.036) and was most apparent in the proximal (t₁₉ = 2.78; P = 0.014) and mid-intestine (t₁₅ = 2.53; P = 0.023). Nominal area per cm was also higher in cricket eaters (ANCOVA F₁,₉ = 13.7; P = 0.006; Fig. 1) at every intestinal position (diet × position

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![Graphs showing intestinal mass, d-glucose uptake, intestinal area, and L-proline uptake](image-url)

**FIG. 1.** Intestinal wet mass, nominal area, carrier-mediated d-glucose uptake at 50 mM and total L-proline uptake at 50 mM as a function of intestinal position for northern bobwhite fed either chow (open circle, dashed line; n = 9) or crickets (filled circle, solid line; n = 8). Intestinal positions are P = proximal, M = mid and D = distal. Vertical bars are SE.
interaction \( P > 0.9 \), with trends also for differences at certain intestinal positions according to sex (\( F_{1,8} = 4.26; P = 0.073 \)) and body mass (\( F_{1,8} = 5.14; P = 0.053 \)).

Diet effects were opposite in the cecum, where chow-feeders had greater nominal surface area (ANOVA \( F_{1,9} = 9.34; P = 0.014 \)), and length and mass tended to be greater, though not significantly so (respectively, \( F_{1,8} = 2.45; P = 0.15 \); \( F_{1,8} = 3.42; P = 0.10 \) Table 1). None of the cecal measures varied significantly with either sex (all \( P's > 0.36 \) or body mass (all \( P's > 0.24 \).

**Nutrient Absorption**

Uptake rates were normalized to centimeter of intestine (Fig. 1) but also can be normalized to wet mass or nominal area using conversion factors in Fig. 1.

Mediated glucose uptake/centimeter varied within bobwhites with intestinal position (ANOVA \( F_{1,28} = 7.27; P = 0.003 \)) but not among them according to either diet (\( F_{1,14} = 0.1; P = 0.8 \)) or sex (\( F_{1,14} = 2.73; P = 0.12 \)) (Fig. 1). Similarly, total l-proline uptake varied with position (\( F_{1,28} = 26.6; P < 0.001 \)) but not with diet (\( F_{1,14} = 0.2; P = 0.68 \)) or sex (\( F_{1,14} = 2.9; P = 0.11 \)) (Fig. 1). The apparent permeability coefficient measured with l-glucose in the mid-intestine was similar in the cow-feeders (2.94 ± 0.26 \( \mu \)l min⁻¹ cm⁻¹) and cricket-feeders (3.01 ± 0.14) (ANOVA \( F_{1,14} = 0.2; P = 0.64 \)) and did not vary with sex (\( F_{1,14} = 1.8; P = 0.2 \). When uptakes were normalized to intestinal mass the diet comparisons were still not significant for \( \alpha \)-glucose and l-glucose, but the l-proline uptake normalized to milligram wet mass was significantly lower in the cricket-feeders (\( F_{1,14} = 8.7; P = 0.01 \). We note that this is opposite the pattern originally predicted, and could result simply from heavier nonabsorbing tissue (e.g., muscle layer) in that group contributing to a larger denominator. Had the predicted changes occurred we would have observed a higher ratio of glucose uptake to proline uptake in chow feeders, whereas we observed no significant difference in this ratio between diet groups (\( F_{1,14} = 0.12; P = 0.7 \)). Also, the whole-intestine-summed uptake capacities for glucose and proline did not differ with diet (respectively, \( F_{1,14} = 0.02 \)) and 0.25; Table 1).
table in cecum and small intestine (19) the combined intestinal and cecal uptake would be perhaps a third higher. Total mediated glucose uptake seems far too low to account for the glucose absorption at the whole-animal level. For example, considering the bobwhite's food intake rate (11.9 g/d), and assuming a dietary nonfiber carbohydrate content of 60% of which most (>90%) is digestible, bobwhites were probably absorbing glucose at a rate of at least 35 mmol/d. Granted that we did not quantify every variable, but in order to rationalize the in vitro and in vivo measures, one must assume extraordinarily low starch or glucose in the diet, or extraordinarily low digestive efficiency for those substrates. The latter seems unlikely considering that bobwhites are at least 85% efficient digesting corn (8).

For these reasons, we rejected the prediction that the transport capacity of the apical glucose transporter is matched to meet metabolic demands with some provision for a safety margin. Furthermore, we note that in rainbow lorikeets the glucose transporter capacity in vitro was 10% of glucose absorbed at the whole animal level, and in cedar waxwings it was even lower (10). According to Diamond (2), tests of the spare capacity hypothesis, with regard to carbohydrate absorption, have been positive for mice, rats, rabbits and chickens, and for cats for amino acid absorption. What explains the difference between these two sets of studies, the former in which transporter capacity did not match up to intake and the latter in which it did?

Reevaluation and New Directions

The lower uptake capacity in the former group can be ascribed both to lower tissue-specific-mediated glucose uptake rate and to lower amount of intestinal tissue (Table 2). We did not quantitate the differences because the need for a body size correction is uncertain in the case of tissue-specific uptake, and the appropriate scaling factor for intestinal mass is unknown. Both these topics deserve further attention.

With regard to intestinal tissue mass, there has possibly been selection for intestinal tissue mass reduction in flying organisms. A unit increase in body mass causes a larger increase in the power requirements for aerial locomotion than terrestrial locomotion (4). A comparison within birds of volant and nonvolant species would be interesting.

With regard to tissue-specific uptakes, there is possibly some bias in the everted sleeve technique (lower tissue viability?) that resulted in relatively low uptake rates in waxwings, lorikeets and bobwhites. On the other hand, the relatively low uptake rate might result from regulated low glucose transporter site density. This idea might be tested by measuring relative transporter site density immunochemically or with phosphoridin binding (6, 7).

It is not apparent that lower tissue viability could account for our rejection of the adaptive modulation hypothesis because this bias would apply equally to both diet groups. An implicit assumption underlying that hypothesis, as well as the spare capacity hypothesis, is that mediated absorption is the primary pathway of glucose absorption and thus its regulation is the object of natural selection. Pappenheimer (21) argued that passive absorption is relatively more important, but the topic is hotly debated (2, 22). We cannot directly estimate passive absorption in northern bobwhites using the measured glucose permeability coefficient, because the luminal concentration is unknown. There is direct evidence of substantial passive glucose absorption in both lorikeets (10) and waxwings (17). In rainbow lorikeets feeding normally it was estimated that 80% of glucose was absorbed passively. Thus, we would predict that there is substantial passive glucose absorption in northern bobwhites. Levey and Cipollini (17) present evidence to this effect. Furthermore, we suggest that the spare capacity hypothesis cannot be effectively tested in some animals without taking into account this other absorption pathway. Possibly, if passive absorption predominates, the adaptive modulation hypothesis might not apply.

<table>
<thead>
<tr>
<th>Species</th>
<th>Mass (g)</th>
<th>Mass (g)</th>
<th>Tissue-specific uptake (μmol/min · g)</th>
<th>Uptake capacity (μmol/min)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedar waxwing</td>
<td>34.5</td>
<td>1.82</td>
<td>1.16</td>
<td>2.12</td>
<td>Karasov and Levey (13)</td>
</tr>
<tr>
<td>Rainbow lorikeet</td>
<td>122</td>
<td>2.95</td>
<td>1.13</td>
<td>5.34</td>
<td>Karasov and Gork (10)</td>
</tr>
<tr>
<td>Northern bobwhite</td>
<td>154</td>
<td>3.51</td>
<td>1.47</td>
<td>5.30</td>
<td>This study</td>
</tr>
<tr>
<td>Chicken</td>
<td>2917</td>
<td>71.5</td>
<td>2.69</td>
<td>206</td>
<td>Obst and Diamond (19)</td>
</tr>
<tr>
<td>White mouse</td>
<td>23.5</td>
<td>2.2</td>
<td>8.0</td>
<td>17.6</td>
<td>Karasov et al. (14)</td>
</tr>
<tr>
<td>Laboratory rabbit</td>
<td>335</td>
<td>10.05</td>
<td>4.44</td>
<td>45.0</td>
<td>Tolecz and Diamond (25)</td>
</tr>
<tr>
<td>Laboratory rabbit</td>
<td>3868</td>
<td>65.7</td>
<td>1.15</td>
<td>75.5</td>
<td>Buddington and Diamond (1)</td>
</tr>
</tbody>
</table>
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References