Archives of Osteoporosis (2019) 14:72
https://doi.org/10.1007/s11657-019-0617-y

SHORT COMMUNICATION

Restricting vitamin A intake increases bone formation in Zambian children with high liver stores of vitamin

S. A. Tanumihardjo[1] · B. M. Gannon[1,2] · C. Kaliwile[3] · J. Chileshe[4] · N. C. Binkley[5]

Received: 1 April 2019 / Accepted: 4 June 2019

Abstract

Summary This analysis was performed in Zambian children who had a high prevalence of hypervitaminosis A, defined as ≥ 1.0 μmol retinol/g liver. Bone parameters included markers of bone formation (P1NP), bone resorption (CTX), parathyroid hormone, calcium, vitamin A, and vitamin D. Low dietary vitamin A intake increased P1NP.

Purpose Vitamin A (VA) interacts with bone health, but mechanisms require clarification. In countries where multiple interventions exist to eradicate VA deficiency, some groups are consuming excessive VA. Bone metabolism and inflammatory parameters were measured in Zambian children who had high prevalence of hypervitaminosis A determined by ^{13}C-retinol isotope dilution.

Methods Children ($n = 143$), 5 to 7 years, were recruited into a placebo-controlled biofortified orange maize feeding study for 90 days. Bone turnover (P1NP and CTX) and inflammatory (C-reactive protein (CRP) and alpha-1-acid glycoprotein) biomarkers were measured in fasting blood samples before and/or after intervention with the following: (1) VA at the recommended dietary allowance (400 μg retinol activity equivalents/day (as retinyl palmitate)), (2) maize enhanced with the provitamin A carotenoid β-carotene (2.86 mg/day), or (3) a placebo. Parathyroid hormone, calcium, and 25(OH)-vitamin D were measured at end line.

Results Bone formation, as measured by P1NP, increased ($P < 0.0001$) in the placebo group who consumed low preformed VA during the intervention. Bone resorption, measured by CTX, was not affected. P1NP and CTX were negatively associated with inflammation, most strongly with CRP. Serum calcium did not differ among groups and was low (7.29 ± 0.87 μg/dL). Serum 25(OH) D did not differ among groups (54.5 ± 15 nmol/L), with 91% < 75 nmol/L and 38% < 50 nmol/L.

Conclusions Reduction of dietary preformed VA in Zambian children for 4 months improved bone formation. Chronic consumption of preformed VA caused hypervitaminosis A and may impair bone formation. In children, this could be associated with failure to accrue optimal peak bone mass.

Trial registration The NIH Clinical Trial registry number is NCT01814891; https://clinicaltrials.gov/ct2/show/NCT01814891.

Keywords Calcium · CTX · P1NP · PTH · Vitamin a · Vitamin D · Zambia

S. A. Tanumihardjo and B. M. Gannon contributed equally to this work.

✉ S. A. Tanumihardjo
sherry@nutrisci.wisc.edu

[1] Department of Nutritional Sciences, University of Wisconsin-Madison, 1415 Linden Dr., Madison, WI 53706, USA

[2] Present address: Cornell University, Ithaca, NY, USA

[3] National Food and Nutrition Commission of Zambia, P.O. Box 32669, Lusaka, Zambia

[4] Tropical Diseases Research Center, Ndola, Zambia

[5] Osteoporosis Clinical Research Program, University of Wisconsin-Madison, Madison, WI, USA

Abbreviations

RID Retinol isotope dilution
VA Vitamin A

Introduction

Vitamin A (VA) status can vary within a population from deficiency through toxicity depending upon dietary intake, supplementation, and public health interventions [1]. The main VA public health goal is obtaining optimal status, which is defined as 0.1 to 0.7 μmol retinol/g liver [1]. Vitamin A

Published online: 28 June 2019

deficiency can lead to blindness, anemia, suppression of the immune system, and increased risk of mortality [2]. Current methods to alleviate VA deficiency are periodic high-dose supplementation, fortification with preformed VA, and dietary diversification. In Nicaragua, VA status was evaluated in children after implementing a sugar fortification program and found that many of them developed hypervitaminosis A, defined as ≥ 1 μmol/g liver [3]. Similarly, Zambia adopted both high-dose VA supplements and fortified sugar [4]. In a cohort of Zambian children, a high prevalence of hypervitaminosis A [5], elevated serum retinyl esters and provitamin A carotenoids [6], and hypercarotenodermia occurred [4].

The effect(s) of VA on bone are complex and appear to depend on calcium status. High dietary intake of preformed VA, a form that is efficiently absorbed, can increase bone resorption and cause hypercalcemia, bone loss [7], and periosteal calcification [8]. Dietary provitamin A carotenoids do not have the same adverse skeletal effects because their absorption and bioconversion are regulated by VA status [9]. Calcium intake may have protective effects on bone even when preformed VA intake is high [10]. Dietary analysis of the Zambian children participating in a provitamin A biofortified maize intervention [5] revealed that they had low intakes of dietary calcium and adequate VA intake [11]. Hypervitaminosis A with low calcium intakes may cause adverse bone consequences. Therefore, the purpose of this study was to evaluate the effect of dietary VA change on bone remodeling markers in these Zambian children. Additional bone health biomarkers including serum calcium, parathyroid hormone (PTH), and 25-hydroxyvitamin D_3 (25(OH)D) concentrations were assessed.

Study population and methods

Subjects and ethics

This trial was conducted in 2012 in Nyimba District of the Eastern Province of Zambia in preschool children ($n = 143$ initial enrollment, aged 71.5 ± 6.9 months) because of high prevalence of low serum retinol concentrations in a prior survey [12]. Inclusion criteria were apparently healthy children aged 5–7 years living in the study area and not enrolled in school. Height and weight were measured using digital scales and stadiometers (Seca, Hamburg) [5]. Children needed to have weight-for-age and weight-for-height z scores > -3 based on WHO guidelines [13], hemoglobin > 7.0 g/dL, no clinical infection at recruitment causing fever, antihelminthic treatment the week before treatment, and not having received a 200,000 IU retinyl palmitate supplement in the past 6 months.

Procedures involving human subjects were approved by the Tropical Disease Research Center's Ethics Review Committee in Zambia and University of Wisconsin-Madison's Health Sciences Human Subjects Institutional Review Board. Written informed consent was obtained from parents or caregivers. This trial was registered with Clinicaltrials.gov as NCT01814891.

Study design

Children were individually randomized into three groups, blocked by site, by randomly picking an opaque envelope containing a colored sticker corresponding to their treatment group. Treatment groups consisted of a negative control group (VA−, $n = 47$), who ate white maize that was not provitamin A biofortified and received daily placebo oil; the test group (orange, $n = 46$), who consumed provitamin A biofortified orange maize (average 2.86 mg β-carotene/day) and received daily placebo oil; and a positive control group (VA+, $n = 47$), who ate white maize and received a daily VA dose (retinyl palmitate, 400 μg RAEs (current U.S. Recommended Daily Allowance for children this age)) in oil. The internally calculated bioefficacy factor was 10.4 μg β-carotene equivalents consumed from the maize to 1 μg retinol formed in the body [5]; the orange group was obtaining a VA equivalent of 275 μg RAE/day. Oil doses were identical in appearance, given with a positive displacement pipette onto a serving spoon, and administered immediately before the lunch meal by the study investigators. Four feeding sites throughout the district were used. Children ate breakfast, lunch, and dinner 6 days/week at the feeding sites. All meals consisted of soft porridge for breakfast or stiff porridge made from maize with side dishes for lunch and dinner. All food intakes were measured with battery-operated scales to the nearest 1 g. Children consumed orange maize in a separate room to avoid food sharing.

After a baseline blood draw, an oral VA tracer dose was given to assess total body stores. After 14 days, a second blood draw was obtained. This was followed by the intervention for 90 days of feeding, a washout period of 1 week, and a similar end line assessment consisting of two blood draws 14 days apart [5]. The final end line blood draw was used for the third P1NP measurement. Samples were obtained fasting. Blood was clotted and stored on ice until centrifugation the same day. Serum was transported either in liquid nitrogen tanks or on dry ice and stored at − 80 °C until analysis.

Laboratory variables

Technicians were blinded to treatment groups. After determining that this cohort of children had a high prevalence of hypervitaminosis A [5], defined as ≥ 1.0 μmol retinol/g liver [1], using retinol isotope dilution (RID), markers of bone remodeling were investigated. Serum CTX was analyzed as a marker of bone breakdown after the intervention. Serum P1NP, a

marker of bone formation, was measured at three timepoints: baseline, immediately after the intervention at the same time as CTX, and after the washout period. PTH was analyzed at the end of the trial post-intervention. CTX was analyzed using ELISA (CTX: Serum CrossLaps), P1NP by RIA (Orion Diagnostica), and PTH by immunoradiometric assay (Scantibodies). The intra-/interassay CVs of these assays were 3%, 3.5–4%, and 3%, respectively.

25(OH) D analysis was adapted from a published procedure [14]. Serum (350 μL) was mixed with 80:20 methanol:isopropanol and extracted three times with hexanes. Dodecanophenone was the internal standard. Supernatant was dried under nitrogen and reconstituted in 30 μL methanol. Five microliters was injected onto an Acquity H Class ultra-performance liquid chromatograph® (UPLC) equipped with a photodiode array detector and a BEH C18 column (1.7 μm, 2.1 × 100 mm; Waters, Milford, MA). Mobile phases were 67:33 (vol:vol) methanol:water with 10 mmol ammonium acetate/L as solvent A and 75:25 (vol:vol) methanol:isopropanol as B. The gradient was run at 0.45 mL/min: (1) start 90% A and 10% B, (2) a 6-min linear gradient to 60% A, (3) a 2-min hold at 60% A, (4) a 3-min linear gradient to 5% A, (5) a 4-min hold at 5% A, and (6) a 1-min linear gradient to 90% A. Serum 25(OH) D concentration was quantified with a 25(OH) D standard (Enzo Life Sciences, Inc.).

Vitamin A metabolites and esters were analyzed post-intervention following a published procedure using C23-β-*apo*-carotenol as internal standard [15]. After extraction, samples were reconstituted in 100 μL 90:10 (vol:vol) methanol:dichloroethane and 50 μL was injected onto a Waters Sunfire™ C18 column (5 μm, 4.6 × 250 mm; Waters, Milford, MA) equipped with a guard column. The HPLC system consisted of a 1525 binary pump, 2707 autosampler, and 2998 photodiode array detector. Mobile phase was 70:30 (vol:vol) methanol:water as solvent A and 80:20 (vol:vol) methanol:dichloroethane as solvent B both with 10 mmol ammonium acetate/L. At 0.9 mL/min, the gradient was as follows: (1) 100% A, (2) 20-min linear gradient to 100% B, and (3) 1-min transition to 100% A.

Serum calcium was measured as part of serum zinc analyses (reported in [5]) using inductively coupled plasma optical emission spectrometry by University of Wisconsin-Madison Soil Testing Laboratories. Serum C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) as measures of the acute phase response were quantified by ELISA; malaria was diagnosed by blood smear.

Statistics

Data are reported as median (Q1, Q3), mean ± SD, predicted population margins (LS mean ± standard error), or Pearson correlation coefficients (r). Data were analyzed using Statistical Analysis System (SAS Institute, version 9.4). Mixed models were used with a random effect of child for repeated measures analysis and fixed effects of age, sex, CRP, and AGP. Normality of residuals was assessed, and variables were log-transformed if necessary. A Tukey-Kramer adjustment was used for comparisons and P values; significance was defined as $P \leq 0.05$.

Results

Correlations among biomarkers of bone metabolism and inflammation

The bone remodeling markers P1NP and CTX were positively correlated ($r = 0.42$, $P < 0.001$). Inflammatory biomarkers CRP and AGP were positively correlated ($r = 0.54$), and both were associated with malaria diagnosis ($r = 0.31$ and 0.23, respectively), all $P \leq 0.001$. P1NP was negatively associated with CRP, AGP, and malaria ($r = -0.17$, -0.17, -0.20, respectively; $P \leq 0.022$). CTX was negatively associated with CRP, AGP, and malaria ($r = -0.24$, -0.18, -0.21, respectively; $P \leq 0.045$). Positive malaria smears were identified in 15% of children at baseline and 1.5% at end line, and neither time differed by treatment group ($P \geq 0.07$).

Markers of bone turnover in response to intervention

Serum P1NP concentrations did not differ at baseline but showed a treatment effect 1 week after the intervention ceased (Fig. 1; $P < 0.0001$). The group that had received the orange β-carotene enriched maize was intermediate, while the group receiving the VA supplement had the lowest values. This change was primarily driven by higher values in the orange and VA− groups. In order to tease out whether this was due to

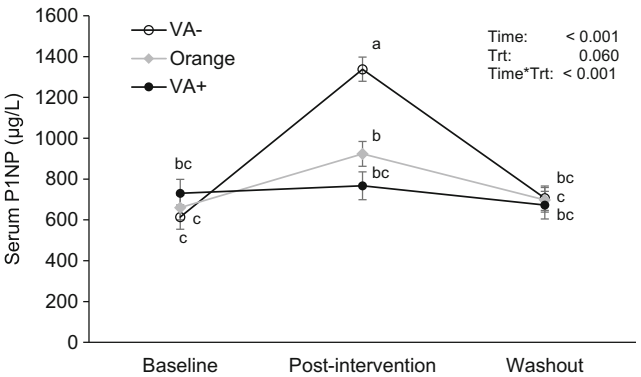

Fig. 1 P1NP measurements in Zambian children ($n = 71$) at three timepoints displayed an effect of time ($P < 0.0001$), a trending treatment effect ($P = 0.060$), and a treatment by time interaction ($P < 0.0001$). The time interval between post-intervention and washout was 2 weeks between the blood samples, and all children were on the same diet during this period. Data are predicted population margins ± standard errors; values without a common letter differ: a > b > c

the intervention or the increased VA stores, PINP measurements were repeated after the prolonged washout period. Considering all three timepoints, ($n = 71$), P1NP had a time effect ($P < 0.0001$), a trending treatment effect ($P = 0.060$), and a treatment-by-time interaction ($P < 0.0001$), with no effect of sex, age, or any interaction with sex or age ($P \geq 0.12$).

Serum CTX concentrations did not differ among the three treatment groups after the intervention (Table 1; $P = 0.31$), indicating that the VA supplement did not change the rate of bone breakdown. CTX (μg/L) did not vary by age, sex, AGP, or malaria status ($P \geq 0.11$) but remained significantly associated with CRP (mg/L) in the final model ($\beta = -0.035 \pm 0.013$; $P = 0.006$).

Considering baseline and end line timepoints (with CRP and AGP analyses), P1NP (μg/L) did not vary by age, AGP, or malaria ($P \geq 0.60$) but remained significantly associated with CRP (mg/L) in the final model ($\beta = -3.32 \pm 2.64$; $P = 0.025$). P1NP had a sex-by-time interaction, with females having significantly higher P1NP at the end line timepoint than males (1146 ± 53 vs. 906 ± 46 μg/L, $P = 0.019$), but no difference at other timepoints.

Related indicators

Serum calcium concentrations did not differ by treatment, age, or sex (Table 1; $P \geq 0.46$). The serum calcium reference range for this age group is 8.8–10.7 μg/dL [16]. The range in these children was 4.94–9.23 μg/dL, and 94% were below the reference range. The prevalence of low serum ferritin (< 12 μg/L) at baseline was 9.3, 14, and 17.5% in the VA+, orange, and VA− groups, respectively, and did not differ [5]. Hemoglobin and prevalence of anemia (hemoglobin < 110 g/L) did not show effects of time, treatment, or a time-by-treatment interaction ($P \geq 0.09$); overall, the mean hemoglobin was 117 ± 11 g/L and prevalence of anemia was 23%.

PTH differed by treatment group ($P = 0.0089$) and was higher in the orange maize fed group than the positive and negative control groups. The reference range for PTH is 10–65 pg/mL [17]. Over 65 pg/mL, hyperparathyroidism is a concern and no child was above this value. All but two children were within the reference range. PTH did not have an effect of age ($P = 0.58$), but differed by sex, with females having greater PTH than males (29.8 ± 1.4 vs. 24.1 ± 1.2, $P = 0.0021$). After the washout, P1NP and PTH were significantly correlated ($r = 0.27$, $P = 0.025$), but calcium was not associated with either P1NP or PTH ($P \geq 0.22$).

Serum 25(OH) D was analyzed post-intervention (mean 54.5 ± 15.0 nmol/L) and did not have an effect of treatment, age, or sex ($P \geq 0.24$). However, according to a currently used cutoff for 25(OH) D of < 75 nmol/L for deficiency [18], 91% of these children were deficient. Using a more conservative value of < 50 nmol/L, 38% of these children would be classified vitamin D deficient. Vitamin D did not have a significant effect or interaction with treatment for either CTX or P1NP ($P \geq 0.13$).

No significant effects of treatment, age, or sex were found for total retinyl esters, 13-*cis* retinoic acid, or all-*trans* retinoic acid ($P \geq 0.11$) (Table 1). 4=Oxo retinoic acid and 4=oxo retinol were not quantifiable.

Discussion

This was an analysis of bone markers and nutritional indicators related to optimal bone health in a group of children with a high prevalence of hypervitaminosis A. We observed that

Table 1 Final vitamin A, vitamin D, and other bone markers evaluated in Zambian children who were enrolled in a high β-carotene (orange) maize intervention study

Parameter	Negative control	Orange maize	Positive control	*P*
Total body vitamin A stores (μmol)	665 (509, 818) [44]	806 (586, 1024) [44]	811 (621, 1136) [45]	0.0040
Total liver reserves (μmol/g liver)	0.97 (0.71, 1.17) [44]	1.09 (0.85, 1.49) [44]	1.17 (0.86, 1.74) [45]	0.0042
Serum retinol concentration (μmol/L)	0.974 (0.840, 1.20) [43]	0.944 (0.808, 1.31) [43]	0.972 (0.789, 1.04) [43]	0.39
Serum total retinyl esters (μmol/L)	0.024 (0.015, 0.031) [38]	0.025 (0.016, 0.034) [35]	0.023 (0.017, 0.029) [37]	0.40
Serum 13-*cis*-retinoic acid (nmol/L)	1.96 (ND, 5.7) [38]	ND (ND, 5.30) [35]	1.0 (ND, 4.4) [37]	0.69
Serum all-*trans*-retinoic acid (nmol/L)	13.6 (11.8, 15.7) [38]	13.4 (10.4, 15.0) [35]	13.2 (12.0, 15.3) [37]	0.51
Serum P1NP (μg/L)	1274 (826, 1676) [40]	835 (663, 1207) [42]	747 (618, 917) [34]	0.0001
Serum CTX (μg/L)	2.36 (1.85, 2.78) [42]	2.02 (1.76, 2.51) [43]	2.19 (1.86, 2.53) [43]	0.31
Serum 25(OH) D (nmol/L)	57.6 (50.2, 66.1) [37]	49.3 (42.3, 59.6) [36]	54.8 (38.5, 63.8) [39]	0.24
Serum parathyroid hormone (pg/mL)	23.5 (18.3, 30.3) [37]	31.2 (21.2, 35.2) [41]	24.2 (15.8, 33.2) [40]	0.0072
Serum calcium (μg/dL)	7.45 (6.91, 7.95) [40]	7.16 (6.78, 7.85) [38]	7.21 (6.64, 7.89) [41]	0.69
Serum C-reactive protein (mg/L)	0.753 (0.286, 1.83) [41]	0.373 (0.171, 1.07) [43]	0.587 (0.228, 2.15) [37]	0.35
Serum alpha-1-acid glycoprotein (mg/mL)	1.78 (1.31, 2.39) [41]	1.46 (1.07, 2.24) [41]	1.75 (1.24, 2.42) [42]	0.16

Results are expressed as median (Q1, Q3) [*n*]. The *n* is different depending on the amount of serum that was left from other analyses

withdrawing VA from these children's diets for several months increased bone formation. This unexpected finding requires more research but suggests that overzealous efforts to rectify VA deficiency might produce adverse skeletal effects. It only took 3 weeks for P1NP to return to baseline after these children were on the same VA intakes.

Currently, there are only sparse data on bone turnover markers in children. The P1NP values in these children were higher than those reported in older Gambian children (7.90 ± 1.27 years; range 470 to 487 μg/L) [19]. It is important to note that P1NP did not change in the Gambian children by season, supporting the treatment-by-time effect in this study. A zinc intervention study in premenarcheal girls in the USA reported an increase in P1NP with zinc treatment [20]. One could speculate that this may be important as zinc, VA, and provitamin A carotenoid metabolism are related and perhaps changing preformed VA shifted other nutrient metabolism leading to an increase in bone formation.

The Zambian CTX values (range 2.11 to 2.30 μg/L) were similar to older Gambian boys (range 2.03 to 2.24 μg/L), which did vary by season [19]. No changes were determined by treatment in this study indicating that osteoclast activity was not affected. On the other hand, the Zambian CTX values are much higher than those reported in Spanish children (6.8 ± 0.2 years) with a value of 1.22 ± 0.04 μg/L [21]. Thus, populations may differ and considering the universal high VA stores in these Zambian children, bone markers should be further evaluated with measures of VA status in different groups of children.

We observed inverse associations between markers of bone turnover and inflammatory biomarkers, as observed in the Gambian study [19]. In this study, CRP, AGP, and malaria were all correlated, but CRP remained significantly associated with CTX and P1NP in the final models accounting for treatment, time, age, and sex. This indicates that multiple factors are influencing bone metabolism, including inflammation and VA intake.

The low serum calcium concentrations are notable. Dietary intake of children in this community was documented to be low using a database for US foods [11], but Zambia does not have a good reference for calcium in their local foods that would allow adjusting this database. Furthermore, vitamin D status was suboptimal. The combination of low calcium and vitamin D does not support optimal bone health and could affect markers of bone remodeling.

In conclusion, in these children with hypervitaminosis A, reduction of dietary VA intake increased a marker of bone formation. This suggests that VA excess may impair bone formation; in children, this could be associated with failure to accrue optimal peak bone mass. Finally, the potential effect of VA withdrawal on the skeleton of these children with hypervitaminotic A liver stores requires further studies in randomized, controlled trials.

Acknowledgments The authors would like to thank Kiersten Olsen and Michael Grahn for analyzing samples and Devika Suri for assistance with statistical evaluation. HarvestPlus (www.harvestplus.org) is a global alliance of agriculture and nutrition research institutions working to increase the micronutrient density of staple food crops through biofortification.

Funding information The work was supported by HarvestPlus contract number 8256. HarvestPlus provided funding on the basis of study design and recommended additional assays to perform. Other support was from an endowment to Tanumihardjo entitled, "Friday Chair for Vegetable Processing Research" and Global Health Funds at the University of Wisconsin-Madison.

Compliance with ethical standards

The Tropical Disease Research Center's Ethics Review Committee in Ndola, Zambia approved the study. The consent form and related materials were translated into English and the University of Wisconsin-Madison's Health Sciences Human Subjects' Institutional Review Board approved the study.

Disclaimer The views expressed do not necessarily reflect those of HarvestPlus.

Conflicts of interest None.

References

1. Tanumihardjo SA, Russell RM, Stephenson CB, Gannon BM, Craft NE, Haskell MJ, Lietz G, Schulze KJ, Raiten DJ (2016) Biomarkers of nutrition for development (BOND) - vitamin A review. J Nutr 146:1816S–1848S
2. World Health Organization (2009) Global prevalence of vitamin A deficiency in populations at risk 1995–2005: WHO global database on vitamin A deficiency. [Accessed 8 October 2018] **Available from:** http://apps.who.int//iris/handle/10665/44110
3. Ribaya-Mercado JD, Solomons NW, Medrano Y, Bulux J, Dolnikowski GG, Russell RM, Wallace CB (2004) Use of the deuterated-retinol-dilution technique to monitor the vitamin A status of Nicaraguan schoolchildren 1 y after initiation of the Nicaraguan national program of sugar fortification with vitamin A. Am J Clin Nutr 80:1291–1298
4. Tanumihardjo SA, Gannon BM, Kaliwile C, Chileshe J (2015) Hypercarotenodermia in Zambia: which children turned orange during mango season? Eur J Clin Nutr 69:1346–1349
5. Gannon B, Kaliwile C, Arscott SA, Schmaelzle S, Chileshe J, Kalungwana N, Mosonda M, Pixley K, Masi C, Tanumihardjo SA (2014) Biofortified orange maize is as efficacious as a vitamin A supplement in Zambian children even in the presence of high liver reserves of vitamin A: a community-based, randomized placebo-controlled trial. Am J Clin Nutr 100:1541–1550
6. Mondloch S, Gannon BM, Davis CR, Chileshe J, Kaliwile C, Masi C, Rios-Avila L, Gregory JF 3rd, Tanumihardjo SA (2015) High provitamin A carotenoid serum concentrations, elevated retinyl esters, and saturated retinol-binding protein in Zambian preschool children are consistent with the presence of high liver vitamin A stores. Am J Clin Nutr 102:497–504
7. Genaro PDS, Martini LA (2004) Vitamin A supplementation and risk of skeletal fracture. Nutr Rev 62:65–67
8. Binkley N, Krueger D (2000) Hypervitaminosis A and bone. Nutr Rev 58:138–144

9. Tanumihardjo SA (2013) Vitamin A and bone health: the balancing act. J Clin Densitom 16:414–419
10. Whiting SJ, Lemke B (2003) Brief critical reviews: excess retinol intake may explain the incidence of osteoporosis in Northern Europe. Nutr Rev 57:423–426
11. Schmaelzle S, Kaliwile C, Arscott SA, Gannon BM, Masi C, Tanumihardjo SA (2014) Nutrient and nontraditional food intakes by Zambian children in a controlled feeding trial. Food Nutr Bull 35:60–67
12. Hotz C, Chileshe J, Siamusantu W, Palaniappan U, Kafwembe E (2012) Vitamin A intake and infection are associated with plasma retinol among pre-school children in rural Zambia. Public Health Nutr 15:1688–1696
13. World Health Organization. WHO Anthro (version 3.2.2, January 2011) and macros [Internet]. 2011 [Accessed 19 March 2019]. Available from: http://www.who.int/childgrowth/software/en/
14. Turpeinen U, Hohenthal U, Stenman UH (2003) Determination of 25-hydroxyvitamin D in serum by HPLC and immunoassay. Clin Chem 94:1521–1524
15. Penniston KL, Thayer JC, Tanumihardjo SA (2003) Serum vitamin A esters are high in captive rhesus (*Macaca mulatta*) and marmoset (*Callithrix jacchus*) monkeys. J Nutr 133:4202–4206
16. Roizen JD, Shah V, Levine MA, Carlow DC (2013) Determination of reference intervals for serum total calcium in the vitamin D-replete pediatric population. J Clin Endocrinol Metab 98:E1946–E1950
17. Saliba W, Barnett O, Rennert HS, Lavi I, Rennert G (2011) The relationship between serum 25(OH) D and parathyroid hormone levels. Am J Med 124:1165–1170
18. Binkley N, Ramamurthy R, Krueger D (2010) Low vitamin D status: definition, prevalence, consequences and correction. Endocrinol Metab Clin N Am 39:287–301
19. Munday K, Ginty F, Fulford A, Bates CJ (2006) Relationships between biochemical bone turnover markers, season, and inflammatory status indices in prepubertal Gambian boys. Calcif Tissue Int 79:15–21
20. Berger PK, Pollock NK, Laing EM, Chertin V, Bernard PJ, Grider A, Shapses SA, Ding KH, Isales CM, Lewis RD (2015) Zinc supplementation increases procollagen type 1 amino-terminal propeptide in premenarcheal girls: a randomized controlled trial. J Nutr 145:2699–2704
21. Prats-Puig A, Puig T, Sitjar C, Mas-Parareda M, Grau-Cabrera P, Soriano-Rodríguez P, Montesinos-Costa M, Diaz M, de Zegher F, Ibáñez L, Bassols J, López-Bermejo A (2012) Soluble fatty acid synthase relates to bone biomarkers in prepubertal children. Osteoporos Int 23:2053–2058