Dietary regulation of intestinal ascorbate uptake in guinea pigs

WILLIAM H. KARASOV, BRUCE W. DARKEN, AND MICHAEL C. BOTTUM
Department of Wildlife Ecology, University of Wisconsin, Madison, Wisconsin 53706

KARASOV, WILLIAM H., BRUCE W. DARKEN, AND MICHAEL C. BOTTUM. Dietary regulation of intestinal ascorbate uptake in guinea pigs. Am. J. Physiol. 260 (Gastrointest. Liver Physiol. 23): G108–G118, 1991.—We measured ascorbic acid (AA) uptake across the intestinal brush border in vitro in intact tissue from guinea pigs fed maintenance AA (200 mg/kg diet) or made hypervitaminotic (5,000 mg/kg diet) or hypovitaminotic (chronically and acutely). Total uptake per centimeter ileum was 25–50% lower in hypervitaminotic juvenile, adult male, and lactating guinea pigs compared with their respective controls, whereas carrier-mediated d-glucose uptake and Na+-independent AA uptake were similar. High dietary ascorbate specifically reduced the V_{max} for carrier-mediated AA uptake. Hypovitaminosis had no significant effect on uptake of AA or other solutes. We performed diet-switching experiments (high-AA diet to maintenance diet) with young and adult guinea pigs to determine the reversibility of the downregulation. In adult guinea pigs, the downregulation of AA uptake was reversible within 7 days. In the young of mothers fed high AA during pregnancy and lactation, and which fed on high AA for 14 days after weaning, the downregulation was reversible within 14 days. Thus regulation of AA uptake is reversible and therefore probably does not play a significant role in the development of vitamin C dependency in human adults, or their young, after ingestion of megadoses of ascorbic acid.

development; glucose transport; hypervitaminosis; deficiency; scurvy; vitamin C

MOST INTESTINAL nutrient transporters have been proven to be regulated by dietary levels of their substrates (5) including, apparently, the transporter for ascorbic acid (24). If intestinal absorption of vitamin C [ascorbic acid (AA)] is modulated, it could have at least two health consequences. First, it could change the extraction efficiency for a given residence time of digesta in the gut and hence affect AAs bioavailability. AA is one of many nutrients whose extraction efficiency is <100% and for which the intestine may have a limited absorptive capacity (16, 20).

Second, if intestinal ascorbate absorption were reduced after consumption of high dietary ascorbate, as reported by Rose and Nahrwold (24), and if the reduction were irreversible, then this could contribute to the condition known as rebound scurvy. In this condition, human adults or infants previously exposed to high dietary ascorbate display deficiency symptoms when later consuming lower normal levels of ascorbate (25). Could reduced bioavailability of dietary ascorbate due to reduced intestinal absorption be a cause of rebound scurvy? An answer to this question is important considering the current interest in the possible therapeutic and prophylactic use of pharmacological levels of ascorbic acid. Indeed, we are generally ignorant of effects of megadoses of any vitamin on gut structure and function.

We report here on experiments that confirm the finding of Rose and Nahrwold (24) that feeding guinea pigs high dietary ascorbate reduced intestinal uptake of AA. Guinea pigs and humans share a need for dietary vitamin C and also a Na+-dependent carrier-mediated mechanism for its absorption in the intestine (23). Our study was designed 1) to test for this dietary effect at several stages in the life cycle, 2) to determine more exactly the mechanism for this effect (e.g., change in surface area, electrochemical gradient for Na+-coupled AA transport, or brush-border AA transporters), and 3) to determine how reversible this effect is in adults or whether AA uptake in young animals can be influenced in any irreversible way by their early nutrition in utero or postweaning. An irreversible effect on young animals might result from critical period programming (14).

Because vitamins are essential nutrients that are not significant as a source of calories, it has been predicted (4, 12) that their intestinal absorption should be downregulated in hypervitaminosis and possibly upregulated in hypovitaminosis. This prediction has been tested for only a few vitamins (5), and so we also included in our study experiments with ascorbate-deficient animals. Because we tested animals from weaning through reproduction, our study also contributes to a growing body of knowledge about regulation of nutrient transport during development.

MATERIALS AND METHODS

Animals, Diets, and Feeding Protocols

Guinea pigs (short-haired strain from Sprague-Dawley) were housed individually (except for mothers and their young) with ad libitum water and initially were fed standard guinea pig chow (Teklad) and then were switched over the course of several days to synthetic diets with high, maintenance (19), or deficient levels of AA [added at 5,000, 200, or 0 mg/kg, respectively, to the base diet (Table 1)]. The base diet was purchased in powder form from ICN Nutritional Biochemicals, and we added the ascorbate (Sigma). Diets were stored in refrigerators.

Hypervitaminosis experiments. Experiments relating to high dietary ascorbate were performed with adult
TABLE 1. Composition of basal diet

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% Dry Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground bran</td>
<td>15.00</td>
</tr>
<tr>
<td>Ground oats</td>
<td>35.29</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>8.00</td>
</tr>
<tr>
<td>Skim milk powder</td>
<td>20.00</td>
</tr>
<tr>
<td>Vitamin-free casein</td>
<td>10.00</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>5.00</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>1.50</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.04</td>
</tr>
<tr>
<td>Magnesium sulfate-7 H₂O</td>
<td>1.52</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>1.15</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.50</td>
</tr>
<tr>
<td>Mineral mix, mg/kg of diet</td>
<td>100</td>
</tr>
<tr>
<td>ZnCO₃</td>
<td></td>
</tr>
<tr>
<td>MnSO₄·H₂O</td>
<td>160</td>
</tr>
<tr>
<td>CuSO₄·5 H₂O</td>
<td>40</td>
</tr>
<tr>
<td>Ferric citrate</td>
<td>350</td>
</tr>
<tr>
<td>KI</td>
<td>2</td>
</tr>
<tr>
<td>Na₂SeO₃</td>
<td>1</td>
</tr>
<tr>
<td>CrK(SO₄)₂-12 H₂O</td>
<td>22</td>
</tr>
<tr>
<td>+ I CN vitamin diet fortification mixture − AA</td>
<td></td>
</tr>
</tbody>
</table>

* Ascorbic acid (AA)-deficient diet for guinea pigs, from Rose and Nahrwold (24).

males, females at the end of lactation, and the latter’s progeny.

In experiments with adult males, guinea pigs initially were pair-fed high-ascorbate diet (5,000 AA mg/kg diet) or the maintenance diet (200 mg/kg). Some animals from each dietary group were killed at least 14 days (range 14–21 days) to measure intestinal uptake of AA. Subsequently, those animals originally fed high dietary ascorbate were then switched to the maintenance diet at day 14, while those originally pair-fed the maintenance diet remained on the diet. At days 7 and 14 after the diet switch, AA uptake was measured in control and experimental groups of guinea pigs (Fig. 1A).

In experiments with lactating females and their progeny (Fig. 1B), guinea pigs ~30-days pregnant were fed either the maintenance diet or the high-ascorbate diet ad libitum through birth (~30 days) and lactation. Young guinea pigs were allowed to feed ad libitum on their mother’s diet (weaning occurs at ~2 wk), and at 4 wk past birth some animals were killed for measurement of AA uptake. Also at this time (~day 14 postweaning) the remaining young that had been raised on high-ascorbate diet were switched to the maintenance diet, while young raised on the maintenance diet were left on the maintenance diet. Fourteen days later, AA uptake was measured in these two groups. The guinea pig mothers were separated from their young at 21 days postbirth and killed for measurement of AA uptake also.

Hypovitaminosis experiments. In experiments relating to ascorbate deficiency, we sought to study the effect of acute and chronic ascorbate deficiency. Previous studies of acute deficiency found that AA uptake in vivo (22) and in vitro (24) was decreased. But at 28 days acutely scurvy deficient guinea pigs can lose 30% of their weight and deaths begin to occur; hence the results could reflect generalized decreases in physiological function. Thus a goal of this experiment was to test for changes in AA uptake in chronic deficiency in the absence of wholesale degeneration of the body, and gut in particular.

We used the procedure of Ginter et al. (6) to induce chronic hypovitaminosis C. In the first 2 wk of the experiment, experimental guinea pigs were given the ascorbate-deficient diet (0 mg/kg). [Within this period, the level of AA shows a rapid fall in the tissues, but the course of body weight change is not influenced (6).] Then the scorbutogenic diet was supplemented by a maintaining dose of AA: 3.5 mg/wk administered in the morning Mondays (1 mg), Wednesdays (1 mg), and Fridays (1.5 mg). The dose was administered by feeding the guinea pigs 5 or 7.5 g of a diet containing 200 mg/kg (Table 1). The guinea pigs always ate all of this small meal and were then provided in the afternoon with ad libitum quantities of the ascorbate-deficient diet. Control guinea pigs in this study were pair-fed the ascorbate-deficient diet, but they received a sustaining dose 10 times higher in AA in their morning meal, all of which was consumed (hence 35 mg/wk).

We followed this feeding protocol for at least 8 wk. Early signs of deficiency that we noted included swelling in hind legs in association with limping and hopping, and crepitation. We kept the guinea pigs on their diets if they had these symptoms, but the day after they first
displayed reduced food uptake (measured daily) we killed them for measurement of AA uptake. Six of the pigs showed these deficiency signs and were killed at weeks 8, 10, 11, 13, and 14, and three others did not but were killed after 19–20 wk. One control guinea pig was killed each time an experimental pig was killed (with one exception).

We also performed one experiment with acutely deficient guinea pigs. Guinea pigs that had been raised on the maintenance diet were switched to ascorbate-free diet ad libitum for 27 or 29 days and then were killed for measurement of AA uptake. Controls were left on maintenance diet ad libitum throughout.

Uptake Measurements

The preparation for measuring solute uptake across the brush border (not transepithelial flux) was an excised, everted sleeve of intestine 1-cm long secured to a solid glass rod with the intestinal mucosa pointing outward (13). Measurements were begun around noon to reduce effects of any dirurnal rhythm in uptake. Guinea pigs were anesthetized (methoxyflurane) and the small intestine was isolated and perfused with ice-cold Ringer solution. Solution composition was (in mM) 128 NaCl, 20 NaHCO₃, 4.7 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, and 1.2 MgSO₄, gassed with 5% CO₂-95% O₂ to yield pH 7.3–7.4, osmolality 290 mosM. Sleeves were kept in ice-cold mammalian Ringer bubbled with 5% CO₂-95% O₂ until an uptake measurement was made (typically, uptake measurements were performed 90–180 min after dissection). After a precirculation for 5 min in Ringer at 37°C, the sleeve was incubated for 1–4 min at 37°C in a stirred (1,200 revolutions/min) and bubbled solution containing radioactive tracers. Incubation times were chosen as 1 min for D-glucose and 4 min for L-glucose on the basis of our previous studies with small mammals (11, 13), and 2 min for AA on the basis of experiments described in the APPENDIX.

In studies of total uptake (i.e., sum of carrier-mediated and passive uptake) of L-[carboxyl-¹⁴C]AA (10–20 mCi/mm; Amersham), [1,2-³H]polylethylene glycol (PEG; mol wt 4,000, 0.5–2 mCi/g; Du Pont) was used to correct for AA in adherent fluid. In studies of carrier-mediated uptake of D-[6-³H]glucose (20–40 Ci/mm; Amersham), L-[¹⁴C]glucose (20–40 mCi/mm; Amersham) at tracer concentration was used to correct simultaneously for D-glucose in adherent fluid and for D-glucose taken up passively. In studies of passive L-[¹⁴C]glucose uptake, [1,2-³H]PEG was used to correct for L-glucose in adherent fluid.

After incubation for measurement of D-glucose uptake, tissues were rinsed for 20 s in 30 ml Ringer solution at 2°C to reduce the amount of radiolabeled glucose in adherent fluid (13). Because PEG has a much lower diffusion coefficient than either AA or L-glucose, tissues used to measure uptake of those solutes were blotted to accomplish the same goal. After rinsing or blotting, 1-cm lengths of tissue were removed from the rods for weighing, solubilization, and scintillation counting (13).

Uptake at 0.5 and 0.05 mM AA was measured in ileum of most guinea pigs. These concentrations (which bracket the apparent Kₘ for AA uptake; see below) were chosen for routine measurements because (as will be shown subsequently) they are low enough so that the majority (61–66%) of uptake is due to carrier-mediated transport but high enough to be measured reliably. Additionally, in some groups we also measured AA uptake at other concentrations or at other intestinal positions. To determine whether alterations in uptake were specific for AA, we performed two tests. We used measures of passive D-glucose uptake as a general probe for altered surface area, a possible explanation for altered AA uptake. Carrier-mediated D-glucose uptake at 50 mM was used as a general probe for AA altered Na⁺-coupled transport, another possible explanation for altered AA uptake. At this concentration, uptake is nearly Vₘₐₓ, because with the everted sleeve preparation the apparent Kₘ is ≤5 mM in rodents (9).

Minimization of errors caused by autoxidation of ascorbic acid. AA reversibly oxidizes to dehydroascorbic acid (DHAA), which in turn undergoes an irreversible hydrolysis to its immediate product, diketogulonic acid. Thus the use of radiolabeled AA to measure epithelial transport of AA requires special procedures for preserving AA in its reduced form and ensuring that one is indeed measuring AA transport and not transport of its oxidation products.

L-[carboxyl-¹⁴C]AA was received as a freeze-dried solid guaranteed to be 97% pure. It was dissolved in 15% HPO₃ (0.025 μCi AA/μl) and stored in a nitrogen environment at -20°C (R. C. Rose, personal communication). We used paper chromatographic procedures (Gelman Sciences ITLC-SA, solvent system acetonitrile-butylcyronitrile-7% metaphosphoric acid in water (60:30:2:10 by volume)) (7) to confirm its purity and to study its rate of autoxidation in our oxygenated Ringer solution. Our analysis revealed >94% purity. In Ringer solution at 37°C bubbled with 5% CO₂-95% O₂, labeled AA at tracer concentration decreased linearly with time at a rate of 0.7 ± 0.1%/min (n = 9 time points, r = -0.95, P < 0.001). Diketogulonic acid increased linearly, whereas no measurable diketogulonic acid was recovered. Winkler (30) also found that most AA proceeded quickly to diketogulonic acid under similar conditions.

Subsequent experiments using L-[carboxyl-¹⁴C]AA to measure uptake were executed in a manner in which labeled AA was never thawed and in solution >6 min, thereby holding the extent of autoxidation to <5%. Several hours before experiments concentrated mixtures (120 μl) of unlabeled and labeled AA were prepared (including 7% HPO₃) and then immediately refrozen. Sixty seconds before an incubation, the solution was thawed and mixed with 8.0 ml Ringer labeled with [³H]PEG to yield the desired final concentration of AA in the 8-ml solution (0.5 μCi AA and 4 μCi PEG/8 ml). Then two aliquots (50 μl) were removed for scintillation counting and tissue incubations were performed.

As judged from our transport measurements, these procedures were effective at enabling us to study regulation of transport of AA, distinct from its oxidation products. This is so because we found alterations in Na⁺-dependent AA uptake (see RESULTS), and only the reduced form is known to be transported across the brush
border by a Na⁺-dependent mechanism (1). It is possible that Na⁺-independent uptake measured by our methods includes some uptake of oxidation products.

**Morphometric Measurements**

To test for changes in intestinal morphology that may have resulted from hyper- or hypovitaminosis and affected nutrient uptake, we measured four intestinal parameters in all guinea pigs: 1) intestinal length (to the nearest 0.5 cm) from pylorus to the ileal-cecal junction; 2) intestinal wet mass per centimeter (±0.1 mg); 3) proportion that mass of scraped mucosa bears to mass of whole intestinal thickness, measured by cutting a 2-cm piece of intestine and scraping off the mucosa with a glass slide and determining its dry mass (±0.1 mg) and that of the remaining tissue; and 4) nominal surface area (i.e., excluding contribution of villi) per centimeter, measured by cutting open a 2-cm sleeve of intestine and measuring its width to the nearest millimeter. We report most nutrient uptake rates normalized to a 1-cm length of intestine, which we consider physiologically most meaningful. Our data, however, can be compared with those of other studies that normalize uptake rates to mass or surface area through the use of the morphometric measures.

**Statistical Analysis and Presentation of Data**

Results are given as means ± SE. Statistical differences as a function of diet were determined by several tests (29), depending on the data set. For comparisons of single measures between control and experimental guinea pigs, t tests were used. Statistical differences within an entire data set (i.e., among several experiments, as a function of diet) were determined by an analysis of variance (ANOVA) followed by Tukey's least significant difference test. Simultaneous tests for effects of intestinal position on uptake within guinea pigs, and diet effects on uptake between guinea pigs, were made by repeated measures ANOVA (29). For fitting uptake rates to kinetic models to derive kinetic parameters, and for comparing kinetics of uptake rates in different diet groups, we used the procedures recommended by Motulsky and Ransnas (17). The P < 0.05 level was considered significant.

**RESULTS**

**Effects of Hyper- and Hypovitaminosis C on Food Intake, and Mass Change**

There was no significant difference in food intake between guinea pigs eating high-ascorbate diet or maintenance diet (P = 0.76, ANOVA), whether intake was ad libitum (the case for juveniles and pregnant/lactating females) or controlled (i.e., paired intake in adult males). Guinea pigs fed high ascorbate did not display significantly different patterns of body mass change when compared with those fed maintenance diet (P = 0.56, ANOVA) (Table 2). Regardless of diet, juveniles increased in mass faster than medium-sized adults, large adult males showed no change in body mass, and females over the interval of pregnancy to the end of lactation showed small decreases in body mass (Table 2).

Chronically deficient guinea pigs fed low ascorbate were pair-fed with their controls and so food uptakes were not significantly different (P > 0.3), but their rate of mass gain was significantly lower (P < 0.001) (Table 2). Acutely deficient guinea pigs fed ascorbate-free diet ad libitum had both significantly lower feeding rates (P = 0.01) and rates of mass change (P < 0.001) than those eating maintenance diet (Table 2). After 4 wk guinea pigs in the former group appeared weak and sickly.

**Effects of Hyper- and Hypovitaminosis on Morphometric Measurements**

Hyper- and hypovitaminosis had no significant effect on intestinal length (P = 0.95 by ANOVA). In the maintenance vs. high-ascorbate diet comparison, small intestinal lengths (and corresponding body masses) were, respectively, younger males and females, 142 ± 4 cm (n = 9 on maintenance, 296 ± 15 g body mass) vs. 141 ± 2 cm (n = 6 on high ascorbate, 338 ± 11 g); adult males,

**TABLE 2. Food intake and body mass change of guinea pigs fed different diets**

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Intake</th>
<th></th>
<th>Body Mass</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean, g/day</td>
<td>P*</td>
<td>Initial, g</td>
<td>Change, %/wk</td>
</tr>
<tr>
<td><strong>High ascorbate vs. maintenance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>Maintenance</td>
<td>27±1 (6)</td>
<td>&gt;0.5</td>
<td>838±31 (6)</td>
<td>-1±3 (6)</td>
</tr>
<tr>
<td></td>
<td>High ascorbate</td>
<td>27±1 (6)</td>
<td></td>
<td>830±45 (6)</td>
<td>0±2 (6)</td>
</tr>
<tr>
<td>Females, pregnancy through lactation</td>
<td>Maintenance</td>
<td>41±7 (5)</td>
<td>&gt;0.5</td>
<td>927±51 (7)</td>
<td>-4±1 (5)</td>
</tr>
<tr>
<td></td>
<td>High ascorbate</td>
<td>46±7 (6)</td>
<td></td>
<td>881±56 (7)</td>
<td>-1±1 (3)</td>
</tr>
<tr>
<td>Younger males and females</td>
<td>Maintenance</td>
<td>16±1 (5)</td>
<td>&gt;0.5</td>
<td>84±5 (9)</td>
<td>65±4 (9)</td>
</tr>
<tr>
<td></td>
<td>High ascorbate</td>
<td>16±1 (5)</td>
<td></td>
<td>96±6 (9)</td>
<td>65±5 (9)</td>
</tr>
<tr>
<td><strong>Chronic deficiency vs. maintenance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>Maintenance</td>
<td>24±1 (8)</td>
<td>&gt;0.5</td>
<td>369±4 (8)</td>
<td>8±1 (8)</td>
</tr>
<tr>
<td></td>
<td>Low ascorbate</td>
<td>25±1 (9)</td>
<td></td>
<td>407±13 (8)</td>
<td>4±1 (9)</td>
</tr>
<tr>
<td><strong>Acute deficiency vs. maintenance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males and females</td>
<td>Maintenance</td>
<td>24±2 (5)</td>
<td>0.01</td>
<td>296±15 (9)</td>
<td>15±2 (11)</td>
</tr>
<tr>
<td></td>
<td>Ascorbate free</td>
<td>16±1 (6)</td>
<td></td>
<td>288±11 (9)</td>
<td>1±1 (6)</td>
</tr>
</tbody>
</table>

Values are means ± SE (no. of guinea pigs). * P value is for comparison of mean intake or change in body mass by diet group.
Dietary Regulation of Intestinal Ascorbate Transport

153 ± 4 cm (n = 6, 814 ± 20 g) vs. 161 ± 5 cm (n = 6, 848 ± 22 g); females (pregnancy through lactation), 192 ± 7 cm (n = 5, 765 ± 45 g) vs. 186 ± 5 cm (n = 5, 816 ± 50 g). In the maintenance vs. deficiency comparisons, intestinal lengths (and corresponding body masses) were 155 ± 2 cm (n = 8 maintenance males, 634 ± 35 g) vs. 157 ± 3 cm (n = 9 chronically deficient males, 586 ± 28 g); 145 ± 4 cm (n = 12 maintenance males and females, 379 ± 17 g) vs. 144 ± 2 cm (n = 6 acutely deficient males and females, 304 ± 12 g). Notable differences not related to diet were that small intestinal length of juveniles was less than that of adults and that intestinal length of females was not significantly different from that of males at 2–4 wk postweaning (P = 0.73), but intestinal length of females at the end of lactation was considerably longer than in similar-sized adult males (P < 0.001).

Hypervitaminosis had no significant effect on gut weight per centimeter, proportion-scrapable mucosa, or nominal surface area per centimeter in any region of the gut at any stage of development that we tested (Fig. 2). Gut mass per centimeter was significantly lower in ileum (the only region tested) of acutely deficient guinea pigs compared with guinea pigs fed maintenance diet (P = 0.024). In contrast, in guinea pigs made chronically deficient, gut mass per centimeter in all regions was not significantly different from that in guinea pigs fed maintenance diet (Fig. 2). Acute and chronic hypovitaminosis had no significant effect on proportion-scrapable mucosa or nominal surface area.

Overall, dietary effects on morphometric measures of the small intestine were generally lacking, but ontogenic changes were very apparent. In the DISCUSSION, we consider how the generalized increases in these measures with increasing body mass, and in lactating females, permit the intestine to adapt to the higher feeding rates concomitant with growth and reproduction.

Effects of Hypervitaminosis on AA Uptake

For adult males and pregnant/lactating females, total ileal AA uptake was significantly lower in guinea pigs fed high dietary ascorbate than in guinea pigs fed maintenance diet (ANOVA, P < 0.025 for both groups; Fig. 3). Figure 3 also shows the AA uptake of the young born to the females, before the young’s diet switch. The expected reduction in total ileal AA uptake in the hyper-vitaminotic juveniles was observed (ANOVA, P = 0.016). For AA uptake measured in adjacent tissues in the absence of Na+, there was no significant diet effect in juveniles (P > 0.14) and adult males (P > 0.25), indicating that the effect was primarily due to reduced Na+-dependent uptake of AA. In the females there was a significant diet effect on Na+-independent uptake (P < 0.015).

There were no significant diet effects on L-glucose uptake in the ileum or on carrier-mediated D-glucose uptake (Fig. 3). Both of these measures were made using tissues from the same guinea pigs, for which there had been significant diet effects on AA uptake.

In lactating females, AA uptake was significantly reduced in midgut also, but not in proximal gut (Fig. 4). As was the case for ileum, altered AA uptake in midgut occurred in the absence of change in D-glucose uptake. In other experiments (described below) midgut AA uptake was significantly reduced in juveniles 2 wk postweaning fed high ascorbate, but not in adult males.

We used the data of Fig. 4 to estimate the total uptake capacity of the whole length of small intestine by interpolating uptake rates linearly between proximal, mid, and distal positions and summing over the intestine's length. In lactating female guinea pigs, those eating the maintenance diet had summed uptake for AA at 0.5 mM of 819 ± 98 nmol/min (n = 5) compared with 543 ± 79 nmol/min (n = 5) among those eating high dietary ascorbate (P = 0.05). This 34% reduction in summed uptake for AA occurred in the absence of any significant change in summed uptake for D-glucose at 50 mM (respectively, 29.7 ± 2.8 vs. 32.7 ± 3.3 μmol/min; P > 0.5). Similarly, juveniles fed high dietary ascorbate exhibited a 27% reduction in summed uptake compared with those fed maintenance diet (P = 0.04) in the absence of any significant change in summed glucose uptake (P > 0.5) (see below, Test for Upregulation in Juveniles).
Ascorbate Acid Uptake as a Function of Concentration in Ileum and Jejunum

To determine the kinetic basis for reduced AA uptake in hypervitaminosis, as well as differences in AA uptake with intestinal position, we performed additional measurements at other concentrations besides 0.05 and 0.5 mM AA. In preliminary experiments (not shown) we found that from 1 to 10 mM, total AA uptake was linear with concentration (n = 17 measurements at 4 concentrations, r = 0.99, P < 0.001), whereas below 1 mM, total uptake increased sublinearly with concentration. Because AA transport was nearly saturated at concentrations >1 mM, we performed more detailed kinetic determinations at concentrations ≤1 mM.

In adult male guinea pigs, Na+-independent uptake was measured at two to four concentrations in every guinea pig in either jejunum or ileum, and the apparent passive permeability coefficient (P*) was estimated in each pig from either the slope of uptake vs. concentration (Fig. 5) or the ratio of uptake to concentration. The latter procedure proved valid because in 12 guinea pigs the value of P* calculated from the slope was not significantly different from that calculated using the ratio; P > 0.5.) In both ileum and jejunum there was no significant effect of diet on P* (P > 0.25) (Fig. 5), nor was there a significant difference between the regions in P* (P* in jejunum was 6.0 ± 0.8 vs. 4.2 ± 0.3 μl/min in ileum; P = 0.08).

Total AA uptake measured in the presence of Na+ increased sublinearly in both ileum and jejunum, indicating the involvement of a carrier-mediated process (Fig. 5). Indeed, these data were better fit to a model of diffusion plus a carrier-mediated process than to one of simple diffusion alone (e.g., for ileum, F0.58 = 31.8, P < 0.001) and could not be fit to a model with two carrier-mediated processes. In ileum there was a significant effect of diet on total uptake measured in the presence of Na+ (P < 0.01). V_{max} of guinea pigs fed high AA diet (1.7 ± 0.4 nmol·min^{-1}·cm^{-2}; n = 5) was reduced by ~50% compared with control guinea pigs (2.6 ± 0.3; n = 5), whereas apparent K_{m} values (K_{m*}) were similar (respectively, 0.21 ± 0.05 vs. 0.23 ± 0.05 mM). In jejunum of adult males there was no significant effect of diet on carrier-mediated uptake (F_{2,08} = 0.11, P > 0.25).

On average, the V_{max} for AA uptake per centimeter in
were switched back to the maintenance diet and were killed at days 7 and 14 for measurement of AA uptake at 0.05 and 0.5 mM (see Fig. 1A). To reduce the effect of variability among batches of guinea pigs, uptake rates of experimental guinea pigs (those whose diet was switched) were expressed relative to uptake rates of control guinea pigs (those on the maintenance diet throughout) from the same batch and measured on the same day (Fig. 6). Whereas guinea pigs that had been fed high-ascorbate diet 2 wk had significantly reduced uptake rates at both concentrations, guinea pigs that were then switched to the maintenance diet for 7 or 14 days had AA uptake rates not significantly different from those measured simultaneously in control guinea pigs (for 7 days, \( P = 0.133 \) by ANOVA; for 14 days, \( P > 0.5 \) by ANOVA). Thus AA uptake decreased reversibly with dietary ascorbate level.

**Test for Upregulation in Juveniles Born of Mothers Fed High Dietary Ascorbate**

To determine whether AA uptake in young animals can be influenced in any irreversible way by their early nutrition in utero or postweaning, juveniles eating high-ascorbate diet and born of mothers that were fed high-ascorbate diet during pregnancy and lactation were switched to the maintenance diet 14 days after weaning (see Fig. 1B). Before the diet switch, guinea pigs that had been raised on high-ascorbate diet, and were born of mothers fed high-ascorbate diet, had significantly reduced AA uptake rates at 0.5 mM in mid- and distal gut with no significant difference in proximal gut (Fig. 7). In contrast, guinea pigs that were then switched from the high-ascorbate to the maintenance diet for 14 days had AA uptake rates at all intestinal positions not significantly different from those measured simultaneously in

---

**Fig. 5.** Total uptake (±SE) of L-ascorbic acid in the absence (squares) and presence (circles) of Na\(^+\), as a function of concentration in midgut (A; \( n = 6 \) in each case) and ileum (B; \( n = 5 \) in each case) of adult male guinea pigs fed for 2 wk the maintenance diet (open symbols) or the high-ascorbate diet (solid symbols). Line of dots in both figures are linear least-squares fits of Na\(^+\)-independent uptake. Solid and dashed curves are nonlinear least-squares fits of uptake in the presence of Na\(^+\) to the equation: uptake = \( P^* \times \text{conc} + [(V_{\text{max}} \times \text{conc})/(K_{\text{m}} + \text{conc})] \), where \( P^* \) is the respective slope of the Na\(^+\)-independent line. Overall, uptake by ileum of guinea pigs fed high-ascorbate diet (○—○) was significantly lower than that for guinea pigs fed maintenance diet (■—■) (\( P < 0.01 \) by ANOVA), but there was no effect of diet in midgut. * Significant differences at each concentration (\( P < 0.05, t \) test). Fitted kinetic parameters for these curves are presented in text.

jejunum (4.5 ± 0.3 nmol·min\(^{-1}·cm\(^{-1}\); \( n = 12 \) in both diet groups) was almost twice as high as in ileum, and \( K_{\text{m}}^* \) (0.23 ± 0.05 mM) was similar. Although the measurements on jejunum and ileum shown in Fig. 5 were made in different guinea pigs, the apparent positional difference holds up when values from the same individuals are compared. At 0.01 mM, for example, AA uptake (in nmol·min\(^{-1}·cm\(^{-1}\)) was 0.22 ± 0.015 in midgut vs. 0.153 ± 0.008 in distal gut of guinea pigs fed maintenance diet (\( n = 6 \)), and 0.201 ± 0.016 in midgut vs. 0.104 ± 0.008 in distal gut of guinea pigs fed high dietary ascorbate (\( n = 6 \)) (\( P < 0.005 \) for comparison of mid- and distal gut and \( P < 0.005 \) for comparison by diet in distal gut).

Thus the kinetic studies indicated that the basis for differences in AA uptake according to diet, or position in the gut, was altered \( V_{\text{max}} \), with no change in \( K_{\text{m}}^* \) or \( P^* \).

**Time Course for Upregulation**

To study the reversibility of the downregulation of AA uptake, guinea pigs initially fed the high-ascorbate diet

**Fig. 6.** Time course for effect of change in dietary ascorbate level on total AA uptake at 0.05 mM (triangles) and 0.5 mM (squares) in ileum of adult males. To reduce the effect of variability among batches of guinea pigs and experimental days, uptake rates of experimental guinea pigs (those whose diet was switched) are expressed relative to uptake rates of control guinea pigs (those on the maintenance diet throughout) from the same batch and measured on the same day. Dashed line designates relative uptake (=1.0) if uptake in both groups were the same. Vertical bars give SE of means (\( n ≥ 5 \) in each case). At time 0, guinea pigs that had been fed high-ascorbate diet 2 wk (open symbols) had significantly reduced uptake rates at both concentrations (at 0.5 mM, \( P < 0.01 \); at 0.05 mM, \( P < 0.025 \)). Guinea pigs that were then switched to the maintenance diet for 7 or 14 days (solid symbols) had AA uptake rates similar to those measured simultaneously in control guinea pigs (for 7 days, \( P = 0.133 \) by ANOVA; for 14 days, \( P > 0.5 \) by ANOVA). Thus AA uptake decreased reversibly with dietary ascorbate level.
control guinea pigs raised on maintenance diet and born of mothers fed maintenance diet (Fig. 7). After the diet switch, summed uptake over the entire length of small intestine was not significantly different in experimentals and controls both for AA at 0.5 mM [respectively, 409 ± 28 (n = 10) vs. 386 ± 29 nmol/min (n = 12); P > 0.9] and for D-glucose at 50 mM (respectively, 16.9 ± 2.3 vs. 15.8 ± 1.9 μmol/min; P > 0.9). Thus, even in juveniles raised on high dietary ascorbate, the reduction in AA uptake was reversible within 2 wk.

Our measurements with juveniles included some made on littersmates and therefore did not constitute entirely independent measures of nutrient transport. However, when we pooled the data according to litter and then tested for an effect of diet, the differences apparent in Fig. 7 were still statistically significant (ANOVA 1-tailed, P = 0.034).

Effects of Hypovitaminosis on AA Uptake

There was no significant difference in ileal uptake of AA, L-glucose, or D-glucose between guinea pigs eating the maintenance diet and those chronically or acutely deficient (Fig. 8), nor were there significant effects on uptake in other regions of the gut in chronically deficient guinea pigs (Fig. 9). Summed uptake rates for maintenance-fed (n = 8) and chronically deficient (n = 9) guinea pigs were, respectively, for AA uptake at 0.5 mM, 458 ± 28 vs. 441 ± 31 nmol/min (P = 0.68); and for D-glucose at 50 mM, 26.7 ± 1.1 vs. 26.1 ± 1.3 μmol/min (P = 0.76).

**DISCUSSION**

Our results confirm the finding of Rose and Nahrwold (24) that high dietary ascorbate causes a reduction of up to 50% in ileal uptake of AA in adult guinea pigs. Our findings extend the original finding to include other stages of the life cycle (juveniles, reproducing females) and other regions of the gut (midgut). We now discuss this phenomenon in relation to its human health significance, and as an example of regulation of an intestinal nutrient transporter according to its substrate levels and according to whole animal requirements during growth and reproduction.

**Role of Downregulation of AA Transport in Rebound Scurvy**

In human infants, scurvy can occur despite an adequate daily uptake of vitamin C if the mother has re-
ceived large supplements of the vitamin during pregnancy. Cochrane (3) ascribed this to an inductive “ascorbic acid dependency.” Adults have also been found to be subject to this phenomenon, which has been called rebound scurvy (25). This condition can be reproduced in offspring of pregnant guinea pigs fed high dietary ascorbate (21) and in adult guinea pigs (27).

Theoretically, downregulated intestinal AA absorption could contribute to the development of rebound scurvy by reducing the bioavailability of typically normal inputs of vitamin C. Because the human intestine extracts <90% of ingested ascorbate at intakes of ≤200 mg/day (8, 15), presumably the intestine’s capacity for absorption is not excessive in comparison to normal uptake of ascorbate. In this situation, a reduction in rate of absorption would cause a proportionally equal reduction in extraction efficiency (hence bioavailability) because the latter is the product of rate of absorption and the mean retention time of digesta in the small intestine.

Despite this theoretical possibility, the following calculations indicate that downregulation of AA absorption is probably not a major factor in the development of rebound scurvy, at least in adults. If humans experienced a one-third downregulation of total intestinal uptake capacity after ingestion of high doses of ascorbate, as did the guinea pigs, then the bioavailability of a lower recommended dose of vitamin C (60 mg/day; see Ref. 18) might be reduced to ~45%. While 27 mg/day (i.e., 0.45 × 60 mg/day) is less than the daily rate of catabolism of AA (34–60 mg/day; see Ref. 18), the amount of time before the normal body pool (~1,500 mg; see Ref. 18) was reduced to a level where scurvy would occur (300 mg; see Ref. 18) would be at least a month (i.e., [1,500 mg – 300 mg]/[60 mg/day catabolized – 27 mg/day absorbed] = 36 days). Because the reduction in absorption in guinea pigs was reversible already by a week, presumably in humans intestinal absorption of vitamin C would return to normal higher levels well before the onset of scurvy.

We hasten to point out, however, that our conclusion was based on several assumptions: that downregulation in response to ingestion of high dietary ascorbate occurs and is reversible in humans as in guinea pigs. Also, in juveniles, body stores are apparently depleted more rapidly (at least in guinea pigs; Norkus and Rosso (21)) and this would increase the likelihood that decreased absorption of dietary ascorbate might play a role in development of rebound scurvy in juveniles of mothers ingesting high dietary ascorbate.

If decreased bioavailability of dietary ascorbate is not the major cause of rebound scurvy, what is? In addition to reduced intestinal ascorbate absorption, both adult and juvenile guinea pigs exposed to high dietary ascorbate have increased rates of catabolism, as measured by 14CO2 excretion (21, 27). In adults, the accelerated catabolism was not reversible after more than two months on subnormal uptake of ascorbate. Thus the most significant alteration in guinea pigs exposed to high dietary ascorbate is probably an induction of AA-metabolizing enzymes. Future research might focus on the time course for downregulation of these enzymes.

Regulation of the Intestinal Ascorbate Transporter by Its Substrate

Knowledge about regulation of intestinal transporters can be organized according to the patterns, signals, kinetic mechanisms, and time courses of regulation (5, 12). We know more about regulation of transport of AA than any other water-soluble vitamin.

Pattern of regulation. It has been predicted that a vitamin transporter should be downregulated by its substrate if the biosynthetic and other costs of synthesizing and maintaining the transporter exceed the benefits the transporter provides (5, 12). Intestinal AA transport provides a nice illustration of this maxim of “enough but not too much” (5). Because ascorbate requirements, and concentrations in foods, are low in comparison with macronutrients, the number of AA transporters is far below that for the glucose transporter (Vmax values differ by almost an order of magnitude). Also, as described above, the human intestine’s capacity to absorb ascorbate is not excessive in comparison to normal intakes of the vitamin. When uptake is increased to supernormal levels over a period of time, carrier-mediated transport rate is reduced, though AA continues to be absorbed passively. It might seem surprising that AA transport is not downregulated even more than 50%. But it seems to be the typical pattern for vertebrate intestinal nutrient transporters to have peak-to-base activity ratios of ≤2 (5).

One might also predict (4, 12) that the vitamin absorption rate would be upregulated in deficiency. However, if extraction efficiency is already nearly complete (as in humans for ascorbate), there is little to be gained. Thus it is perhaps not surprising that in guinea pigs acutely or
chronically deficient in ascorbate, AA uptake rate was unchanged or slightly reduced (probably due to deterioration of intestinal mucosa).

**Kinetic mechanism.** The kinetic basis for the difference in AA uptake according to diet was an altered $V_{\text{max}}$ with no change in apparent $K_m$ or passive permeation (Fig. 5). This is the commonly observed mechanistic basis for regulatory changes in intestinal nutrient transporters by their dietary substrates (5). The simplest interpretation for reduced $V_{\text{max}}$ for AA uptake is a decreased number of AA transport sites per cell, due either to decreased rate of synthesis or increased rate of degradation. Other explanations for a change in $V_{\text{max}}$ are possible: change in electrochemical gradient for Na$^+$ during Na$^+$-coupled transport, changes in membrane lipid, and altered membrane area or number of cells. Such explanations are rendered implausible by the fact that dietary ascorbate specifically reduced carrier-mediated AA uptake while carrier-mediated (Na$^+$-dependent) D-glucose uptake was unchanged, as was Na$^+$-independent AA uptake (with the exception of pregnant/lactating females). In addition, we failed to observe dietary differences in any measure of quantity of absorptive tissue.

**Time course.** The downregulation of intestinal AA uptake was reversible in adult males within ≤7 days (Fig. 6). This is consistent with previous findings of adaptation of brush-border nutrient transporters within 1–8 days (5, 12). Even in juveniles born of females fed high-ascorbate diet during pregnancy and lactation, and which were themselves fed high-ascorbate diet postweaning, the downregulation of intestinal AA uptake was reversible within at most 14 days (Fig. 7). This is consistent with another study (14) that failed to find evidence that intestinal transport of sugars and amino acids could be fixed irreversibly at some critical point in early life.

**Proximate signal.** Does downregulation of AA transport occur directly in response to high-ascorbate concentration in the intestinal lumen or blood? Rose and Nahrwold (24) found that AA uptake was reduced by intramuscular administration of AA, suggesting that the transport mechanism may respond to circulating levels of the vitamin. There have been no studies as to whether an intermediate signal such as a hormone is involved.

In summary, AA downregulates its brush-border transporter, as predicted for an essential nutrient that does not yield calories. The simplest explanation is alteration in number of transporters occurring over the course of days in response to ascorbate levels in the blood.

**Regulation of Intestinal Transport in Growth and Reproduction**

We prepared a composite of the response of the entire small intestine in guinea pigs (as opposed to just the ileum) to increased food intake during growth and reproduction by drawing on data from the tables and figures in RESULTS for those guinea pigs fed the maintenance diet (Table 3). Among guinea pigs classified as juveniles (2–4 wk postweaning, body mass <400 g), adult (body mass >600 g), and lactating, the range in body mass, food intake, intestinal nominal surface area, and summed uptake of glucose and AA was about twofold. From these

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Mass, g</th>
<th>Food Intake, g/day</th>
<th>Surface Area, cm$^2$</th>
<th>Ascorbic Acid Uptake, mmol/min</th>
<th>d-Glucose Uptake, mmol/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subadult</td>
<td>296$^*$</td>
<td>16$^*$</td>
<td>130$^*$</td>
<td>386$^*$</td>
<td>15.8$^*$</td>
</tr>
<tr>
<td>Adult</td>
<td>644</td>
<td>24$^*$</td>
<td>191$^*$</td>
<td>458</td>
<td>26.7$^*$</td>
</tr>
<tr>
<td>Lactating</td>
<td>765$^*$</td>
<td>41$^*$</td>
<td>261$^*$</td>
<td>819$^*$</td>
<td>29.7$^*$</td>
</tr>
</tbody>
</table>

Values are means and were drawn from text, tables, and figures. Within a column, values that do not share a common superscript are significantly different ($P < 0.05$ or smaller) by Tukey's least-significant difference test (29).

Data one can make at least two inferences.

First, the growth of the intestine, and not increased uptake per unit tissue, is the main reason why intestinal uptake capacity for d-glucose and AA increases with age (size) or reproduction. Uptake capacity normalized to intestinal surface area did not differ significantly between the three groups in Table 3 ($P > 0.109$ or greater by Tukey's multiple-range test). Indeed, except for the fact that AA uptake was downregulated in jejunum of lactating females and juveniles but not adult males (compare Figs. 4, 5, 7), we observed little ontogenetic variation in nutrient absorption at the tissue level. The uptake per square centimeter nominal area of another vitamin, choline, also did not significantly change during growth (26) and reproduction (10) in rats and mice. Increased intestinal growth is the major explanation for increased sugar and amino acid absorbing capability during growth and reproduction in other mammals as well (2, 12).

Second, considering how well matched the intestine's growth and transport capacity is to the increases in food intake during growth and reproduction, one might predict that extraction efficiency (i.e., the proportion of ingested nutrients digested and absorbed) will be fairly independent of age or reproductive status in mammals. Although data are generally lacking to test this idea for particular nutrients such as glucose and ascorbic acid, it is known that in growing and reproducing small mammals, increases in daily food intake of two to three times are achieved without any decrease in digestive efficiency of the entire diet (28).

**APPENDIX**

**Selection of Incubation Time**

We incubated tissues for various times in Binger solution containing tracer PEG and AA (0.05 mM) and determined tissue uptake after blotting the tissue. Adjacent tissues from the same animals were incubated to minimize interindividual variation; one piece incubated for 2 min was a control, and the adjacent piece was incubated for 1 or 4 min.

Uptake proved to vary linearly with time to at least 2 min, as evidenced by uptakes at 2 min that were 1.89 ± 0.20 times uptakes at 1 min ($n = 9$ pairs of tissues). Uptakes at 4 min, however, were significantly less than double the uptakes at 2 min (ratio = 1.55 ± 0.09, $n = 8$ tissue pairs; $P < 0.005$ for difference from 2.0). PEG space, which was used to correct measured AA uptake for AA in the adherent fluid, was similar at 1 min (0.22 ± 0.02 μl/mg), 2 min (0.23 ± 0.01 μl/mg), and 4 min (0.23 ± 0.02 μl/mg). Thus the PEG space had equilibrated...
by 1 min. Therefore, in subsequent studies of AA uptake, we used 2-min incubations to ensure AA uptakes large enough to measure accurately but still in the linear uptake-vs.-time range.

One-minute incubations were used to measure D-glucose uptake, and 4-min incubations were used to measure L-glucose uptake. The incubation times for glucose were chosen because they have been validated in studies with seven other species of rodents (e.g., 11, 13).

We thank R. C. Rose for advice during the initial phase of this project.

This material is based on work supported by the Cooperative State Research Service, US Department of Agriculture under Agreement No. 87-CR-1-2311, and the National Science Foundation (BIB8453089).

Address for reprint requests: W. H. Karasov, Univ. of Wisconsin, 226 Russell Labs, 1630 Linden Dr., Madison, WI 53706.

Received 24 May 1990; accepted in final form 30 August 1990.

REFERENCES


