Intestinal transport of monosaccharides and amino acids during postnatal development of mink

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1Department of Biological Sciences, Mississippi State University, Mississippi State, Mississippi 39762; 2Membrane Transport Research Group, Department of Physiology, University of Montreal, Montreal, Quebec, H3C 3J7 Canada; and 3Fur Animal Science, Department of Animal Science and Animal Health, DK-1870 Frederiksberg C, Denmark

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Buddington, Randal K., Christiane Malo, Per T. Sangild and Jan Elnif. Intestinal transport of monosaccharides and amino acids during postnatal development of mink. Am J Physiol Regulatory Integrative Comp Physiol 279: R2287–R2296, 2000.—Intestinal development is typically studied using omnivores. For comparative purposes, we examined an altricial carnivore, the mink (Mustela vison). In mink, intestinal dimensions increase up to 8 wk after birth and then remain constant (length) or decrease (mass) into maturity despite continuing gains in body mass. Rates of glucose and fructose transport decline after birth for intact tissues but increase for brush-border membrane vesicles (BBMV). Rates of absorption for five amino acids that are substrates for the acidic (aspartate), basic (lysine), neutral (leucine and methionine), and imino acid (proline) carriers increase between birth and 24 h for intact tissues before declining, but increase after 2 wk for BBMV. The proportion of BBMV amino acid uptake that is \( Na^+ \)-dependent increases during development but for aspartate is nearly 100% at all ages. Tracer uptake by BBMV can be inhibited by 100 mmol/l of unlabeled amino acid, except for lysine. BBMV uptake of the dipeptide glycyl-sarcosine does not differ between ages, is not \( Na^+ \)-dependent, and is only partially inhibited by 100 mmol/l unlabeled dipeptide. Despite the ability to rapidly and efficiently digest high dietary loads of protein, rates of amino acid and peptide absorption are not markedly higher than those of other mammals.

INTESTINAL STRUCTURE AND FUNCTIONS change during ontogenetic development of mammals, either in anticipation of, or in response to, shifts in diet composition. The changes in intestinal structure and functions that occur at weaning are the best-known examples. These include a decrease in villus height, increase in crypt depth, increase in gastric proteases, and the well-known reciprocal shift in the intestinal activities of lactase and sucrase (19). The magnitude and timing of the age-related changes are the result of dietary inputs interacting with genetic determinants. Corresponding with this, age-related changes do not occur at the same time for all species or for the various digestive functions. For example, sucrase and lactase are expressed early in gestation of human fetuses, and although lactase is detected before birth in species such as the rat and mouse, sucrase does not develop until the time of weaning (19).

Patterns of development for the intestinal apical transporters also vary among species and solutes (3, 12). For example, apical transporters for glucose and amino acids appear during gestation at the time the enterocytes differentiate, whereas carrier-mediated fructose transport does not develop until the time of birth in most precocial species, such as pigs, and not until weaning in altricial rodents, such as rats and mice. Another dramatic example is the appearance of the bile acid transporters at weaning, with expression restricted to the distal ileum.

Development of digestive functions are much better known for omnivores compared with carnivores, which undergo a markedly different shift in dietary inputs at weaning. The few carnivorous mammals that have been studied (dog and cat) are born at a relatively advanced stage of development. Mink provide an interesting comparison for three reasons. First, mink are born altricial, much like laboratory rats and mice are being comparison for three reasons. First, mink are born altricial, much like laboratory rats and mice are
though food transits the intestine rapidly [3–4 h in adults (2, 14)], protein digestibility is high (21).

From these interesting characteristics of the mink, we set out to characterize age-related changes in the rates and regional distribution of nutrient absorption. The expectation was that because of the carnivorous diet, rates of absorption for amino acids and peptides would be higher for mink compared with omnivores, and also relative to other carnivores because of the rapid and efficient digestion of the high dietary loads of protein. This was examined by measuring from birth to maturity rates of absorption along the entire length of the intestine during the first 1 wk of life for small intestine. Older mink were sedated with ketamine hydrochloride (Ketominol Vet; Veteraria, Zurich; 50 mg/kg im) and placed within 1 min in cold (2–4°C) Ringer solution that contained 25 mmol/l of unlabeled amino acid. The selection of 25 and 50 mmol/l for mink was based on Michaelis constant (K_m) of 1–10 mmol/l that we and others have measured using the same method to study other mammals (15, 16; unpublished data for pigs). The expectation was that because of the carnivorous diet, rapid and efficient digestion of the high dietary loads of protein. This was examined by measuring from birth to maturity rates of absorption along the entire length of the intestine during the first 1 wk of life for small intestine. Older mink were sedated with ketamine hydrochloride (Ketominol Vet; Veteraria, Zurich; 50 mg/kg im) and placed within 1 min in cold (2–4°C) Ringer solution that contained 25 mmol/l of unlabeled amino acid. The selection of 25 and 50 mmol/l for mink was based on Michaelis constant (K_m) of 1–10 mmol/l that we and others have measured using the same method to study other mammals (15, 16; unpublished data for pigs). The expectation was that because of the carnivorous diet, rapid and efficient digestion of the high dietary loads of protein. This was examined by measuring from birth to maturity rates of absorption along the entire length of the intestine during the first 1 wk of life for small intestine. Older mink were sedated with ketamine hydrochloride (Ketominol Vet; Veteraria, Zurich; 50 mg/kg im) and placed within 1 min in cold (2–4°C) Ringer solution that contained 25 mmol/l of unlabeled amino acid. The selection of 25 and 50 mmol/l for mink was based on Michaelis constant (K_m) of 1–10 mmol/l that we and others have measured using the same method to study other mammals (15, 16; unpublished data for pigs). Therefore, it was speculated that if the amino acid transporters of mink are similar to those of other mammals, 25 mmol/l should be sufficiently high to study other mammals (15, 16; unpublished data for pigs). Therefore, it was speculated that if the amino acid transporters of mink are similar to those of other mammals, 25 mmol/l should be sufficiently high to↓

**Animals and Their Care**

Mink of the mahogany strain were obtained from the Department of Animal Science and Animal Health of the Danish Royal Veterinary and Agricultural University (Copenhagen). The use of the animals followed the guidelines approved by the Member States of the Council of Europe. The mink were housed in conventional external facilities and exposed to ambient temperatures and light conditions. A standard production diet formulated with animal by-products and with 56%, 35%, and 9% of the calories from protein, fat, and carbohydrate, respectively (unpublished findings from proximate analyses), was provided to the mink twice each day. To reduce variation for onset of solid food ingestion, small amounts of the adult diet were placed in the nest boxes when the kits were 4 wk old. The kits were allowed to suckle until 6 wk of age at which time they were separated from the females and thereafter received only the production diet.

**Sampling**

Mink kits of both sexes were obtained immediately after birth before suckling (0 h; n = 17), after suckling for 1 day (n = 17), at 1 (n = 12), 2 (n = 12), 4 (n = 12), and 6 (n = 12) wk of suckling, and at 8 wk after weaning (n = 8). Adult females were studied at least 6 wk after lactation had finished (n = 6). At each age, all of the kits originated from different litters. The 0-h, 1-day, and 1-wk-old mink were killed by decapitation. Older mink were sedated with ketamine hydrochloride (Ketominol Vet; Veteraria, Zurich; 50 mg/kg im) and xylazine (Rompun; Bayer, Leverkusen; 10 mg/kg im) before decapitation was performed (11).

After death, the entire gastrointestinal tract was removed and placed within cold (2–4°C) Ringer solution that had been aerated with a mixture of O_2 and CO_2 (95%/5%). The intestine was freed from the associated mesentery, and the length (from the pyloric sphincter to the ileocolonic junction) was measured on a table top in a relaxed state. Between 24 h and 4 wk only milk was present in the stomach and intestine, whereas both milk and the solid food were present at 6 wk.

For the 0-h, 1-day, and 1-wk-old mink, two segments of small intestine were used for measurements of sugar and amino acid transport by intact tissues. A proximal segment of 12–15 cm was taken beginning from the end of the attached pancreas. A second segment of 10–12 cm was taken at about 67% of small intestinal length. The distal-most region of the neonate intestine was too friable and small in diameter for evertion and measuring uptake by intact tissues. For all other ages the intestine was separated into three regions of equal length, which were designated as proximal, mid, and distal, and these were used to measure rates of uptake by intact tissues. For BBMV studies (2 wk and older), the small intestine was cut into proximal and distal halves to ensure availability of adequate amounts of tissue. The two regions were frozen intact in liquid nitrogen and stored at −70°C until shipped on dry ice to the University of Montreal, where they were again held at −70°C until used.

**Intact tissue measurements.** Following a previous study (4), we measured nutrient uptake from 45 min and 100 min after the animal was killed by incubating 1-cm everted sleeves for 2 min in mammalian Ringer with 50 mmol/l for each of the sugars and amino acids. At each age, rates of absorption for all nutrients were measured using a minimum of six animals that originated from different litters. Accumulation of glucose and fructose by the tissues was quantified by adding trace levels of 14C-labeled D-isomers of the sugars to the incubation solutions. L-[^3]H]glucose was also added for simultaneous correction of sugar adherent to the tissue and absorbed independent of carriers. For measuring amino acid absorption, the ^3H-labeled L-isomer was used and [14C]polyethylene glycol (4,000 mol wt) was added for correction of amino acid associated with the adherent fluid. The uptake solutions were aerated with the gas mixture to maintain tissue viability and stirred (1,200 rpm) to minimize unstirred layer effects. After the incubation, tissues exposed to the sugars were rinsed for 20 s in cold Ringer, but not those incubated in the amino acid solutions. After tissue mass was recorded, solubilizer (Optisol, Wallach Biochem) and scintillant (Optisafe 2, Wallach Biochem) were added, and radioactivity was measured by liquid scintillation counting. Calculated rates of transport (15) were normalized to tissue mass. Presented values for glucose and fructose represent carrier-mediated uptake, whereas those for the amino acids are the sum of the carrier-mediated and carrier independent components of absorption.

The regional distribution of absorption was examined by incubating sleeves from each of the different regions in 50 mmol/l solutions of the nutrients. The relationships between glucose and fructose concentrations and rates of uptake were defined by incubating tissues from the proximal small intestine (region of highest sugar uptake) (4) in solutions containing tracer alone and in the presence of 0.1, 1, 10, and 50 mmol/l of unlabeled sugar for mink kits between 0 and 4 wk of age and 0.5, 5, 25, and 50 mmol/l for animals 6 wk and older. Because of the limited number of tissues that could be prepared from the short intestine, rates of amino acid uptake by the midintestine were measured at only three concentrations (tracer alone and in the presence of 25 and 50 mmol/l unlabeled amino acid). The selection of 25 and 50 mmol/l concentrations of amino acid was based on Michaelis constant (K_m) of 1–10 mmol/l that we and others have measured using the same method to study other mammals (15, 16; unpublished data for pigs). Therefore, it was speculated that if the amino acid transporters of mink are similar to those of other mammals, 25 mmol/l should be sufficiently high to...
saturate the carriers, and the slopes of the lines between 25 and 50 mmol/l would approximate the carrier-independent influx for the amino acids.

Osmolarity of all nutrient solutions was maintained at 290 mosmol/l by an isosmotic reduction of the NaCl (maximum reduction of 25 of the 117 mmol/l), and pH was 7.4 when aerated with the gas mixture. It was necessary to adjust the pH of the aspartate solution by adding NaOH.

To determine whether a saturable component of absorption was present, we compared accumulation of tracer sugar and amino acid when present alone compared with when added to 50 mmol/l of unlabeled sugar and amino acid (accumulation ratios). We assumed that if transporters were present in limited numbers (a saturable component is present), the presence of 50 mmol/l unlabeled sugar or amino acid would reduce the accumulation of tracer due to competition. As a consequence, accumulation ratios greater than a value of 1.0 were considered to indicate the presence of a saturable component. In contrast, values not different from 1.0 would indicate that tracer influx is independent of the concentration of unlabeled nutrient and that absorption is largely by simple diffusion. This could occur if the transporters are absent or are present in very low densities.

BBMV studies. BBMV were prepared using a standard protocol (26). Accumulation of tracer by the BBMV was measured using a fast-sampling, rapid filtration device programmed to collect nine samples over the first 2.7 s of incubation for methionine, leucine, and glycyl-sarcosine; 3.6 s for aspartate, and 4.5 s for proline, lysine, and glucose. The different time periods were selected so that accumulation could be measured during the linear phase. Accumulation of tracer was studied in the presence of 0 and 200 mmol/l NaCl to determine whether uptake is sodium dependent and with 200 mmol/l NaCl and 100 mmol/l unlabeled nutrient to determine whether there is competition for transporter sites. Osmolarity of internal and external solutions was maintained constant by varying mannitol concentrations. Initial rates of BBMV uptake were calculated by linear regression analysis. When a curve deviated from linearity, the initial rate was estimated from the first-degree coefficient of the second-degree polynomial.

Chemicals

All reagents used to prepare solutions were purchased from Sigma (St. Louis, MO) and were of the highest purity available. Radiolabeled compounds were purchased from New England Nuclear (Amherst, MA: D-[^14]Cglucose, L-[^3]Hglucose, D-[^14]C]fructose, [^14]C]polyethylene glycol, and Mississaugua, ON, Canada: D-[1-^3]Hglucose) or Amersham (Oakville, ON, Canada; glycyl-[N-methyl-^3]Hsarcosine, L-[^2,3,4,5-^3]Harginine, L-[^methyl-^3]Hmethionine, L-[^4,5-^3]Hleucine, L-[^4,5-^3]Hlysine, D-[2,3-^3]H]aspartic acid, and L-[2,3,4,5-^3]H]proline).

Data Analysis and Statistics

Values presented in the text, tables, and figures are means ± SE. The main effects of intestinal region and age on rates of absorption by intact tissues were evaluated using the PROC GLM procedure of SAS (Statistical Analysis Systems, version 6.11). When a significant effect was detected, specific differences were identified by Duncan’s test. The PROC Univariate procedure of SAS was used to determine whether the accumulation ratios exceeded a value of 1.0. If so, this was considered to be indicative of competition between the tracer and unlabeled amino acid for a limited number of transporter sites, and that a portion of absorption was via a saturable pathway. For all analyses, a value of P < 0.05 was accepted as the critical level of significance.

Kinetics of sugar transport by intact tissues were defined using the Enzfitter nonlinear regression analysis program (Biosoft, Elsevier, UK). Glucose data were fit to model equations that included one and two transporters to estimate maximum rates of absorption (Vmax) and apparent affinity constants (Km). The model equation providing the best fit of the data (based on examination of the residuals) was used for estimating the kinetic parameters. Fructose data were initially fit to a linear regression and then to a model equation for a single transporter to determine whether this improved the fit. The limited number of concentrations used to study amino acid uptake precluded kinetic analysis.

RESULTS

Body Weights and Intestinal Dimensions

Age influences. Mean body mass increased from 11 ± 0.4 g at birth to 367 ± 17 g at 6 wk when the kits were weaned and was 719 ± 36 g at 8 wk. The adult females weighed 1,183 ± 101 g. Intestinal length increased from birth to 8 wk of age, but did not increase between 8 wk and maturity (Fig. 1A), despite the 65% increase in body mass. Intestinal length normalized to body mass.
mass (cm/kg) declined during development ($P < 0.05$). Double-log plots of intestinal length and body mass (data not presented) revealed a highly significant correlation ($r^2 = 0.97; P < 0.0001$) with a slope of 0.41 ± 0.008. This is greater than the value of 0.33 predicted from dimensional analysis ($P < 0.05$) (17).

Total intestinal mass (estimated by multiplying the average for weights of the 1-cm sleeves from the different regions times the length of the intestine) increased between birth and 8 wk and then actually declined 20% between 8 wk and maturity (Fig. 1B). Intestinal mass normalized to whole body mass (g/kg), doubled during the first 24 h after birth, remained stable for the next 2 wk, was higher at 4 and 6 wk, and then began to decline with the lowest values recorded from mature animals. Double-log plots of body and intestinal mass revealed a highly significant relationship ($r^2 = 0.96; P < 0.0001$) with a slope of 1.02 ± 0.02. This value does not differ from the predicted value of 1.0 (17).

Sugar and Amino Acid Uptake

**Age influences.** There was a significant effect of age on rates of uptake by intact tissues ($P < 0.01$), with the patterns varying among the different sugars and amino acids. Glucose uptake per milligram intestine at 50 mmol/l averaged from the different sites of small intestine (2 for mink 1 wk old and younger and 3 for mink 2 wk and older) was highest at birth and declined during the first 24 h of suckling and between consecutive age groups up to 4 wk (Fig. 2A; $P < 0.05$). Thereafter, rates of glucose uptake remained stable into maturity. In contrast, initial rates of tracer d-glucose uptake by BBMV in the presence of an inwardly directed 200 mmol Na\(^+\) gradient increased during suckling (Fig. 2B).

Fructose uptake by intact tissues did not change during the first 24 h after birth but had declined by 1 wk ($P < 0.05$). Values during suckling, adolescence, and maturity remained lower than those during the neonatal period (0 days to 1 wk). Interestingly, fructose uptake by the adult intestine was greater than at 6 wk ($P < 0.05$).

Rates of amino acid uptake by intact tissues exposed to 50 mmol/l were highest during the neonatal period and showed an increase during the first 24 h after birth. Values declined thereafter, with the onset of the decline varying among the four different classes of amino acids. Compared with values at 24 h after birth, the decline was already significant at 1 wk for aspartate, at 2 wk for proline (Fig. 4A), and at 4 wk for lysine, leucine, and methionine (Figs. 5A–7A). Rates of amino acid absorption at 8 wk and maturity were comparable to those measured at 6 wk, indicating that weaning to the production diet did not induce higher rates of absorption. Rates of amino acid absorption by midintestine at tracer concentration and at 25 mmol/l showed similar patterns of postnatal declines ($P = 0.0004$ and 0.0001) followed by stable values between 6 wk and maturity.

Initial rates of BBMV uptake measured in the presence of a 200 mmol/l inwardly directed Na\(^+\) gradient also varied among the different ages for each amino acid. Peak tracer accumulation was recorded at 4 weeks for aspartate (Fig. 8, B and C), at 6 weeks for methionine (Fig. 7, B and C), and after weaning for...
lysine and leucine (Figs. 5, B and C and 6, B and C). BBMV proline uptake rates increased between birth and maturity (Fig. 4, B and C), with the exception of high rates measured in the distal small intestine at 4 wk.

BBMV uptake of the dipeptide glycyl-sarcosine did not differ significantly among ages for the proximal small intestine but increased between 2 and 8 wk in the distal intestine (Fig. 9).

**Regional distribution.** A significant declining proximal-to-distal gradient of glucose uptake by intact tissues was present only during suckling (birth to 4 wk) with values in the proximal intestine averaging 1.9-fold higher (±0.3) than in the distal intestine (data not shown). From 6 wk into maturity, regional differences were not detected. The BBMV data (Fig. 2, B and C) provided a contrasting scenario in that from 2 to 8 wk initial rates of glucose uptake by BBMV prepared from proximal intestine averaged ~60% of values measured for distal intestine ($P < 0.05$). Between 8 wk and maturity there was a redistribution of BBMV glucose uptake resulting in rates by the proximal intestine exceeding those for the distal intestine by 2.3-fold ($P < 0.05$).

Rates of fructose uptake by intact tissues did not differ between regions at birth and 24 h and from week 6 to maturity. However, from week 1 to 4 there was a declining proximal-to-distal gradient for rates of fructose uptake.

A redistribution of intact tissue absorption along the length of the small intestine was detected for all five amino acids. At birth and during early neonatal development (up to 1 wk), rates of absorption averaged for all five amino acids did not differ between the two segments studied. During the remainder of suckling (weeks 2–6), rates of amino acid absorption declined from the proximal to distal regions. Before or at the time of weaning, there was a shift such that at 8 wk
and in maturity, rates of absorption were higher in the distal intestine compared with the proximal segment.

Rates of tracer accumulation by BBMV did not differ between proximal and distal intestine at any age for aspartate (Fig. 8, A and B) and glycyl-sarcosine (Fig. 9, A and B), whereas lysine uptake was higher in distal intestine at 6 and 8 wk (Fig. 5, A and B; P < 0.05). In contrast, BBMV leucine uptake was higher in the proximal intestine at 6 wk and maturity (Fig. 6, A and B; P < 0.05) but was higher for the distal intestine at all other ages. Rates of BBMV methionine uptake were higher in the proximal segment at all ages, except maturity (Fig. 7, A and B). BBMV proline uptake shifted from being higher in the distal intestine at 2 and 4 wk to becoming higher in the proximal intestine at 6 wk and older (Fig. 4, A and B).

Kinetics of sugar uptake. During the first week after birth, accumulation ratios for D-glucose uptake by intact tissue of the proximal intestine averaged 111-fold (±17) (P < 0.01). The accumulation ratios declined during the remainder of suckling (data not shown), but from 6 wk into maturity the ratios were relatively stable and averaged 6.2 ± 0.4. These data suggest that throughout development, tracer and unlabeled D-glucose compete for a limited number of transporters and provide evidence for a saturable component of carrier-mediated D-glucose uptake.

At all ages, the concentration-uptake data for glucose did not show obvious saturation kinetics (presence of distinct plateau region) and best fit a model equation for two transporters (Table 1). A high-affinity system (K_m averaged 0.55 ± 0.18 mmol/l for all ages) was evident during suckling (birth to 4 wk), but V_max values were negligible during and after weaning (from week 6 to maturity). The second system was characterized by a lower affinity (58 ± 21 mmol/l for all ages), which made it difficult to accurately define age-related changes in kinetic characteristics based on the concentrations used.

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Rates of tracer accumulation by BBMV did not differ between proximal and distal intestine at any age for aspartate (Fig. 8, B and C) and glycyl-sarcosine (Fig. 9, B and C), whereas lysine uptake was higher in distal intestine at 6 and 8 wk (Fig. 5, B and C; P < 0.05). In contrast, BBMV leucine uptake was higher in the proximal intestine at 6 wk and maturity (Fig. 6, B and C; P < 0.05) but was higher for the distal intestine at all other ages. Rates of BBMV methionine uptake were higher in the proximal segment at all ages, except maturity (Fig. 7, B and C). BBMV proline uptake shifted from being higher in the distal intestine at 2 and 4 wk to becoming higher in the proximal intestine at 6 wk and older (Fig. 4, B and C).

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Fructose accumulation ratios for intact tissues averaged 2.8 ± 0.3 for the first 2 wk after birth. During this period, the uptake-concentration data best fit a model for a single, low-affinity transport system ($K_m$ averaged 68 ± 32 mmol/l). For the remainder of suckling and at 8 wk, accumulation ratios averaged 0.6 ± 0.1. This value does not exceed 1.0, indicating there is a lack of competition between tracer and unlabeled fructose for carriers. Corresponding with these findings, fitting the uptake-concentration data for 4, 6, and 8 wk to an equation with a saturable component did not improve the fit over that obtained for linear, nonsaturable uptake. Accumulation ratios for adults (1.5 ± 0.2) were >1.0 ($P < 0.05$), and analysis of the uptake-concentration data suggested the presence of a carrier with a $K_m$ of 24 mmol/l.

**Characteristics of amino acid transport by intact tissues and BBMV.** Accumulation ratios for intact tissues from the midintestine exceeded 1.0 for all amino acids during early development (from birth to 2 wk; $P < 0.05$) and for adults ($P < 0.05$). The ratios were lower at 4, 6, and 8 wk, and during this period ratios did not differ from 1.0 for lysine and proline. BBMV accumulation of tracer aspartate, leucine, methionine, and proline was inhibited in the presence of 100 mmol/l of the corresponding unlabeled form at all ages and in both proximal and distal halves of the small intestine (Figs. 4, 6, 7, 8; B and C). Accumulation of tracer lysine by both proximal and distal intestine was only partially inhibited by 100 mmol/l unlabeled lysine (Fig. 5, B and C), with similar findings for glycy1-sarcosine (Fig. 9). Collectively, the findings from the intact tissues and BBMV suggest low-affinity amino acid carriers (not saturated at 50 mmol/l) may be present and that the increase in rates of absorption between 25 and 50 mmol/l may not be indicative of carrier-independent, diffusive influx.

BBMV uptake of tracer aspartate and proline was virtually entirely Na$^+$ dependent in both proximal and distal small intestine from 2 wk to maturity, whereas significant proportions of lysine, leucine, and methionine uptake were not Na$^+$ dependent. Accumulation of tracer glycy1-sarcosine did not differ when measured in the presence or absence of an inwardly directed Na$^+$ gradient (Fig. 9), with the exception of the proximal

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Fig. 8. Rates of total L-aspartate absorption (carrier mediated and carrier independent) by intact tissues (A) from birth to maturity for mink. Values are the average for the proximal, mid, and distal small intestine, with significant differences ($P < 0.05$) between age groups indicated by different letters. Accumulation of tracer L-aspartate by BBMV prepared from the proximal (B) and distal (C) halves of the small intestine from 2 wk to maturity. BBMV aspartate accumulation was measured with an inwardly directed 200 mmol/l Na$^+$ gradient (first bar), 0 mmol external Na$^+$ (second bar), and in the presence of an external solution containing 200 mmol/l Na$^+$ and 100 mmol/l unlabeled aspartate (third bar).

Fig. 9. Accumulation of tracer glycy1-sarcosine by BBMV prepared from the proximal (A) and distal (B) halves of the small intestine mink from 2 wk to maturity. BBMV glycy1-sarcosine accumulation was measured with an inwardly directed 200 mmol/l Na$^+$ gradient (first bar), 0 mmol external Na$^+$ (second bar), and in the presence of an external solution containing 200 mmol/l Na$^+$ and 100 mmol/l unlabeled glycy1-sarcosine (third bar).
The intestines of mink are relatively short throughout ontogeny (10) compared with those omnivorous mammals, which is typical for carnivores (22). However, when normalized to body mass, the intestines of adult mink (123 cm/kg) are longer than those of 3.6-kg adult cats (59 cm/kg; 2). The intestines of mink actually decline in intestinal size, despite a continuing increase in body size. In fact, intestinal mass of mink actually declines between 8 wk and maturity.

DISCUSSION

Intestinal Dimensions

The intestines of mink are relatively short throughout ontogeny (10) compared with those omnivorous mammals, which is typical for carnivores (22). However, when normalized to body mass, the intestines of adult mink (123 cm/kg) are longer than those of 3.6-kg adult cats (59 cm/kg; 2). The lack of change between 6 wk (weaning) and maturity for rates of intact tissue glucose uptake at 50 mmol/l differs from the postweaning decline detected in postnatal omnivores (18, 23, 26). The declines in maximum rates of uptake for both systems and for rates of uptake at 50 mmol/l leading up to weaning are consistent with decreases in the densities of transporters. The lack of change between 6 wk (weaning) and maturity for rates of intact tissue glucose uptake at 50 mmol/l differs from the postweaning decline detected in rats (7, 8), and in several ways is more similar to the pattern reported for the rat. Moreover, the age-related increases in initial rates of BBMV tracer glucose uptake particularly in the proximal intestine are compatible with a shift to a greater proportion of uptake via the high capacity system. When the increases in intestinal weight per centimeter during development are considered, rates of glucose uptake normalized to length or surface area actually increase after weaning. Collectively, these findings suggest that the total number of glucose transporters per unit of intestine actually increase after weaning instead of decline, as would be expected from the decrease in dietary carbohydrates.

Absorption of Amino Acids and Sugars

Ontogenetic changes in intestinal structure and functions are considered to be set by genetic determinants to match anticipated shifts in diet composition and the requirements for energy and nutrients (5). From our findings for the cat (7), the a priori expectation was that during postnatal development of mink, rates of uptake would decline for sugars, whereas absorption of amino acids would increase to match the shift from a milk diet to an adult diet low in carbohydrate and high in protein. The most dramatic changes were expected to occur at birth and again at weaning, when changes in the rates of enterocyte proliferation and replacement alter villus and crypts dimensions and are thought to affect intestinal functions, such as nutrient transport (12) and lactase activity (9).

Sugars. The kinetic data from the intact tissues suggest that throughout postnatal development, glucose is absorbed by two systems that differ in affinity and capacities. These findings contrast with the single high-affinity system for glucose transport detected in postnatal omnivores (18, 23, 26). The declines in maximum rates of uptake for both systems and for rates of uptake at 50 mmol/l leading up to weaning are consistent with decreases in the densities of transporters. The lack of change between 6 wk (weaning) and maturity for rates of intact tissue glucose uptake at 50 mmol/l differs from the postweaning decline detected in cats (7, 8), and in several ways is more similar to the pattern reported for the rat. Moreover, the age-related increases in initial rates of BBMV tracer glucose uptake particularly in the proximal intestine are compatible with a shift to a greater proportion of uptake via the high capacity system. When the increases in intestinal weight per centimeter during development are considered, rates of glucose uptake normalized to length or surface area actually increase after weaning. Collectively, these findings suggest that the total number of glucose transporters per unit of intestine actually increase after weaning instead of decline, as would be expected from the decrease in dietary carbohydrates.

Age-related changes in fructose uptake by intact tissues are even more paradoxical because it is absent or present only in negligible amounts in milk and the natural and production diets consumed by adult wild and domestic mink. However, rates of transport were highest during the neonatal period (birth to 2 wk), when accumulation ratios exceeded 1.0, and it was possible to resolve a low-affinity transport system. The nearly twofold increase in fructose uptake between 8 wk and maturity is similar to the pattern seen in the rat (23), but very different from the stable values seen in the cat (7, 8), and suggests a reappearance of the low-affinity carrier system.

Table 1. Kinetic constants for the carrier-mediated transport of glucose and fructose by the proximal intestine of mink from birth to maturity. Selected equations represent the lowest order equations that provided the best fit based on residuals.

<table>
<thead>
<tr>
<th>Age, weeks</th>
<th>Glucose</th>
<th>Fructose</th>
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<tbody>
<tr>
<td>0</td>
<td>$V_{\text{max}}$</td>
<td>$K_m^*$</td>
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<tr>
<td>0.16</td>
<td>1.08 ± 0.18</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>1</td>
<td>1.67 ± 0.05</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.90 ± 0.19</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>4</td>
<td>0.56 ± 0.14</td>
<td>1.3 ± 2.8</td>
</tr>
<tr>
<td>6</td>
<td>0.30 ± 0.07</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>8</td>
<td>0.02 ± 0.01</td>
<td>0.2 ± 0.5</td>
</tr>
<tr>
<td>Adult</td>
<td>0.08 ± 0.01</td>
<td>0.5 ± 0.2</td>
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</tbody>
</table>

Values are means ± SE. $V_{\text{max}}$ (maximum rate of transport) is presented as mmol·min⁻¹·mg intact tissue⁻¹; $K_m^*$ (apparent affinity constant) is as mmol/l; $P$ (permeability coefficient) is the linear component of absorption that was not saturable over the concentration range of tracer to 50 mmol/l.
Expressing rates of fructose uptake relative to those for glucose (F/G ratios) provides additional insights into age-related shifts in the abilities of mink to absorb sugars. Rates of glucose uptake exceed those for fructose throughout postnatal life of the mink with F/G ratios ranging from 0.21 at birth to 0.62 at maturity. These values are comparable to those for rats (23) and rabbits (6) but are higher than those for cats, which are stable during development at 0.14 (7, 8). It is interesting that the increase in F/G ratios from 0.26 ± 0.02 at 2 wk to 0.51 ± 0.05 at 4 wk (P < 0.05) is similar to the pattern for rats and coincides with when both species shift to the adult diet.

**Amino acids.** Unlike the significant postweaning declines in rates of amino acid absorption by intact tissues of rats and cats, values for the mink intestine remained relatively constant after 6 wk. The different patterns among species may be partly explained by the composition of the adult diet. Specifically, rats are weaned to commercial diets that are comparatively low in protein (20–25%) and correspondingly exhibit greater proportional declines in rates of amino acid absorption. Not only is the protein content of commercial diets fed to cats (~45%) lower than that of the production diet fed to mink (56%), the plant-based proteins often added to diets fed to cats (e.g., corn gluten) have lower digestibility, hence reducing the concentrations of amino acids and peptides available for intestinal absorption. Despite the high dietary loads of digestible protein consumed by adult mink, rates of amino acid absorption at 50 mmol/l (sum of both carrier-mediated and carrier-independent pathways) normalized to tissue mass were not different from those for weaned rats (23), cats (7), and dogs (8). This contrasts with our speculation that rates of amino acid and peptide absorption would be higher in weaned mink than other species to allow for the rapid and efficient digestion of the high dietary loads of protein.

The characteristics of amino acid absorption are not as well understood as for sugar transport, and most of what is known is for omnivores. The BBMV data indicate that aspartate and proline are transported by a saturable process that is almost entirely Na⁺ dependent, whereas a Na⁺-independent pathway is present for accumulation of leucine, methionine, and lysine. The low accumulation ratios for aspartate, leucine, methionine, and proline based on intact tissues, despite inhibition of initial rates of BBMV uptake by 100 mmol/l unlabeled amino acids could result if the amino acid transporters of mink exist at such high densities that 50 mmol/l is not high enough to cause saturation. However, rates of accumulation of amino acids by BBMV from adults were lower than the rates for glucose. Alternatively and teleologically more reasonable, the amino acid and peptide transporters of mink may have low affinities for their respective substrates, but high capacities for transport, and this may be particularly true for lysine with BBMV accumulation not fully inhibited by 100 mmol/l. The presence of low-affinity systems would allow a fewer number of transporters to rapidly and efficiently absorb the high dietary loads of amino acids, but this needs to be verified.

**Capacities.** The capacities of the entire length of small intestine to absorb glucose and fructose increased from birth to adulthood (Fig. 10), particularly for fructose, and did not scale directly to intestinal dimensions, which would be the case if the majority of uptake was via a paracellular pathway (17). Furthermore, the continuing increases in capacities after 8 wk, despite declines in intestinal mass, provide further evidence that the densities of sugar transporters per unit mass increased. The increase in capacities to transport fructose during the first 24 h, but not for glucose, is consistent with the presence in the apical membrane of at least two different transport systems for sugars.

Amino acid absorption capacities increased twofold or more between birth and 24 h. Slopes for double log plots of absorption capacities versus whole body mass revealed increases between 1 day and 8 wk that were similar to the value of 0.75 predicted from dimensional analysis (17). Interestingly and in sharp contrast to the sugars, the capacities to absorb amino acids declined between 8 wk and maturity, despite increases in whole body mass.

**Perspectives**

There is a need to better understand the types and characteristics of nutrient transporters present in the...
apical membrane of the small intestine and how they are regulated by dietary loads (quantities and composition). Our findings suggest the ability of the short intestine of mink to rapidly and efficiently process large dietary loads of protein is partly dependent on the presence of multiple amino acid transport systems that have different affinities and Na\(^+\) dependency. However, rates of amino acid absorption at 50 mmol/l by intact tissues are not markedly higher for the mink compared with values we and others have measured in omnivores and herbivores using the same methods (6, 16, 23; our unpublished data for pigs). Moreover, dipeptide absorption does not appear to provide a major mechanism of absorption because glycyrl-sarcosine accumulation by BBMV was lower than the uptake of any of the amino acids. Kinetic studies are needed to elucidate the functional characteristics and relative roles of the various amino acid and peptide transport systems at different stages of development and to search for alternative pathways for absorbing the products of protein hydrolysis. Also to be considered, but not well understood, are the concentrations of substrates for the respective carriers that actually exist at the apical membrane.

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REFERENCES