Original Article

Peanut butter increases the bioavailability and bioconversion of kale β-carotene to vitamin A

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Background and Objectives: Kale is a rich source of provitamin A- β-carotene. This study used intrinsically labeled kale [$^2H_9$] β-carotene to determine the effect of peanut butter on the bioconversion of kale β-carotene to vitamin A in preschool children. **Methods and Study Design:** Preschool children (n=37; age 12-36 mo) were randomly assigned to 50 g cooked kale (1.5 mg β-carotene content) with either 33 g peanut butter (PBG) or with 16 g lard (LG) and a reference dose of 1 mg [$^{13}C_{10}$] retinyl acetate capsule. Blood samples were processed to serum and analyzed by Negative Chemical Ionization-Gas Chromatography Mass Spectrometry (NCI-GCMS) for the enrichments of [^{2}H] retinol from kale [$^2H_9$] β-carotene and [$^{13}C_{10}$] retinol from reference dose. **Results:** The area under curves (AUCs) of molar enrichment at days 1, 2, 3, 6, 15, and 21 after the labeled doses was 56.3±10.5 and 84.8±16.2 (nmole) for [^{2}H] retinol from LG and PBG kale [$^2H_9$] β-carotene, respectively. The AUC of [$^{13}C_{10}$] retinol from reference dose was 432.6±54.9 (LG) and 560.3±156.7 (nmole) (PBG), respectively. The calculated β-carotene conversion factors were 13.4±3.1 and 11.0±3.9 to 1 (p>0.05) by weight for LG and PBG, respectively. **Conclusions:** This study showed that peanut butter enhances the vitamin A value of kale.

Key Words: kale, vitamin A, carotenoids, lutein, deuterium, peanut butter

INTRODUCTION

Children under the age of 5 years in sub Saharan African countries such as Zimbabwe are fed starchy complementary foods that often lack essential nutrients such as vitamin A.[1-3] As a result, these children are at risk of developing vitamin A deficiency (VAD). In Zimbabwe, VAD is a public health problem affecting more 40% of children under the age of 5 years.[4] Worldwide, an estimated 250 million preschool children are affected by VAD and most of these children affected are in sub-Saharan Africa.[5] Providing vitamin A to those children could prevent about a third of all under-five deaths, which amounts to up to 2.7 million children that could be saved from dying unnecessarily.[6] Night blindness, a consequence of VAD, is estimated to affect 5.2 million preschool-age children worldwide.[5] VAD also compromises the immune systems of approximately 40 percent of children under five in the developing world, greatly increasing the severity of common childhood infections, often leading to deadly outcomes.[7]

Studies show that most common complementary foods given to children in Africa are starchy gruels made from maize, millet, sorghum or cassava whose nutrient density is very low.[8,9] These infant feeding practices are a result of lack of nutrition education, food availability and cultural practices.[3] In southern Africa, particularly in Zimbabwe, the diet of pre-school children is deficient in vegetables such as kale.[1,10-12] Vegetables and other plant-based foods provide more than 80% of the total vitamin A intake in developing countries.[13,14] This is because poor people in developing countries have limited or have no access to preformed vitamin A rich animal foods such as dairy, meats and poultry.[15,16] However, these people have easy access to fruits and vegetables, which they grow or buy, cheaply from the markets.[17,18] In east and southern Africa, dark green vegetables such as kale are consumed daily as relish accompanying the staple thick maize porridge.[19-24] However, despite the ubiquitous presence of kale and daily consumption by adults in Zimbabwe, this

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provitamin A carotenoid (precursors of vitamin A) rich green vegetable is not used as a complementary food for young children.

Kale has been shown to be very rich in the provitamin A, β-carotene, with contents ranging from 3-15 mg/100 g fresh weight.[25,26] The β-carotene content is even higher than in some carrot varieties and other common green vegetables such as spinach.[26,27] In the developed countries such as the US and in Europe, kale is used as a complementary baby food.[10] Promotion of kale as a complementary food in Zimbabwe will increase the vitamin A intake by children leading to a reduction of VAD. In some cases, peanut butter sauce is added to boiled kale to increase its palatability and nutrient density.[22,28,29] Peanut butter cooked kale can be a very nutritious complementary food, providing children with vitamin A, vitamin E, proteins and oils. Studies show that vitamin E and oil content enhance the bioavailability and bioconversion of β-carotene to vitamin A.[30-32]

In Zimbabwe, the provitamin A carotenoid contents of kale varieties grown are unknown, and also a lack of human studies to date that show the bioavailability of kale provitamin A carotenoids and their conversion to vitamin A value in humans especially in children may limit its utilization as a complementary food. However, kale studies have been conducted in adults in the US. One such study, using [^{13}C] labeled kale β-carotene in humans, showed that kale β-carotene was very bioavailable and was also efficiently converted to [^{13}C] retinol (vitamin A) in the body.[33,34] However, this study was not designed to determine the conversion factor and vitamin A equivalence of kale β-carotene to vitamin A; as a result, the amount of vitamin A formed was not quantitated.

The determination of the absorption and conversion of kale β-carotene to vitamin A is important for designing infant and young child feeding programs in many kale-consuming regions of the world, where VAD is prevalent. In order to accurately assess the vitamin A value from a plant food source, intrinsically labeled plant material (kale) in which the β-carotene is labeled with a low abundance stable isotope is used. In this way it is possible to determine absorption of labeled β-carotene from the kale food matrices and the subsequent conversion of the labeled β-carotene to vitamin A and allowing the quantitative determination of the vitamin A equivalence of β-carotene, as shown in previous studies.[35-37] In order to quantitatively evaluate the absorption and intestinal conversion of kale β-carotene, deuterium labeled kale and [$^{13}C_{10}$] labeled retinyl acetate reference dose were used in this study.

METHODS

Production of deuterium labeled kale and preparation of labeled kale doses

Intrinsically labeled kale was grown and harvested from a hydroponic plant system by growing the plants with heavy water (deuterium oxide, D_2O) at the USDA-Agriculture Research Service Children's Nutrition Research Center in Houston, TX. The procedures of growing the intrinsically deuterated plants have been described in previous publications.[35,38] One batch (2.7 kg) of freshly harvested deuterium labeled kale was shipped overnight under ice packs from the USDA/ARS Children's Nutrition Research Center, Houston, Texas to the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University in March 2012. Samples of the labeled kale were immediately analyzed for carotenoid contents and deuterium enrichment profile by HPLC and LC/MS (Figure 1 and 2). The rest of the labeled kale was weighed, vacuum packed and stored at -80°C.

One week after receiving the kale from Houston Texas, the labeled kale was cooked in the Metabolic Research Unit Kitchen (MRU) at the Jean Mayer USDA Human Nutrition Research on Aging at Tufts University using a

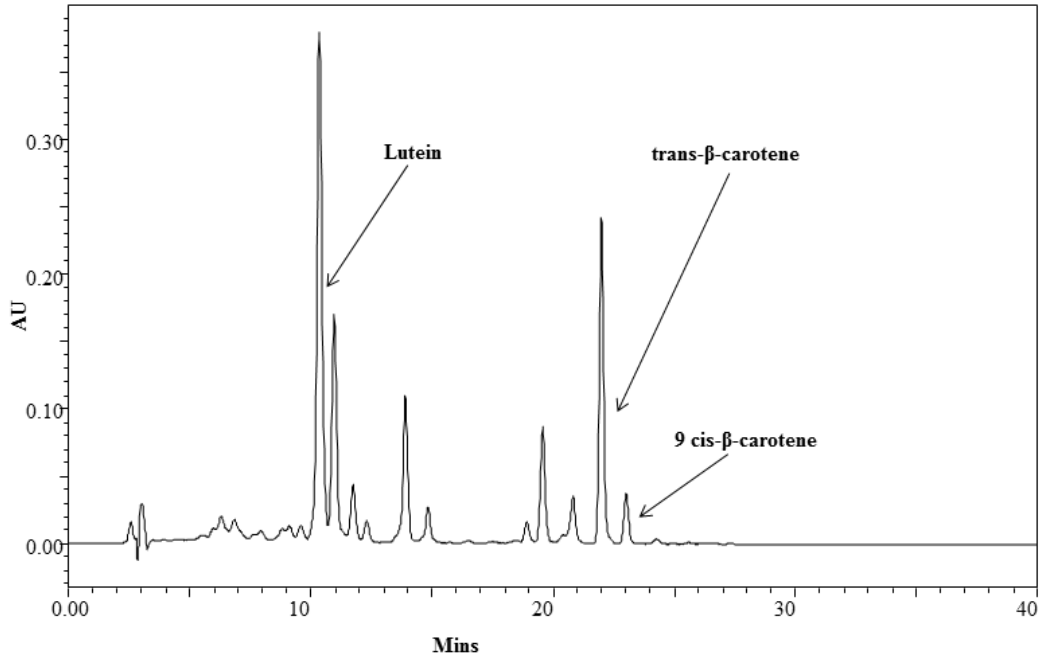

Figure 1. Carotenoid profile of *Brassica oleraceavaracephala* (kale) using C30 HPLC column at 450nm wavelength. The first arrow to the left is pointing at lutein, the second and third arrow to the right are pointing at all-trans β-carotene and 9 cis β-carotene.

previously published protocol for green vegetables.[9] Briefly, kale leaves were steamed for 10 min. After steaming the leaves were pureed by homogenizing using a food processor. After homogenization, the cooked labeled kale was immediately analyzed for carotenoid contents by HPLC (Table 1 and Figure 1). Fifty cooked kale doses weighing 50 g each (containing 1.52 mg of β-carotene content) were vacuum packed and stored at -80ºC for 16 months. In June of 2013, cooked labeled kale samples were analyzed for carotenoid contents before shipment under ice packs for to Bulawayo, Zimbabwe. The kale doses with ice packs were still frozen upon arrival at the National University of Science and Technology (NUST), Bulawayo, Zimbabwe. The labeled kale doses were then stored at -80ºC. On day one of the study, the kale doses were thawed at room temperature. After thawing, 20 x 50 g of the cooked kale doses were each mixed 33 g of peanut butter and were microwaved for 1 min. Another 20 x 50 g cooked kale dose were each mixed with 16 g of lard and microwaved for 1 min. The 33 g of peanut butter contained the same amount of fat as 16 g of lard.

Human participants

Participants were recruited from day care centers in the City of Bulawayo. Forty children aged between 12-36 months of age were screened and enrolled in the study. Thirty-seven participants successfully completed the study. Inclusion criteria was as follows; children not taking vitamin A and carotenoids supplements, who are generally healthy, with no history of liver, gastrointestinal disease, cancers and cardiovascular diseases that would interfere with vitamin A absorption and metabolism. The following situations excluded potential participants from the study: severe and symptomatic cardiac disease, a history of bleeding disorders; chronic history of gastric, intestinal, liver, pancreatic, or renal disease; any portion of the stomach or the intestine removed; history of intestinal obstruction or malabsorption.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki. The ethical Review Committees at the Medical Research Council of Zimbabwe (MRCZ), Ministry of Health and Child Welfare, Zimbabwe Government (MRCZ/B/431) and sample analysis approval Tufts Medical Center Institutional Review Board (IRB) approved the study protocols before enrolment of participants. Written informed consent was obtained from each child's parent or legal guardian.

Study design and procedures

In order to compute the power and sample size, we used changes in serum retinol and standard deviations from our previous studies with dark green vegetables in children.[30] The required sample size was 5 children per group. How-

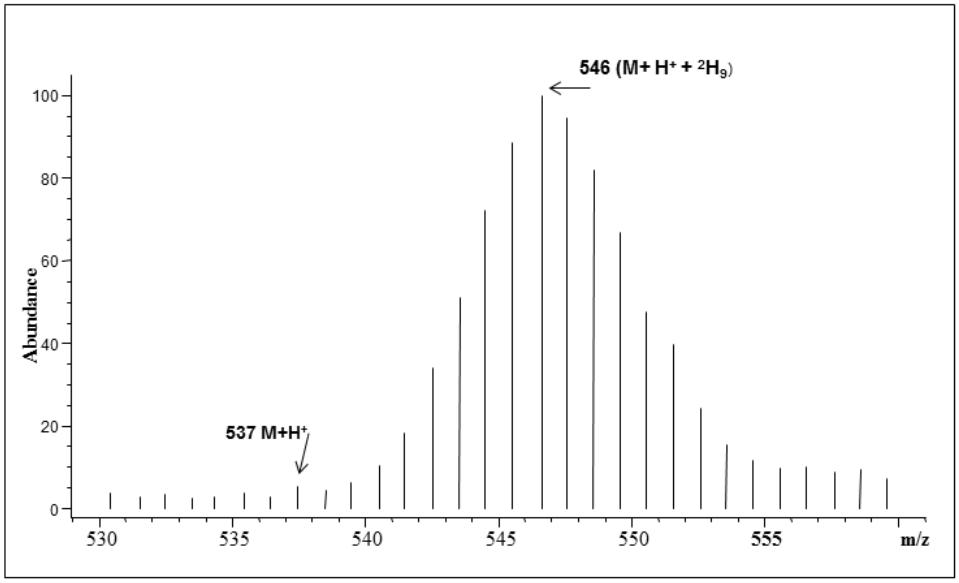

Figure 2. Deuterium enrichment profiles of kale by liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (positive ion mode). The most abundant isotopomer of labeled β-carotene with 9 deuterium atoms is represented by a mass-to-charge ratio (*m/z*) of 546 ($M+H^{+}+^{2}H_9$). The first arrow on each profile points to the 537 peak, showing that the molecular mass of unlabeled β-carotene is 537 (M+H+). The second arrow on each profile points to peak 546 ($M+H^{+}$+2H9), showing the highest abundance of enrichment.

Table 1. The carotenoid contents of raw and cooked kale used in the study. Carotenoid contents of raw labeled kale before cooking and after cooking. Values are means of three independent analysis and concentration are mg/g of fresh weight for raw kale and mg/g wet weight for cooked kale.

Carotenoid	Mean carotenoid content mg/g ±standard deviation	
	Raw kale (fresh weight)	Cooked kale (wet weight)
Lutein	6.23±0.65	2.71±0.16
Trans β-carotene	4.24±0.39	1.52±0.10
9 cis β-carotene	0.68±0.08	0.29±0.06

ever, given difficulty of drawing seven blood draws per child over a 21-day period, we designed the study using the published "Super Child Model" such that each time-point per group will have five randomly assigned participants.[31] Each participant contributed a total of 3 (3-5 mL) blood draws for the 21-day duration of the study.

Six day care centers selected to participate in the study were randomly assigned to either Peanut Butter Group (PBG) or the Lard Group (LG). On day one of the study, participants were assembled at their respective centers and a baseline 3-5 mL of blood (time=0 h) was withdrawn into a no-additive VacutainerTM from a forearm vein by a registered nurse from all participants. The cooked labeled kale doses were thawed and mixed with 33 g (one teaspoon) peanut for the PBG and 16 g lard i.e. animal fat for LG and microwaved for 1 min. After cooling, subjects in each group consumed the labeled kale doses under the supervision of research staff to make sure everything was quantitatively consumed. After consuming the kale doses, participants immediately ingested 1 mg [$^{13}C_{10}$] retinyl acetate (synthesized by the Cambridge Isotope Laboratory, Andover, MA) in 0.5 g corn oil capsule with a glass of water to wash down their mouths. The [$^{13}C_{10}$] retinyl acetate, which converts to [$^{13}C_{10}$] retinol once in circulation, was used as a reference dose to assess the conversion efficiency of the labeled kale β-carotene to retinol. Three to five mL of blood were withdrawn (an intravenous line was inserted for drawing these samples) at 24, 48, 72 hours, days 6, 15, and 21 after the consumption of labeled kale and labeled retinyl acetate doses from 5 participants per time point per group. Participants consumed their normal diets the entire duration of the study.

Serum processing

After blood draws, blood samples were kept in a cooler box, and were then transported by car to National University of Science and Technology (NUST), Department of Applied Biology and Biochemistry Laboratory for processing. The blood samples were centrifuged at 800 g for 30 min at 4°C. The processed serum was collected into 2 mL cryogenic vials, code labeled and stored at -80°C until shipment under ice packs by air to the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University in Boston, MA, US. After an 18 h trip the serum vials were still frozen upon arrival in Boston.

Biochemical analysis of serum samples

The extraction of serum retinol, carotenoids, and tocopherols was conducted using a previously published method.[32] Briefly, 3 mL of chloroform: methanol (2:1, v/v) and 500 uL of saline (0.85%) were added to 100 µL serum sample for the fat soluble components extraction. The chloroform: methanol, saline and serum mixture was vortexed and centrifuged at 800 x g for 10 mins. The bottom layer was extracted; a second extraction with 2 mL hexane was performed on the residue. The combined chloroform: methanol and hexane fraction were evaporated under N_2 gas on an N-EVAP (OrganomationAssociatesInc, South Berlin, MA). After complete dryness the residue was dissolved in 100 µL ethanol forming a clear solution, and 20 µL was injected into an HPLC system equipped with a YMC Carotenoid S C_{30} column (3.0 x 150mm). The HPLC equipment, gradient profile and quality assurance was as described in our previously published work.[32]

Atmospheric Pressure Chemical Ionization (APCI)-MS analysis of deuterium labeled kale

In order to determine the percentage enrichment of labeled β-carotene, 1 g of kale was weighed into a 50 mL test-tube and extracted for carotenoids as described in published methods.[33] An LC-APCI-MS analysis of labeled kale β-carotene mass-to-charge ratios (*m/z*) was conducted as previously described.[34] Our kale β-carotene had the highest enrichment abundance of [$^{2}H_{9}$] β-carotene as 546 (M+ H^{+}+9) (Figure 2), and the deuterium enrichment was randomly distributed to all possible positions of the β-carotene molecule as determined by NMR.[39]

NCI-GC/MS analysis of [^{2}H] retinol from deuterated kale and [$^{13}C_{10}$] retinyl acetate dose

In order to analyze the enrichment of labeled retinol from kale [$^{2}H_{9}$] β-carotene and labeled retinol from [$^{13}C_{10}$] retinyl acetate reference dose, serum samples from all subjects were extracted for retinol as described in published methods.[36] Briefly, 400 µL of the serum sample was extracted for retinol, and the reconstituted concentrated extract (50 µL) was injected into an HPLC apparatus equipped with a C_{18} column (Perkin-Elmer Inc, Norwalk, CT). Retinol fractions were collected using a Gilson Fraction collector FC203B model. The collected retinol fractions were dried under a gentle stream of nitrogen gas to complete dryness. To the dried residue 10 µL N, O-bis(trimethylsilyl) trifluoroacetamide containing 10% trimethylchlorosilane (Pierce, Rockford, IL) was added and heated for 30 min at 70° C to form retinyl trimethylsilyl ether.[36] After cooling, 3 µL was injected into an Agilent 6890 GC instrument equipped with a GC column, Zebron ZB-1MS capillary (PhenomenexInc, Torrance, CA) and 5973 Network Mass Selective Detector. The mass spectrometer was set at a mass to charge ratio (*m/z*) of 260 to 280. The linearity of the GC-MS response and the detection limit of the gas chromatography/electron capture negative ionization-mass spectrometry (GC–ECNCI-MS) system have been previously addressed.[40]

The kale β-carotene enrichment was fifty percent (the *m/z* 544 -548), hence an adjustment factor of 0.5 was used for the total enrichment of retinol formed from the labeled kale β-carotene (Equation 1). The cleavage of [$^{2}H_{9}$] β-carotene as 546 (M+ H^{+}+9) produced labeled $M_{retinol}$ + 4 and $M_{retinol}$ + 5 ($^{2}H_{4}$- and $^{2}H_{5}$- retinol). The percentage enrichments measured by GC-MS and the concentration of retinol in serum were used to calculate the concentration of labeled retinol in the circulation. The mass to charge ratio of $M_{retinol}$ + 4 and $M_{retinol}$ + 5 = *m/z* 272 and 273 could be clearly detected and integrated to represent the formation of retinol from the labeled kale.

Equation 1:

Enrichment of labeled retinol from kale β-carotene:

=(∑ areas of *m/z* 272-273 / 50% / ∑ areas of *m/z* 268–280)[41]

Table 2. Baseline characteristics of the subjects

Variable	Lard group	Peanut butter group
N	20	17
Girls	12	9
Age (months)	29.7±6.5	32±5.7
Weight (kg)	11.9±1.8	12.1±2.0
Height (cm)	79.4±11.0	82.1±11.7
Trans β-carotene (μg/dL)	18.2±8.1	18.6±10.4
Vitamin A (retinol) (μg/dL)	44.7±16.3	44.0±19.8
γ-tocopherol (μg/dL)	192±14.2	218±14.6
α-tocopherol (μg/dL)	1309±640	1241±73.2

Data are presented as mean±standard deviations.

In order to quantitate deuterium labeled vitamin A formed from kale [$^2H_9$] β-carotene, a reference dose differently labeled retinol was used. In this study the reference dose was given as 1 mg of [$^{13}C_{10}$] retinyl acetate capsule that was consumed on day 1 of the study just together with the labeled kale dose. During the GC–ECNCI-MS analysis the retinol m/z values were reduced by the mass of H_2O, which is removed from retinol during ionization in the mass spectrometer.[40] Therefore, $M_{retinol}$ -H_2O equals *m/z* 268 and M [$^{13}C_{10}$] retinol equals m/z 268+10 = *m/z* 278.[40] The total enrichment of labeled retinol was determined by evaluating the negative ions at $M_{retinol}$ [*m/z* 268-270 ($^{13}C_0$-$^{13}C_2$)], $M_{retinol}$ + 4 and $M_{retinol}$+5 [*m/z* 272-273 ($^2H_4$-$^2H_5$)] and Mretinol+10 [*m/z* 278-280 ($^{13}C_0$-$^{13}C_2$].[40] The percentage enrichment of labeled retinol derived from [$^{13}C_{10}$] retinyl acetate was calculated by integrating the peak area under the reconstructed mass chromatogram of the negative ions at *m/z*, 278, 279, and 280, divided by the total area response of labeled and unlabeled retinol fragment ions as shown by,

Equation 2:
Enrichment of 13C retinol from [$^{13}C_{10}$] retinyl acetate:
=(∑ areas of *m/z* 278–280 / ∑ areas of *m/z* 268–280)[37,41]

Retinol equivalence calculations

The area under the curve (AUC) of the total serum [^2H] retinol response from kale [$^2H_9$] β-carotene (mmol vs time) was compared with the AUC of the vitamin A reference dose (1 mg [13C10] retinyl acetate; molecular mass=336) were calculated using the KaleidaGraph Software, (Synergy version 4.1.0 Reading, PA) and by SAS software using proc mixed procedure. The labeled [^2H] retinol derived from kale [$^2H_9$] β-carotene and [$^{13}C_{10}$] retinyl acetate reference dose was calculated as enrichment multiplied (x) by retinol concentration x body weight x 0.0497, where body weight 3 0.0497 L/kg (for children of this age group according to the Blood Transfusion guidelines) was used to determine the total-body serum volume.[42] The retinol equivalence was calculated by comparing the AUC of serum [2H] retinol response from kale [$^2H_9$] β-carotene (nmole) to the AUC of 1 mg [$^{13}C_{10}$] serum response using Equation 3:

Equation 3:
[2H] retinol from [^2H] kale β-carotene (nmole):
=(AUC of [^2H] retinol /AUC of [$^{13}C_{10}$] retinol) x [^{13}C10] retinyl acetate/336*[41]

(*The molecular mass of [$^{13}C_{10}$] retinol=336)

Conversion factor calculations

Equation 4:
The conversion factor of β-carotene to retinol by weight[41]
= β-carotene dose in kale (nmole) x (536)/
[^2H] retinol from ß-carotene dose (nmole) x (286)

Statistical analysis

Statistical analyses were conducted using Statistical Analysis Software (SAS) Inc. (Cary, NC) version 9.3. Descriptive statistics were used to describe the distribution of the variables such as age, weight, and height, serum carotenoids, retinol and tocopherols. Proc t-test was used to determine differences between baseline characteristics of subjects in PBG and LG. Area under the curve (AUC) response for [^2H] retinol from labeled kale β-carotene and [$^{13}C_{10}$] retinol from reference dose was calculated using KaleigdaGraph software (Synagey, PA). The Proc MIXED Procedure of SAS was used to estimate the mean AUC and its standard error (SE) for kale [^2H] retinol, and [$^{13}C_{10}$] retinol enrichments. The delta method was used to estimate the SE of the conversion factors. A total of 37 participants completed the study and each time-point analyzed per group had a minimum of three participants of the required 5 subjects.

RESULTS

There were no significant differences in the distribution of age, sex, weight, height, baseline serum retinol, tocopherols and carotenoids between the PBG and LG as shown in Table 2. The mean serum retinol levels were 44.7±16.3 and 44.0±19.8 μg/dL for the PBG and LG, respectively. The mean serum β-carotene was 18.2±8.1 and 18.6±10.4 μg/dL for the PBG and LG, respectively. All participants had detectable levels of α-tocopherol and γ-tocopherol with the former being more predominant at 1309±640 and 1241±731 μg/dL in the LG and PBG, respectively, compared with 192.4±14.2 and 218±14.6 μg/dL, respectively, for γ-tocopherol. The baseline serum vitamin A for all the participants ranged from 9.5-90.5 μg/dL; three participants were vitamin A deficient with serum retinol less than 20 μg/dL; 32 participants had serum retinol between 20-90 μg/dL, and 2 participants' serum retinol levels were above 90 μg/dL.

The major carotenoids found in kale were lutein, trans β-carotene and 9 cis β-carotene as shown in Table 1 and Figure 1. The trans-β-carotene content of kale before

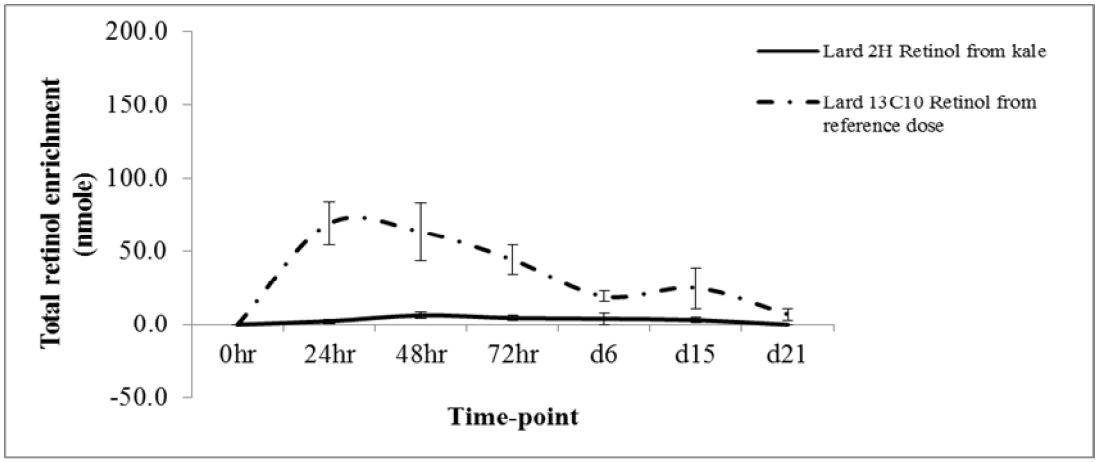

Figure 3. Calculated labeled retinol in the circulation of pooled serum time-points after consumption of kale [$^{2}H_{9}$] β-carotene with 16 g lard (animal fat) and a reference dose of [$^{13}C_{10}$] retinyl acetate on day 1. The continuous line and solid-circle data points show the serum [^{2}H] retinol response after consumption of kale [$^{2}H_{9}$] β-carotene and the dashed line data points show serum [$^{13}C_{10}$] retinol after consumption of a labeled reference dose of [$^{13}C_{10}$] retinyl acetate on day 1of the study. The retinol in circulation measured in nanomoles is shown on the y axis, and time in days is shown on the x axis.

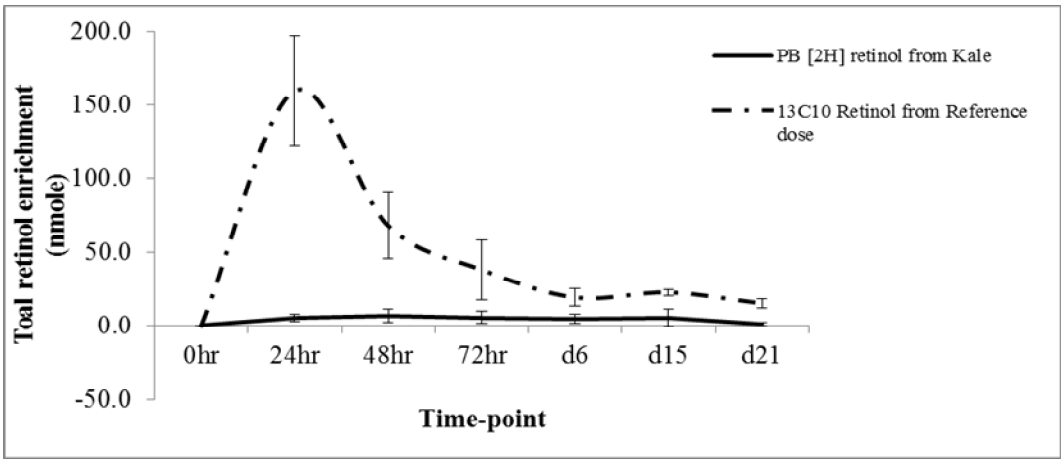

Figure 3. Calculated labeled retinol in the circulation of pooled serum time-points after consumption of kale [$^{2}H_{9}$] β-carotene with 33 g peanut butter and a reference dose of [$^{13}C_{10}$] retinyl acetate on day 1. The continuous line and solid-circle data points show the serum [^{2}H] retinol response after consumption of kale [$^{2}H_{9}$] β-carotene and the dashed line data points show serum [$^{13}C_{10}$] retinol after consumption of a labeled reference dose of [$^{13}C_{10}$] retinyl acetate on day 1of the study. The retinol in circulation measured in nanomoles is shown on the y axis, and time in days is shown on the x axis.

cooking was 4.6±0.4 mg/g fresh weight, and 1.5±0.1 mg/g wet weight after cooking. The β-carotene content in the final cooked labeled kale was 1.52 mg/50 g wet weight. APCI-LC/MS analysis of deuterium labeled kale β-carotene showed the most abundant enrichment peak at molecular mass+9 (Figure 2).

Enrichment peaks of retinol were detected in all blood samples after the ingestion of the [$^{2}H_{9}$] β-carotene and labeled reference dose of [$^{13}C_{10}$] retinyl acetate on day one of the study. Blood samples collected at 24, 48, 72 h, days 6, 15 and 21 after ingestion of labeled kale doses and labeled reference dose were processed into serum and analyzed by GC/MS. The enrichment responses measured in AUCs of the labeled [^{2}H] retinol from labeled kale dose and for the [$^{13}C_{10}$] retinol were determined for LG and PBG as shown by Figure 3 and Figure 4. The calculations of the retinol equivalence of peanut butter cooked kale and lard cooked kale were performed by comparing the AUCs of labeled [^{2}H] retinol formed from the labeled kale β-carotene, to that of 1 mg [$^{13}C_{10}$] retinol (Equation 1 and 2). The labeled β-carotene conversion factors of the LG and PBG kale β-carotene to vitamin A were then determined (Equation 1 and 2). Our study showed that the conversions of LG labeled kale β-carotene (1.52 mg) and PBG labeled kale β-carotene (1.52 mg) to retinol were 13.4±3.1 and 11.0±3.9 to 1 by weight and were not significantly different (p>0.05) (Table 3).

DISCUSSION

VAD is a public health problem in Zimbabwe, where more than 40% of the children under 6-59 months had serum retinol levels <20 µg/dL.[4,43] In this study, only 2 subjects representing 7.4% had VAD. This is not surprising because the participants were recruited from urban day care centers in the city of Bulawayo. Children who attend day care come from working families who can afford to buy vitamin A rich foods compared with children who do not attend day care. In Zimbabwe, children in city day care centers are also fed nutritious foods compared with those at home. Also children in urban centers are exposed to a variety of foods purchased from a variety

Table 3. The calculated AUC for kale [^{2}H] retinol, [$^{13}C_{10}$] retinol for LG and PBG and β-carotene to vitamin A conversion factors by weight

Treatment group	Area under the curve [^{2}H] retinol (nmole)	Area under the curve [$^{13}C_{10}$] retinol (nmole)	β-carotene to retinol conversion factors by weight
Kale lard	56.2±0.53	433±54.9	13.4±3.1
Kale peanut butter	84.8±16.2	560±157	11.0±3.9

Data are presented as mean AUC±standard errors.

of sources as opposed to children in rural areas. The baseline serum carotenoids in the participants were similar to those in western subjects.[44,45] Serum carotenoids are used as a biomarker of fruit and vegetable intake.[46,47] Higher serum carotenoids reflect intakes of fruits and vegetables. The participants also had higher serum levels of α-tocopherol, γ-tocopherols similar to those observed in US participants, which reflects diets that are rich in vegetables, cereals and nuts.[46] This confirms the fact that these urban children were exposed to a variety of foods because of the relatively high income families they came from.

Some studies have used intrinsically labeled plant foods to accurately assess carotenoid absorption and conversion to vitamin A, thus determining the vitamin A value of a food source.[35-37,41] Plant carotenoids are intrinsically labeled with the addition of a hydrogen-stable isotope presented to the roots in the form of heavy water, [^2H_2O] via hydroponic growth on a nutrient solution composed of a fixed ^2H_2O percentage.[39] The isotopic label enables identification and separation of test food carotenoids and their metabolites in serum carotenoids from other serum carotenoids and retinoids. The kale used in this study had [$^2H_9$] β-carotene as the most abundant isotopomer (Figure 2), and this level of enrichment was found to be adequate to detect the labeled retinol metabolites formed from enzymatic cleavage of β-carotene in the body.[37]

Previous studies in the US with adults using [^{13}C] labeled kale showed that kale β-carotene was bioavailable and was converted to vitamin A.[33,34] However, this US study was aimed only at determining the bioavailability of kale carotenoids. Our study was designed to determine the vitamin A value of provitamin A rich kale, and to determine whether cooking it with peanut butter or lard (animal fat) had an effect on the absorption and bioconversion of the β-carotene to vitamin A in children. Peanut butter and lard increased the absorption of kale [$^2H_9$] β-carotene and its conversion to vitamin A [^{2}H] retinol. The kale β-carotene conversion factor to vitamin A was 13.4±3.1 and 11.0±3.9 to 1 by weight for lard and peanut butter cooked kale, respectively. There was no significant difference between the total [^{2}H] retinol enrichment (nmole) of the LG and PBG and the conversion factors (p>0.05). The lack of significant differences in the conversion factors between the lard and peanut butter groups observed maybe because the fat or oil content was matched between the groups 33 g (16 g fat) in peanut butter and 16 g in lard. Another explanation could be the smaller sample size of the study, high variability between subject retinol response kinetics and the better vitamin A status of the participants. It is interesting to note that the PBG had higher response for both [^{2}H] retinol and [$^{13}C_{10}$] retinol compared with the LG although there was no statistical significance (Table 3). This indicates that peanut butter components may increase the bioavailability of vitamin A compared with lard, which contains mostly saturated and monounsaturated fatty acids. It could be that the type of fat (unsaturated fats in peanut butter) affects the bioavailability of vitamin A. More research is required in this area to fully understand the mechanisms. The β-carotene conversion factors of 13.4±3.1 and 11.0±3.9 to 1 by weight in kale are superior to those that have been observed in other studies. One study with Chinese pre-school children fed 275 mg β-carotene from green-yellow vegetables showed that 27 μg β-carotene from vegetables was equivalent to 1 μg retinol.[48] This conversion factor is similar to that reported in another study in Vietnam where participants ingested 5.6 mg β-carotene/d from green leafy vegetables. In this study 1 μg retinol was found to equivalent to 28 μg β-carotene.[13] Other studies also showed the conversion factors of 26 to 1, and 28 to 1 in green vegetables.[49] Our study may have shown better conversion factors because we used advanced techniques of stable isotope labeled kale and labeled reference dose which are accurate methods in determining sources of vitamin A in serum. It is known that the food matrix plays an important role in the bioavailability and bioconversion of β-carotene to vitamin A in humans.[30,50] The green leafy vegetables have thus been shown to have a food matrix that hinders the efficient utilization of β-carotene to vitamin A.[35,49,51] However, cooked kale consumed with fat is better as our study suggests. Another important factor to consider is the vitamin A status of the participants; participants with adequate vitamin A status showed low conversion of β-carotene to vitamin A.42 In our study, 86% of the subject had marginal VAD, which might have contributed to the moderate β-carotene to vitamin A conversion factors of 13.4±3.1 and 11.0±3.9 to 1 by weight. Also genetic factors have been shown to affect the bioavailability and bioconversion of β-carotene to vitamin A in humans.52 Kale β-carotene to vitamin A conversion factors observed in this study are important for the development of complementary feeding guidelines in east and southern Africa where kale is a staple food. This study showed that cooked pureed kale is a good source of vitamin A in children. When kale is cooked with peanut butter, it will not only provide vitamin A, but lutein, protein, vitamin E and fatty acids that are required by growing children.

The main limitation of this study is that we used superchild model approach. This study design was necessitated by an inability to collect multiple blood samples from an individual child for ethical reasons. Therefore, we constructed one AUC for each grouping, with SDs around the various points on the curve. These single group curves

provide estimations for conversion efficiency for a group as a whole, but individually they are not suitable for the statistical analysis of differences in conversion efficiencies between the groups. This approach has been used in other studies successfully.[42]

Conclusion

This the first study to show the conversion of kale β-carotene to vitamin A in children. We showed that kale β-carotene is a good source of vitamin A in pre-school children when consumed with peanut butter and lard. The conversion factors of 13.4±3.1 and 11.0±3.9 to 1 by weight show that kale can be promoted as complementary food for infant and toddlers who are often vulnerable to VAD. However, kale β-carotene cannot be depended on as the sole source of vitamin A, but can be used as part of a dietary diversification strategy to complement vitamin A intake from other source. It is noteworthy that kale is also a good source of lutein.

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AUTHOR DISCLOSURES

The authors would like to report no conflicts of interests.

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