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Vitamin A deficiency reduces uptake of β-carotene by brush border membrane vesicles but does not alter intestinal retinyl ester hydrolase activity in the rat

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Vitamin A deficiency has been reported to result in mild structural and functional changes within the small intestine. The objective of this study was to measure the impact of vitamin A deficiency in the rat on several functional aspects of β-carotene uptake and intestinal retinyl ester hydrolysis. These included uptake of ^{14}C-β-carotene by brush border membrane vesicles (BBMV) and in vitro activity of intrinsic retinyl ester hydrolase (REH). Rats (n = 33) were randomly assigned to receive one of three dietary treatments: vitamin A deficient (−VA), vitamin A sufficient pair-fed (PF), or vitamin A sufficient free access-fed (FA). Liver, serum retinol, and growth data were used to verify clinical vitamin A deficiency. Rats in the −VA group were clinically vitamin A deficient by Day 56 on a vitamin A-free diet and, at that point, all rats were randomly assigned to one of two experimental treatments: BBMV studies or REH activity assays. Uptake of ^{14}C-β-carotene by BBMV was significantly suppressed ($P < 0.05$) in −VA rats when compared to both PF and FA control rats during early passive uptake equilibration (10–20 sec). Uptake was also significantly decreased by BBMV isolated from −VA rats compared to PF controls, but not FA controls, after a 10-min incubation ($P < 0.05$). In vitro activity of REH was not impacted by vitamin A deficiency in rats, although a trend for greater activity from −VA rats was noted. These data suggest that vitamin A deficiency impairs enterocyte membrane uptake of β-carotene without altering the enzymatic activity of intrinsic REH. (J. Nutr. Biochem. 11:436–442, 2000)

Keywords: rat; β-carotene; vitamin A deficiency; retinyl ester hydrolase; brush border membrane vesicles

Introduction

It is estimated that as many as one-half million children every year become partially or completely blind as a result of vitamin A deficiency.[1] Even more children will develop less severe forms of vitamin A deficiency. For example, a strong inverse association has been reported for vitamin A intake and incidence of diarrhea and respiratory infection among Sudanese children.[2] An evaluation of New York City children during an outbreak of measles demonstrated that children with low serum retinol levels during the acute phase of their infection experienced significantly more severe symptoms[3] as assessed by degree and length of fever and hospitalization rates. Research from the World Health Organization suggests that over 70 countries have problems with vitamin A deficiency.[1] Worldwide supplementation programs have existed for many years and have often been extremely beneficial to the populations involved. However, they are costly, not necessarily available to all people, and may not be a permanent solution. The incidence of clinical vitamin A deficiency in the United States is low, although subclinical deficiency in some segments of the population likely exists.

In recent years, the efficacy of β-carotene-containing foods as a source of vitamin A has been studied in human populations. A well-publicized study conducted with lactating Indonesian women with low vitamin A status reported

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that the provision of stir-fried green leafy vegetables did not improve the vitamin A status.[4] In a related study by the same investigators, it was reported that β-carotene-containing fruits were actually more efficacious than β-carotene-containing vegetables.[5] The conclusion of the authors was that the use of β-carotene-containing vegetables to combat vitamin A deficiency should be re-examined.

The effects of vitamin A deficiency on pre-formed vitamin A and provitamin A carotenoid absorption are not well understood. Vitamin A deficiency in the rat increases carotene dioxygenase activity.[6,7] Increased liver retinyl ester hydrolase (REH) activity has been reported during vitamin A deficiency in the rat;[8] however, it is unknown if intestinal hydrolase activity is also increased. Vitamin A deficiency in the rat has also been shown to cause mild structural and functional changes in the small intestine[9] as well as result in the amplification of the destructive effects of methotrexate administration in the gut.[10] Despite reported enhancement of dioxygenase activity, vitamin A deficiency may negatively impact an animal's ability to absorb carotenoids and/or pre-formed vitamin A via impaired intestinal uptake. Our laboratory has previously reported that vitamin A status in the Mongolian gerbil impacts the animal's ability to take up β-carotene, but not retinol, in the in vitro brush border membrane vesicle (BBMV) model.[11] In the current study, we report the impact of vitamin A deficiency in the rat on intestinal uptake of ^{14}C-β-carotene by BBMV prepared from vitamin A-deficient (−VA) rats. We also report the impact of vitamin A deficiency on in vitro activity of REH, the brush border enzyme responsible for hydrolysis of dietary retinyl esters prior to absorption of retinol.

Materials and methods

Animals, diets, and study design

Male Sprague-Dawley rats, 21 days old, were purchased from Harlan (Indianapolis, IN USA). Upon arrival, weanling rats (n = 33) were weighed and randomly assigned to one of three dietary treatments having equal average starting body weights. The three dietary treatments included (1) vitamin A-free pelleted AIN-76 semipurified rodent chow,[12] (2) pair-fed (PF) vitamin A-free diet (same as Diet 1) with 2.2 mg of retinyl palmitate/kg diet, or (3) free-access fed (FA; same diet as Diet 2). The vitamin A level of Diets 2 and 3 was chosen based on the typical content of semipurified rodent diets and meets the minimum requirement for growth in rats established by the National Research Council.[13] All diets were purchased from Research Diets Inc. (New Brunswick, NJ USA) and were made from ethanol-extracted casein to remove naturally occurring vitamin A from this protein source. The fat source in this diet was cottonseed oil, which is extremely low in carotenoids.

Rats were housed in the Edward R. Madigan Laboratory at the University of Illinois, IL USA in an environmentally controlled room at 25°C with a 12-hr light/dark cycle. Rats were caged individually in suspended stainless steel wire mesh units so that average daily food intake could be calculated for the purpose of pair-feeding. All rats were weighed every Monday, Wednesday, and Friday so that average daily growth could be determined and a growth plateau in the −VA rats could be quickly detected.

Rats were sacrificed by cardiac puncture. Serum and liver were analyzed for retinol to establish vitamin A status.

Chemicals

Oleic acid, monooleate, and Na-taurocholate were purchased from Sigma Chemical Corp. (St. Louis, MO USA). Organic solvents (HPLC grade) were purchased from Fisher Scientific (St. Louis, MO USA). ^{14}C-β-carotene was a generous gift from Hoffmann-LaRoche Inc. (Basel, Switzerland). Within 24 hours of use, ^{14}C-β-carotene was purified by high performance liquid chromatography (HPLC). The all-*trans* β-carotene fraction was repeatedly collected from a 250 × 4.6 mm Vydac C18 column (The Separations Group, Hesperia, CA USA) in a mobile phase comprised of 88% methanol, 9% acetonitrile, 3% distilled water with 1% iso-octane added. The pooled collections (100% all-*trans* β-carotene) were evaporated under a stream of argon, reconstituted in hexane containing 0.1% butylated hydroxy toluene (BHT), and stored at −20°C until use.

BBMV

Four rats from each dietary treatment group were sacrificed for isolation of BBMV by methods previously used by our laboratory.[11] The BBMV preparations were aliquoted for single use and stored at −80°C until use in uptake assays. Micelles containing ^{14}C-β-carotene were prepared using 3 mmol/L Na-taurocholate, 1 mmol/L monooleate, 1 mmol/L oleic acid, and up to 0.5 μmol/L β-carotene. The method for micelle preparation has previously been described[11] and is based on the method of Hollander et al.[14] Briefly, fatty acid, monooleate, and radiolabeled carotenoid were measured into a glass test tube and evaporated to dryness under argon. This mixture was then reconstituted in fresh ethyl ether and evaporated again to allow the lipids to form a thin film on the glass. The dried lipids were reconstituted with 12 mmol/L Na-taurocholate in buffer and sonicated for 10 min. Following sonication, the micellar mixture was diluted with buffer to reach a final Na-taurocholate concentration of 3 mmol/L, filtered through a 0.2 μm nylon syringe filter (Nalgene Brand Labware, Rochester, NY USA) and used in uptake assays.

The uptake of β-carotene into the BBMV was determined by the method of Hopfer et al.[15] Micelle preparation (100 μL) was mixed with 10 μL of BBMV (0.04−0.07 mg protein), the mixture vortexed, and allowed to incubate at room temperature with shaking. The incubations were stopped by applying the entire volume to a cellulose acetate membrane attached to a vacuum source and rinsing with 2 mL ice-cold buffer.

To determine uptake during equilibration of passive uptake, incubations were performed in triplicate at 10, 15, and 20 sec. All other incubations were conducted for 10 min. Uptake was determined by liquid scintillation counting using a Beckman L-8000 counter (Beckman Instruments, Fullerton, CA USA) and expressed on both a per mg protein and a per mg phospholipid basis. Bicinchoninic acid protein determination was used (Sigma Chemical Corp., St. Louis, MO USA). Phospholipid content was determined using the Phospholipids B kit from Wako Chemical (Richmond, VA USA).

Retinyl ester hydrolase studies

Seven rats from each dietary group were used for in vitro REH assays. To remove the influence of pancreatic enzymes capable of hydrolyzing retinyl esters, bile duct ligation was performed on the rats 48 hr prior to sacrifice. For −VA rats, the procedure was performed on the second day of growth plateau. An equal number of FA and PF rats underwent bile duct ligation on the same day as −VA rats. Rats were not fasted prior to surgery and food and water was returned upon recovery. Upon sacrifice, the intact ligature was verified and the distal two thirds of the small intestine was removed and rinsed with ice-cold saline. Brush border membranes

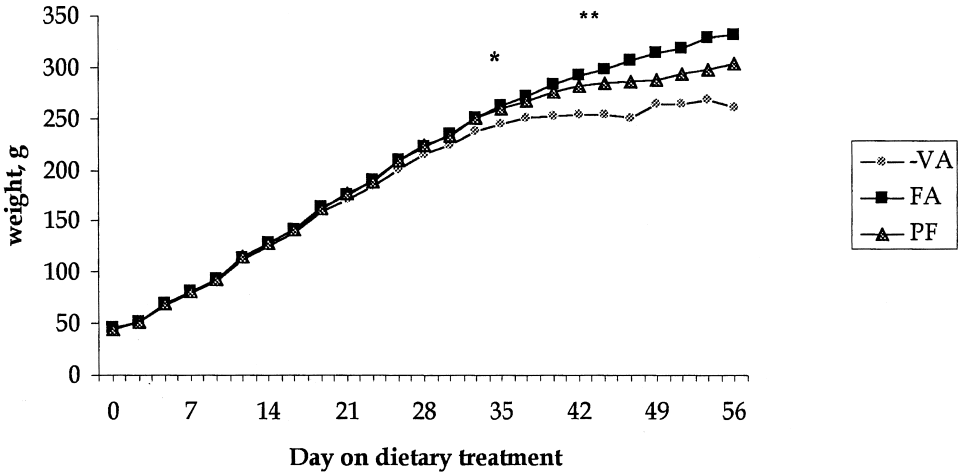

Figure 1 Growth curve for rats fed one of three diets: vitamin A deficient (−VA), vitamin A sufficient pair-fed (PF), and vitamin A sufficient free access-fed (FA). Average group weight in grams is plotted from Day 0 to Day 56 (the conclusion of the study). *Average weight of −VA group is significantly lower than average weight of the FA group ($P < 0.01$). **Average weight of PF group is significantly lower than FA group ($P < 0.01$).

(BBM) were immediately isolated and quick-frozen in liquid nitrogen by the method of Rigtrup and Ong.[16] Briefly, intestinal scrapings were suspended in homogenization buffer (300 mmol/L mannitol, 1 mmol/L Ethylene Glycol tetraacetic acid (EGTA), 2.4 mmol/L Tris, pH 7.1), 15 mL/intestine. Distilled water was added to increase volume to 60 mL/intestine and the mixture homogenized. After the addition of 0.75 mL of 1 mol/L $MgCl_2$, the mixture was incubated on ice for 15 min. The homogenate was then centrifuged for 15 min at 3,000 × *g* and the supernatant at 27,000 × *g* for 30 min. The pellet was resuspended in isolation buffer (60 mmol/L mannitol, 5 mmol/L EGTA, 12 mmol/L Tris, pH 7.1) and the $MgCl_2$ precipitation procedure was repeated. The pellet was resuspended in the final buffer (250 mmol/L mannitol, 20 mmol/L Tris, pH 8.0) and centrifuged at 34,000 × *g* for 30 min. The final pellet was resuspended in final buffer (0.5 mL/intestine), pulled through a 25-gauge needle, and quick-frozen in liquid nitrogen. The samples were stored at −80°C until use in enzyme assays. To monitor enrichment of the BBM fraction, leucine amino peptidase (Sigma Chemical Corp., St. Louis, MO USA) and sucrase activity was measured on both crude homogenates and final BBM fractions. Sucrase activity was measured by the method of Dahlqvist.[17] Protein content of each preparation was determined by bicinchoninic protein determination (Sigma Chemical Corp., St. Louis, MO).

The method of Rigtrup and Ong[16] was used to assay intrinsic REH activity with one modification. Briefly, the reaction was conducted with 0.5% deoxycholate in FTA Hemagluttination buffer, pH 8.0 (Fisher Scientific, St. Louis, MO USA). The natural substrate for intrinsic REH, retinyl palmitate (Sigma Chemical Corp., St. Louis, MO USA) was suspended in ethanol/0.1% BHT. The total volume of the reactions was 1.5 mL. After addition of both buffer (containing 0.5% w/w deoxycholate) and 5 μL ethanol (containing substrate to yield a final concentration of 30 μmol/L retinyl palmitate), 10 μL BBM was added to initiate the reaction. Reactions were incubated for 10 min at 37°C in a shaking water bath (Cole Palmer, Vernon Hills, IL USA). Reactions were stopped by the addition of 2 mL ice-cold ethanol containing 0.1% BHT. Reactions were extracted with 6 mL hexane, evaporated to dryness, reconstituted with 20 μL CH_2Cl_2 and analyzed by HPLC by methods previously described.[11]

Retinyl caproate, used as a negative control, was synthesized from retinol and caproyl chloride by the method of Huang and Goodman.[18] Brush border REH activity is specific for long-chain retinyl esters and has previously been shown to have little activity toward short-chain esters.[16] All incubations with retinyl caproate were performed on BBM that had not previously been thawed. Identical methods were used for both substrates. Activity of intrinsic REH was expressed as nmol retinol liberated per mg protein per min.

Statistical analysis

For the rat BBMV uptake assays, mucosal scrapings from 4 rats were pooled so that one composite BBMV fraction could be used for all assays. Mean uptake and standard error were calculated based on results of assays performed in triplicate. For all data presented, one-way ANOVA was used to determine if a significant difference between the 3 treatment groups existed. An α level of $P < 0.05$ was chosen. When significant differences were identified, Fisher's protected least significant difference[19] was used as a post-hoc comparison. All statistical analysis was performed using StatView™ (Abacus Concepts, Berkeley, CA USA) software program.

Results

Clinical features of vitamin A deficiency

The −VA rats had a greasy appearance to their coats and were more lethargic than the control rats. Their eyes displayed crusty rings on their lids and appeared to have keratitis. Their noses were often bloody and blood droplets often appeared in their cages. No animals suffered from diarrhea during the study.

The rate of growth of the −VA animals became significantly lower than FA rats by Day 31. Growth curves are shown in *Figure 1*. The indices used to verify clinical vitamin A deficiency were liver and serum retinol concentrations. Liver and serum retinol concentrations are shown in *Table 1*. Liver vitamin A in the FA and PF groups was 1,465 ± 127 and 1,667 ± 180 nmol/g, respectively, whereas the −VA group had a mean liver vitamin A level of 24 ± 7 nmol/g (7 μg/g). Several rats had no detectable liver vitamin A; the remaining ranged from 7.0 to 54.8 nmol/g. Liver weights were significantly lower (9.5 ± 0.57 g) in the −VA group. In the FA and PF rats, liver weight was 12.6 ± 0.59 and 12.5 ± 0.53, respectively. Retinol was not detected in serum of any −VA rats. The lower limit of detection for retinol was approximately 0.012 μmol/L. Serum retinol for FA and PF rats was 2.1 ± 0.14 and 2.0 ± 0.18 μmol/L, respectively.

Sucrase activity was not depressed in the −VA rat

Table 1 Liver and serum retinol concentrations and liver weights of rats fed a vitamin A-deficient diet (-VA), a vitamin A sufficient free access-fed diet (FA), and a vitamin A-sufficient pair-fed diet (PF) for 56 days*†

Treatment	Liver weight‡	Liver retinol§	Serum retinol‖
FA	12.6 ± 0.6*	1,465 ± 127*	2.1 ± 1*
PF	12.5 ± 0.5*	1,667 ± 180*	2.0 ± 0.2*
−VA	9.5 ± 0.6**	24 ± 7**	ND**

*Rats in the FA and PF groups ($n = 22$) were fed an AIN-72 pelleted diet that contained 5,000 I.U./kg (1.5 mg/kg) vitamin A as retinyl palmitate. The PF rats were fed to match the average intake of the vitamin A-deficient rats. The −VA group ($n = 11$) was fed the same AIN-72 diet without added retinyl palmitate. All diets were made with ethanol-extracted casein.
†Asterisks represent differences between groups as determined by one-way analysis of variance and Fisher's protected least squares difference. Values with different numbers of asterisks are significantly different at $P < 0.01$.
‡Average weight of rat livers from each group, expressed as grams ± SEM.
§Average concentration of vitamin A in liver tissue from each group, expressed as nmol/g liver ± SEM.
‖Average serum retinol for each group expressed as μmol/L± SEM.
ND— not detected.

BBMV preparation (305.3 U/g) when compared to the PF controls (293.3 U/g). The FA group however, had lower specific activity (185.7 U/g) of sucrase.

BBMV uptake

Uptake of ^{14}C-β-carotene during equilibration of passive uptake was attenuated in BBMV preparations isolated from −VA rats (see *Figure 2*). Uptake, expressed as pmol βC/mg phospholipid, was significantly lower by BBMV isolated from −VA rats at all time points studied ($P < 0.05$). The most dramatic difference was seen in the earliest time point (10 sec). Uptake by BBMV isolated from the −VA rats was only 47 ± 3.7 compared to 69.1 ± 2.6 and 69.5 ± 1.1 by the FA and PF groups, respectively.

Uptake studies were also conducted to characterize uptake achieved by longer incubation times. In these studies, uptake of ^{14}C-β-carotene was determined following 10-min incubations. Data were expressed on protein and phospholipid content of the BBMV preparations. The uptake of ^{14}C-β-carotene was significantly greater in BBMV isolated from PF rats ($P < 0.01$) compared to both FA and −VA rat BBMV in two separate experiments (see *Figure 3*). When BBMV were incubated with 0.07 μmol/L ^{14}C-β-carotene, uptake was 0.11 ± 0.003 nmol/mg protein for the FA group, 0.15 ± 0.003 for the PF group, and 0.12 ± 0.003 for the −VA group. When BBMV were incubated with 0.54 μmol/L ^{14}C-β-carotene, uptake was 0.99 ± 0.03 nmol/mg protein for the FA group, 1.35 ± 0.02 for the PF group, and 1.10 ± 0.03 for the −VA group. In incubations containing 0.54 μmol/L ^{14}C-β-carotene, uptake by BBMV from the −VA group was significantly higher than the FA group ($P < 0.01$) when expressed on a protein basis. When the same data are expressed on a phospholipid basis, it is no longer significant. However, uptake of ^{14}C-β-carotene by BBMV isolated from −VA rats was significantly suppressed ($P < 0.01$) when compared to PF controls when corrected for protein content or phospholipid content.

REH

No significant differences were detected between groups for in vitro REH activity (data shown in *Table 2*). When 30 μmol/L retinyl palmitate (C16 ester, natural substrate) was used as the substrate, the hydrolytic activity was 7.9 ± 0.9 nmol retinol/mg protein/min for the FA group, 5.7 ± 0.5 for the PF group, and 9.1 ± 1.2 for the −VA group. There was a trend ($P = 0.07$ for protected F-test) for the −VA group to have greater activity than the PF group.

When 30 μmol/L retinyl caproate (C6 fatty acyl ester) was used as the substrate, no significant hydrolytic activity was observed in any of the brush border preparations. When BBM preparation was boiled for 2 min prior to assay, no hydrolytic activity could be detected.

Discussion

Based on clinical appearance and growth data, it is apparent that the rats fed the vitamin A-free diets developed clinical

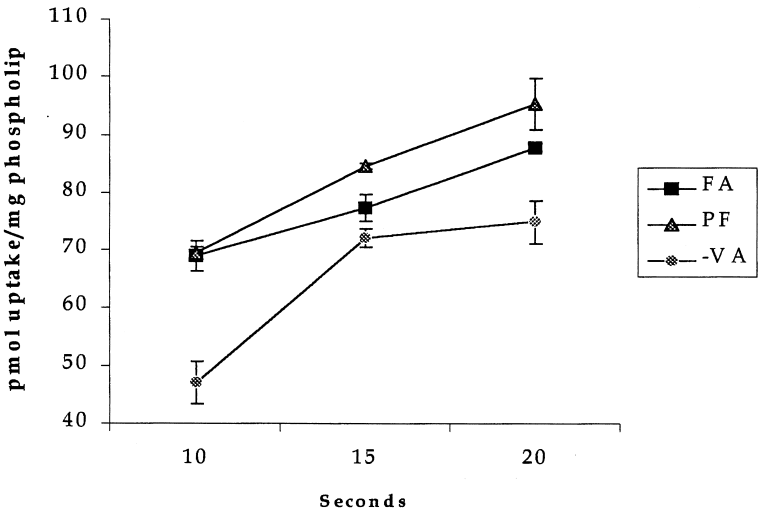

Figure 2 Uptake of ^{14}C-β-carotene by brush border membrane vesicles (BBMV) isolated from three groups of rats: vitamin A deficient (−VA), vitamin A sufficient pair-fed (PF), and vitamin A sufficient free-access fed (FA). BBMV from individual rats were pooled by dietary treatment to yield three pooled groups: FA, PF, and −VA. Micelles containing 0.07 μmol/L ^{14}C-β-carotene were incubated for 10, 15, and 20 sec with each group of BBMV, simultaneously. Each bar represents the mean ± SEM uptake from triplicate incubations, expressed as pmol ^{14}C-β-carotene. Data were normalized for phospholipid content of the vesicle preparation. Uptake into BBMV is significantly depressed in the −VA group at all time points when compared to the FA fed group ($P < 0.05$). The PF group had significantly greater uptake than the −VA group at all time points ($P < 0.05$).

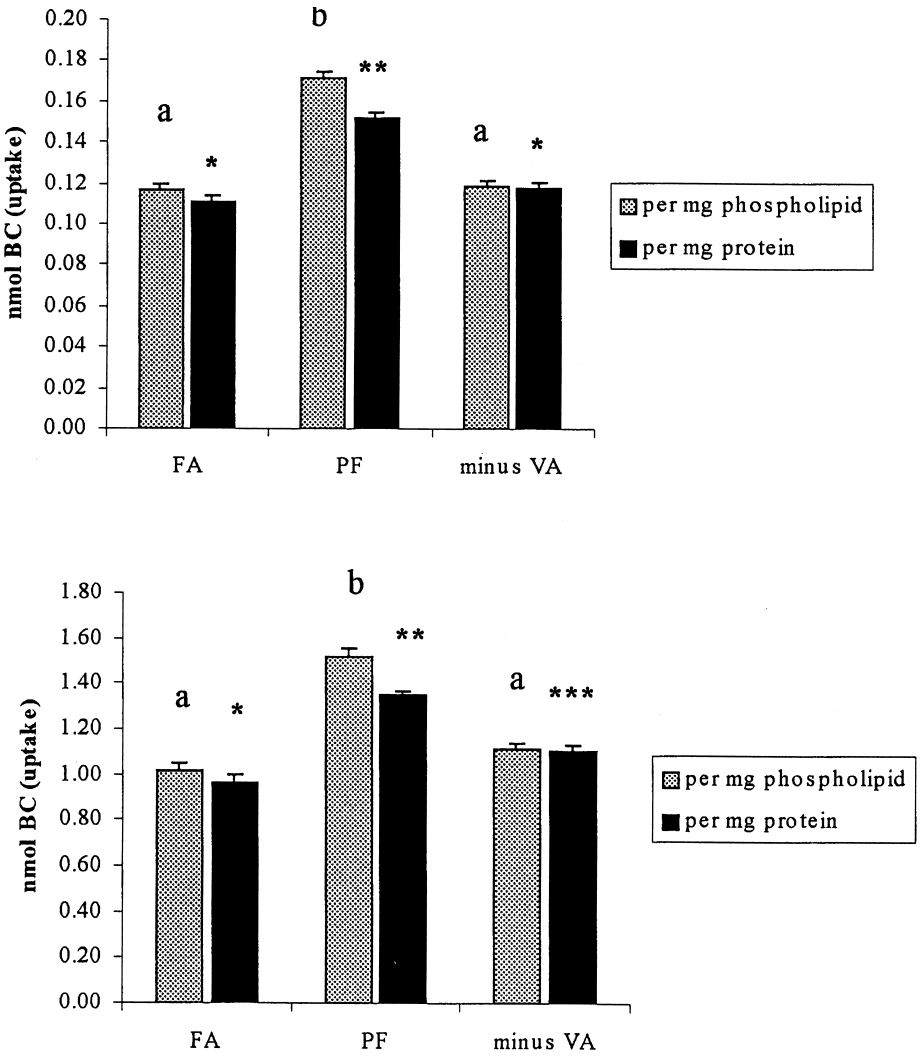

Figure 3 Uptake of ^{14}C-β-carotene by brush border membrane vesicles (BBMV) isolated from three groups of rats: vitamin A deficient (−VA), vitamin A sufficient pair-fed (PF), and vitamin A sufficient free access-fed (FA). BBMV from individual rats were pooled by dietary treatment to yield three pooled groups: FA, PF, and −VA. Micelles containing 0.07 μmol/L ^{14}C-β-carotene (top graph) and 0.5 μmol/L ^{14}C-β-carotene (lower graph) were incubated for 10 min with each group of BBMV, simultaneously. Each bar represents the mean ± SEM uptake expressed as nmol ^{14}C-β-carotene. Data were normalized for both protein content (black bars) and phospholipid content (gray bars). Asterisks represent significant differences at $P < 0.01$ for data expressed on a protein basis. Bars with different letters are significantly different at $P < 0.01$ for data expressed on a phospholipid basis.

vitamin A deficiency. Other investigators have reported nondetectable liver retinol in −VA rats,[9] and liver vitamin A as low as 0.37 ± 0.15 nmol/g in rats fed −VA diets for 7 weeks.[20] Growth data support the development of clinical vitamin A deficiency. Pair-feeding resulted in significantly less growth ($P < 0.01$) when compared to FA rats and tended to increase liver retinol stores. The ability of caloric restriction to increase vitamin A absorption has previously been described.[21]

Table 2 Detection of in vitro REH activity

Treatment group*	Retinyl palmitate† hydrolysis	Retinyl caproate‡ hydrolysis
FA	7.9 ± 0.9	0.1 ± 0.03
PF	5.7 ± 0.5	0.1 ± 0.03
−VA	9.1 ± 1.2	0.1 ± 0.1

*Retinyl ester hydrolase (REH) activity measured in brush border membranes isolated from three groups of rats; vitamin A deficient (−VA), vitamin A sufficient pair-fed (PF), and vitamin A sufficient free access-fed (FA). Bile duct ligation was performed on all rats 48 hr prior to sacrifice to eliminate pancreatic enzymes.
†Mean specific activity of intrinsic REH when retinyl palmitate was used as the substrate. Means are expressed as nmol retinol liberated/mg protein min^{-1} (±SEM), $n = 7$ per group. No significant differences were detected.
‡Mean specific activity of intrinsic REH when retinyl caproate (negative control) was used as the substrate. Means are expressed as nmol retinol liberated/mg protein min^{-1} (±SEM), $n = 7$ per group.

The use of BBMV to study β-carotene uptake in the rat has previously been described by this laboratory.[11] In the current study, a marked delay in early equilibration of passive uptake was observed in the −VA BBMV preparation. This attenuated uptake was noted at all early time points. To further characterize uptake of ^{14}C-β-carotene, 10-min incubations were analyzed. However, by 10 min, the process of passive uptake is complete and the vesicles are saturated. Previous work in our laboratory has demonstrated that by 5 min, rat BBMV has reached equilibration with respect to β-carotene uptake.[11] After a 10-min incubation, uptake also was significantly lower in BBMV isolated from the −VA rats when compared to PF rats. This observation was consistent in all assays. However, uptake by BBMV from the −VA group was not significantly lower than the FA group. Warden et al.[9] reported that mild changes in intestinal morphology and function were only evident when

−VA rats were compared to equally malnourished PF rats. For example, these authors reported decreased sucrase and maltase activities in −VA rats when compared to PF controls but not to FA controls.

From the data in the present study, we conclude that uptake of β-carotene by BBMV isolated from −VA rats may be negatively impacted by vitamin A deficiency, independent of the reduced food intake associated with deficiency. This is also supported by the observation that the BBMV isolated from PF rats exhibited significantly greater uptake of ^{14}C-β-carotene than the FA rats. This is consistent with increased sucrase activity observed in BBMV from PF rats. However, we did not observe a suppression of sucrase activity in the −VA rats when compared to PF rats. The use of pooled BBMV for sucrase determination may explain this. It would have been more appropriate to measure sucrase activity in individual rats that were representative of the individual treatment groups.

The results of this current study agreed with our findings using the Mongolian gerbil; however, the current results were not as profound as those reported earlier.[11] Both studies do support the hypothesis that enterocyte uptake of β-carotene may decrease during vitamin A deficiency. This has implications for the efficacy of using food sources of β-carotene to combat vitamin A deficiency.

The rate of retinyl palmitate hydrolysis by intrinsic REH activity from rat intestine was approximately one half to one third of the rate reported by Rigtrup and Ong.[16] This attenuated rate was not unexpected because the level of BBM purification, as measured by enrichment of leucine amino peptidase activity, was only about one half that reported by these investigators.

There was a trend for greater retinyl ester hydrolysis activity by the −VA rats in comparison to PF control. We did not anticipate finding significant differences in REH activity because it has been reported that the normal rat retains enough activity in its small bowel to hydrolyze an entire day's worth of vitamin A in about 2 min.[22] This estimate is based on the in vitro-derived Vmax for the intrinsic REH activity.

When phospholipase B (a stalked brush border protein) was purified, it demonstrated a striking similarity to the hydrolase activity previously reported in BBM preparations.[23] It is thought that intrinsic REH activity resides on phospholipase B. In addition, these authors previously reported that intrinsic REH activity was only present in the distal two thirds of the rat small intestine.[16] Phospholipase B is localized to the same location in the rat.[23,24]

Using a negative control (retinyl caproate), we were able to validate the efficacy of bile duct ligation. Little to no hydrolytic activity against retinyl caproate in common bile duct ligated rats was also reported by Rigtrup and Ong.[16] Cholesterol ester hydrolase of pancreatic origin exhibits potent activity toward short-chain retinyl esters, the activity being about 3-fold greater toward retinyl caproate (C6:0) than retinyl palmitate (C16:0).[16] In all assays, there was no significant hydrolysis activity by the brush border preparations when retinyl caproate was used as a substrate. Retinyl palmitate is the most common dietary retinyl ester, followed by stearate. It is therefore logical that an intrinsic activity would be designed to hydrolyze physiologically relevant esters.

Uptake of β-carotene by the villus-associated enterocytes may be less efficient during vitamin A deficiency. However, increased activity of β-carotene dioxygenase is expected to compensate for this. Implications do exist for people who are relying on foods that contain β-carotene for nearly 100% of their vitamin A needs. Green leafy vegetables, for example, provide β-carotene, but the bioavailability of β-carotene in this food form is in question. It may be even less bioavailable in persons who have functional manifestations of vitamin A deficiency impacting the small intestine. On the other hand, a supplement containing a more bioavailable form of β-carotene (e.g., water miscible beadlets) or pre-formed vitamin A may be more efficacious in −VA humans.

Acknowledgments

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