NUTRITIONAL STATUS ASSESSMENT AND INTERVENTION WITH A RED PALM OIL BISCUIT AMONG PRE-SCHOOL CHILDREN ATTENDING INFORMAL CRÈCHES, IN THE EASTERN CAPE, SOUTH AFRICA

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ABSTRACT

Little information is available on the nutritional and health status of pre-school children attending informal crèches. The study aimed to determine the status of blood carotenoid, vitamin E, inflammatory markers and omega-3 fatty acids in children attending crèches in the Eastern Cape, South Africa. Baseline measurements were followed by an intervention to determine the effect of a biscuit baked with red palm oil (RPO) shortening on these parameters. Forty-seven (n=47) children were randomly assigned to a RPO (Group A) (n=25) and non-RPO (Group B) (n=22) group. Group A received biscuits providing carotenoids, tocotrienols and α-tocopherol while Group B biscuits provided only tocotrienols and α-tocopherol. Biscuits were distributed daily during the school week. Blood samples were obtained at baseline, 3 months and 6 months during intervention as well as 6 months post intervention. At baseline, children showed a mild (10%) vitamin A deficiency, severe vitamin E deficiency (69%) while 98% presented with omega-3 fatty acid indexes below 8%. For vitamin E data was pooled as both groups received similar amounts of vitamin E while no significant differences were observed between the groups during the intervention. Significant increases ($p \leq 0.05$) in α-tocopherol levels were observed in both groups at 3-, 6- and 6- months post intervention. Deficiency levels declined to 10% and 0% at 6- and 6-months post, respectively. In group A, plasma α-carotene levels ($p \leq 0.05$) increased significantly from baseline (0.04 µmol/L; 95% CI: 0.03 – 0.06) at 3 (0.36 µmol/L; 95% CI: 0.29 – 0.45) and 6 months (0.48 µmol/L; 95% CI: 0.39 – 0.60). Similar significant ($p \leq 0.05$) results were observed for plasma β-carotene levels (3 months (0.31 µmol/L; 95% CI: 0.27 – 0.40) and 6 months (0.33 µmol/L; 95% CI: 0.28 – 0.40). Consumption of a biscuit baked with RPO can play a significant role in alleviating vitamin E deficiency and can significantly increase plasma α- and β-carotene levels in pre-school children.

Key words: Vitamin A, carotenoids, vitamin E, CRP, IL-6, fortification, omega-3 fatty acids

INTRODUCTION

Among other nutrients, vitamin A, vitamin E and omega-3 fatty acids are fundamental in the foundation of a child's physical growth, emotional and cognitive development. The role of these nutrients during childhood development is well established [1–3]. In 2013, results from the South African National Health and Nutrition Examination Survey (SANHANES-1) reported that 43.6% of children under 5 years of age were vitamin A deficient (serum retinol <0.7 µmol/L) [4] which, according to the World Health Organisation (WHO) criteria, places children in the severe public health concern category [5]. The deficiencies from the SANHANES study were observed despite the mandatory fortification of staple foods promulgated in the South African Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act no. 54 of 1972) No. R7634 since 2003 [6,7]. Vitamin A deficiency holds serious health consequences which include among others an impaired immune response with concomitant lowered resistance against infection [8].

Red palm oil (RPO) which contains α- and β-carotene, has been used for a number of years to combat vitamin A deficiency. Both carotenoids are vitamin A precursors. Research reports indicated that the consumption of RPO- containing biscuits meaningfully improved the vitamin A status of school children who were vitamin A deficient [9,10]. Other studies have reported significant increases in serum retinol concentrations in pre-school children receiving RPO supplementation in the form of RPO and carrot noodle [11,12].

Inflammatory markers, which form part of innate immunity pathways, are known to be indicative of infections and general state of health. Evidence exists indicating an inverse relationship between serum/plasma concentrations of α-carotene, β-carotene, lycopene and C-reactive protein (CRP) levels [13]. Although previous studies illustrated the ability of these carotenoids to prevent inflammation [14,15], it was believed that the action of these carotenoids could rather be ascribed to their aforementioned conversion to vitamin A.

Red palm oil is also a rich source of vitamin E. One of the most important functions of vitamin E is the chain-breaking, lipid soluble antioxidant characteristics active in body tissues and is considered the first line of defence against lipid oxidation. Vitamin E further plays an important role from control of inflammation to regulation of gene expression and cognitive performance [16].

The ability of vitamin E to enhance both blood and cell mediated immune function via supplementation in humans is well established. According to Pekmeczi [17],

even a marginal deficiency of vitamin E can impair the immune response. Results from a clinical trial with vitamin E derived from RPO showed the beneficial enhancing effect on the immune response of healthy human subjects following an immunogenic challenge such as vaccination [18]. Vitamin E is also effective in reducing inflammation as reported by two recent meta-analyses. Saboori *et al.* [19] indicated a significant inverse association between vitamin E tocopherol supplementation and CRP while Asbaghi *et al.* [20] illustrated a reducing effect on serum levels of CRP and interleukin 6 (IL-6) with vitamin E supplementation. Although the prevalence of vitamin E deficiency has been described in developing countries [16], no information is available for South African children.

As the brain consists of more than 60% lipids, the maintenance of the lipid composition of neuronal membranes is essential for optimal brain functioning [21]. The essential omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), play an important role as components of the phospholipids that form the structures of cell membranes. Docosahexaenoic acid is vital for brain function as it sustains the state of neural membranes by enabling fluidity and thickness of membranes which in turn supports cell signalling [22]. A number of studies indicated important links between omega-3 fatty acid levels and cognition in children [23,24]. Although there are no epidemiological studies on the omega-3 fatty acid status of South African children, the majority of randomised controlled trials reported omega-3 indexes below 6% [25,26]. An index above 8% is recommended [27].

Even though relatively comprehensive information is available on the health and nutritional status of preschool children in South Africa [4], limited information is available on the status of specifically children who attend informal crèches in an urban setting. As a result, the researchers decided to conduct baseline evaluations on the nutritional status of a group of pre-school children attending informal crèches in the Eastern Cape of South Africa by assessing plasma vitamin A (retinol) levels, α- and β-carotene levels, vitamin E (α-tocopherol) levels, inflammatory status (CRP and IL-6), red blood cell (RBC) omega-3 fatty acid and the presence of wasting and stunting. Baseline evaluations were followed by a nutritional intervention study over a 6-month period where children received RPO fortified biscuits with subsequent assessment of vitamin A, E, carotenoids and inflammation.

MATERIALS AND METHODS

The study was conducted among pre-school children attending the Fundani, Eagle Babes, Kuvukuland and Nomthunzi informal crèches. Informal crèches are typically not registered at the South African Department of Social Development [28] and also do not receive any funding from government. The four crèches are situated 20 km northwest of Gqeberha in the town of Bethelsdorp, which serves communities characterized by low socio-economic status. The trial was a randomised controlled, parallel intervention trial and was single blinded as only the principal researcher was aware of the allocation of the feeding intervention. Pre-school children attending the creches between the ages of 0 and 4 years old were recruited for the study. The study population was stratified by school and by age. Sixty (n = 60) children were recruited for the study of which n = 53 remained after baseline to participate in the feeding intervention trial. Forty-seven (n=47) children completed the study. Parents leaving the area was the main reason for children not completing the study.

After baseline evaluations each age group was randomly assigned to one of two groups using random tables whereafter a 6-month feeding and 6-month washout period followed. Group A (n=25) received biscuits containing both carotenoids and vitamin E while the group B (n=22) biscuit contained only vitamin E. Children received 2 x 20 g biscuits per day which provided 1.1 mg carotenoids (± 35% of NRV for vitamin A), 4 mg tocotrienol/tocopherol (± 27% of NRV for vitamin E) to group A and 4 mg tocotrienol/tocopherol to group B. Children received the biscuit five times a week except during holidays for a total of 109 days. A record of attendance and daily biscuit consumption was also kept for each child.

The shortening for the biscuits was obtained from Carotino SDN BHD Malaysia and the biscuits baked by a bakery in Cape Town. Commercially available fortified bread flour was used for baking the biscuits. Fortificants present in the bread flour were vitamin A, iron, zinc, thiamin, riboflavin, nicotinamide, pyrodoxine and folic acid. Additional fortificants were added so that all biscuits contained 50% of the nutrient reference values for vitamin A, iron, zinc, copper, manganese and selenium.

Biscuits were distributed daily during the school week. A record of attendance and daily biscuit consumption was kept for each child. A primary health care nurse examined them at baseline, 3 months and 6 months and collected blood samples at each of these examinations. The feeding intervention was then terminated after 6 months and the children were examined again at 6 months post intervention.

Ethical clearance (Ref. 13/2013) for the study was obtained from the Cape Peninsula University of Technology's Research Ethics Committee. Further permission for the study was obtained from the Department of Social Development, the managers of the respective crèches, as well as local community leaders. Informed consent was attained from the parents or guardians of all participants.

Anthropometric measurements and blood sampling
Weights were measured on an electronic scale, weighing to the nearest 100g. Heights were measured with a sliding board in the standing position (±1 cm). Ages were recorded for all children who presented their signed consent form confirming their participation in the project. Children were examined by the primary health care nurse who also took their weight and height.

Blood analysis
Blood was drawn from a forearm vein with the child in the supine position. Ethylenediaminetetraacetic acid (EDTA) was used as an anti-coagulant. Blood samples were centrifuged on site to separate plasma and red blood cells (RBC). After separation, the RBC were washed twice with an equal volume of saline. Plasma and RBC were frozen and air-freighted on dry ice to the analytical laboratories in Cape Town.

Alpha- and β-carotene, retinol and α-tocopherol were extracted from plasma and analysed by HPLC. Alpha- and β-carotene were analysed according to the method described by McGeachin and Bailey [29] and vitamin E as described by Rizzo et al. [30]. Human C-reactive protein (CRP) and IL-6 activity were determined on plasma samples with elisa kits (Millipore Cat. No. CYT 298, Cat. No. EZHIL6 and Cat No EZHTNFA, respectively). Fatty acid analysis was performed as described by Opperman et al. [31]. The omega-3 index was calculated as the sum of EPA and DHA expressed as percentage of total RBC fatty acids as obtained from the gas-liquid chromatography analytical results.

Statistical analysis
All statistical analyses were performed using the NCSS statistical analysis package (NCSS 9 Statistical Software (2013) NCSS, LLC.). For baseline, data was analysed to obtain mean values and standard deviations of all the measurements. Prevalence (where applicable) were calculated for parameters where cut-off values for normal/elevated levels were available. Pearson correlations were calculated on log-transformed data to indicate the relationship between α- and β-carotene levels and retinol.

Generally, the analyses were performed on log-transformed data and then back transformed for illustration purposes to show geometric means and confidence intervals. The 95% confidence intervals were calculated throughout. To determine whether a transformation was required, descriptive analyses were performed on both untransformed and log-transformed data and the transformation decision based on the results.

RESULTS AND DISCUSSION

Nutritional status assessment

The nutritional status assessment was conducted first and data was also used as baseline measurements. Anthropometric data and blood measurements are depicted in Tables 1 and 2, respectively. No statistical difference was found in the anthropometric data and blood measurements between the two groups and data was therefore pooled for statistical analysis.

According to the height and weight for age measurements, using cut-off levels as recommended by World Health Organisation (WHO) [32], the prevalence of stunting and underweight in this group of children for height-for-age z-score (HAZ) was 6.4% and 1.6% for weight-for-age z-score (WAZ) which can be regarded as low.

In terms of the biochemical parameters, the prevalence of vitamin A deficiency in this study, although lower than that reported in the SANHANES, was still about 10% which can be regarded as mild/moderate [4]. The South African National Health and Nutrition Examination Survey reported a very high prevalence (43.6%) of vitamin A deficiency in South Africa in a similar age group.

Plasma α- and β-carotene levels, are indicative of a habitual fruit and vegetable intake [33]. Considering the low socio-economic status of the community, the children's baseline carotene levels were in line with low fruit and vegetable intakes among other low socio-economic areas as described in Malawi [34], rural Guatemala [35] and Lagos [36]. Diets low in vegetables and fruits among children are probably low in water soluble vitamins and substances like phytochemicals increasing the risk for several diseases later in life. Fruits and vegetables most strongly associated with multiple disease risk reduction include green leafy, cruciferous and yellow/orange fruit and vegetables [37]. Both green leafy and yellow/orange fruit and vegetables are rich in α- and β-carotene.

A significant observation of the baseline results, is the high prevalence of vitamin E deficiency where 69% of children displayed vitamin E levels of less than 12 µmol/L. In general, plasma α-tocopherol concentrations below 12µmol/L are associated with increased infection, anaemia, stunting of growth and poor health outcomes in children [38]. These results indicate that vitamin E deficiency can exist in the absence of severe malnutrition.

For inflammatory markers, a cut-off value of 3 mg/L for CRP and 5 pg/ml for IL-6 (as per the kit's manufacturer guide) was used. Results indicated that 29% (CRP) and 24% (IL-6) of children displayed values exceeding the upper limits. Whether the vitamin E deficiency found in these children had an effect on the prevalence of the moderately elevated levels of CRP and IL-6 is not known. However, statistical analysis suggested that the number of observations were probably too small to show any correlations between the two parameters.

The omega-3 index was proposed by Harris and Von Schacky [39] and reflects the EPA + DHA content of RBC expressed as a percentage of total RBC fatty acids. The index can be used as surrogate for the assessment of tissue EPA + DHA levels. In populations following a Western diet with low fish intakes the EPA + DHA RBC levels typically range between 3-5%. An omega-3 index range of 8%-11% is recommended [27].

The relatively low RBC omega-3 fatty acid content of the majority of children is indicative of a low omega-3 dietary intake (deep sea fatty fish) [39]. Low levels of omega-3 fatty acids in children have been linked to conditions such as attention deficit /hyperactivity disorder [40] and slower processing speed [24]. These conditions may affect brain development and concomitant scholastic performance. For this reason, it is vital that increased omega-3 consumption among this group of children is prioritised.

Intervention study
Mean compliance, indicating the number of days a child received the biscuit out of the possible number of potential biscuit days, expressed as a percentage, was 90.2% for the RPO group and 89.3% for the non-RPO group. This compliance was considered as satisfactory since 100% compliance was considered ideal and less than 50% considered as poor.

Vitamin A
Figure 1 shows the changes in the plasma retinol levels during intervention. As no statistical difference was found between the two groups, data was pooled for

statistical analysis. A significant (p ≤ 0.05) increase in plasma retinol between baseline (0.96 µmol/L; 95% CI: 0.90 – 1.01) and 3 months (1.11 µmol/L; 95% CI: 1.04 – 1.17) was observed. Vitamin A deficiency during this time declined from 9.8% to 3.9%. Commercially fortified bread flour was used for baking the biscuits and the effect on the vitamin A status of the children can clearly be seen in both groups as deficiency levels declined considerably during the biscuit feeding period. However, at 12 months plasma retinol returned to levels below baseline while deficiency prevalence increased to levels above baseline (20.5%). This observation strongly supports the contention that vitamin A intake in both groups was inadequate before and after intervention. This further suggests that cessation of intervention with the fortified biscuit can prolong vitamin A deficiency. It also stresses the importance of continuation of the fortification process and even more important, a continued consumption of the fortified product once started.

Additionally, even though previous studies showed that RPO can alleviate vitamin A deficiency in school learners [10] and pre-schoolers [41] results from the present study did not show any significant effect of RPO on the plasma retinol (group A vs group B) levels. This suggest that when vitamin A is available from food (fortification), the role of pro-vitamin A (α- and β-carotene) as pre-cursor for vitamin A is less important. This is supported by the observation that plasma retinol concentration was not affected by the presence of pro-vitamin A in the biscuits which indicates that pro-vitamin A carotenes are converted to vitamin A only on demand [42].
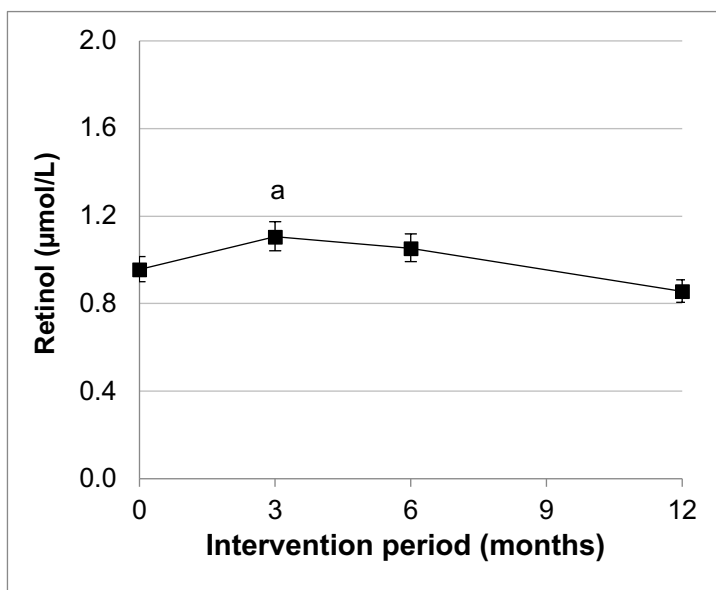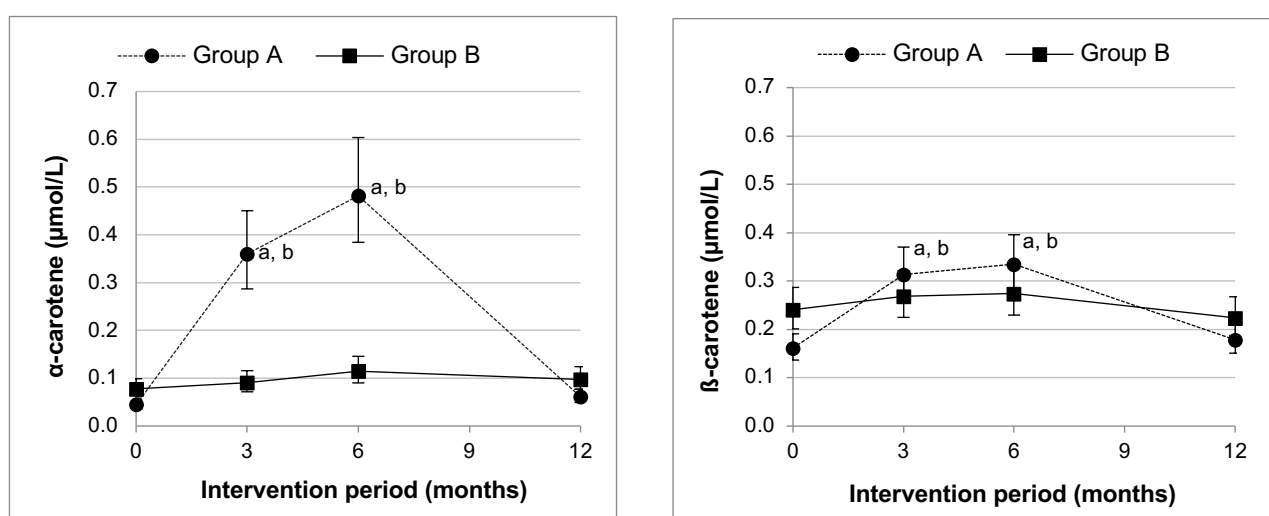

Figure 1: Geometric means and confidence intervals for retinol
a = Significantly different from baseline at p ≤ 0.05

Plasma α and β-carotene levels

In group A, a significant ($p \leq 0.05$) increase in plasma α-carotene levels from baseline (0.04 µmol/L; 95% CI: 0.03 – 0.06) was observed at 3 (0.36 µmol/L; 95% CI: 0.29 – 0.45) and 6 months (0.48 µmol/L; 95% CI: 0.39 – 0.60) whereas levels at 12 months, decreased to baseline levels. Similar significant ($p \leq 0.05$) results were observed for plasma β-carotene levels at 3 months (0.31 µmol/L; 95% CI: 0.27 – 0.40) and 6 months (0.33 µmol/L; 95% CI: 0.28 – 0.40). No significant changes in plasma α- or β-carotene concentrations from baseline were observed in Group B during and after intervention (Figure 2 a and b).

Figures 2a and 2b: Geometric means and confidence intervals for α- and β-carotene

a = Significantly different from baseline at $p \leq 0.05$
b = Significantly different from group B at $p \leq 0.05$

Consumption of the RPO biscuit lead to significant increases in plasma α- and β-carotene levels. In the past, the main emphasis was on the role of α- and β-carotene as pro-vitamin A carotenoids and their ability to alleviate biochemical vitamin A deficiency. However, results from epidemiological studies in the past decade provided more information on some of the other health advantages of elevated plasma carotene levels. Carotene concentrations were inversely associated with the risk of death from all causes and death from cardiovascular disease and cancer as well as other causes for example diabetes. Carotene concentrations, especially α-carotene, were inversely associated with the risk of death from all causes and death from cardiovascular disease, cancer and all causes other than CVD and cancer. This association was mainly ascribed to the antioxidant activity of carotenes. Carotenoids such as α-and β-carotene show a high antioxidant capacity thus protecting DNA membranes and other cellular

components against oxidative damage [33]. This inverse association was further independent of demographic characteristics, lifestyle habits and traditional health risk factors [43]. Whether this information can be applied to the children in the present study, is not known. However, the results provide a futuristic indication of what could happen if the situation is not addressed at this early stage of life.

Plasma α-tocopherol

As no statistical difference was found between the two groups at baseline, data was pooled for statistical analysis. Plasma α-tocopherol increased significantly ($p \leq 0.05$) from baseline in both groups at each of the time intervals (see Figure 3). Group A increased from baseline (9.26 µmol/L; 95% CI: 8.18 – 10.49), to 22.65 µmol/L (95% CI: 20.00 – 25.66) at 3 months, 18.21 µmol/L (95% CI: 16.07 – 20.63) at 6 months and 19.74 µmol/L (95% CI: 17.43 – 22.36) at 12 months. Group B levels increased from baseline (10.74 µmol/L; 95% CI: 9.40 – 12.66), to 23.25 µmol/L (95% CI: 20.03 – 26.98) at 3 months, 17.39 µmol/L (95% CI: 14.99 – 20.18) at 6 months and 18.50 µmol/L (95% CI: 15.94 – 21.47) at 12 months. No differences were observed in the responses between the two groups.
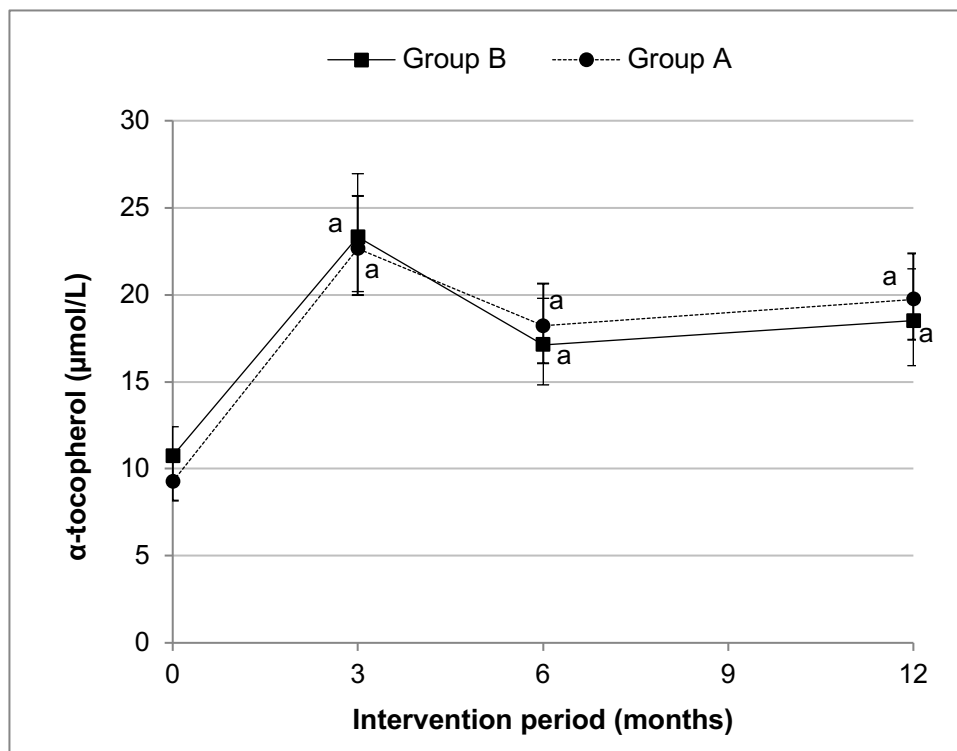

Figure 3: Geometric means and confidence intervals for α-tocopherol
a = Significantly different from baseline at $p \leq 0.05$

In Figure 4, vitamin E data was pooled and indicates the mean prevalence of vitamin E deficiency in the two groups. It has been suggested that a plasma

concentration of ≥ 30 µmol/L is desirable at which beneficial health effects may occur [44]. Using a cut-off value of < 12 µmol/L as indicated by the Institute of Medicine [45], the prevalence at baseline was 69%. Using the same cut-off, the prevalence was 10% at 6 months and 0% at 12 months. Using an arbitrary cut-off of less than 20 µmol/L the respective values were 94.0% (baseline), 61.1% (6 months) and 61.1% (12 months).
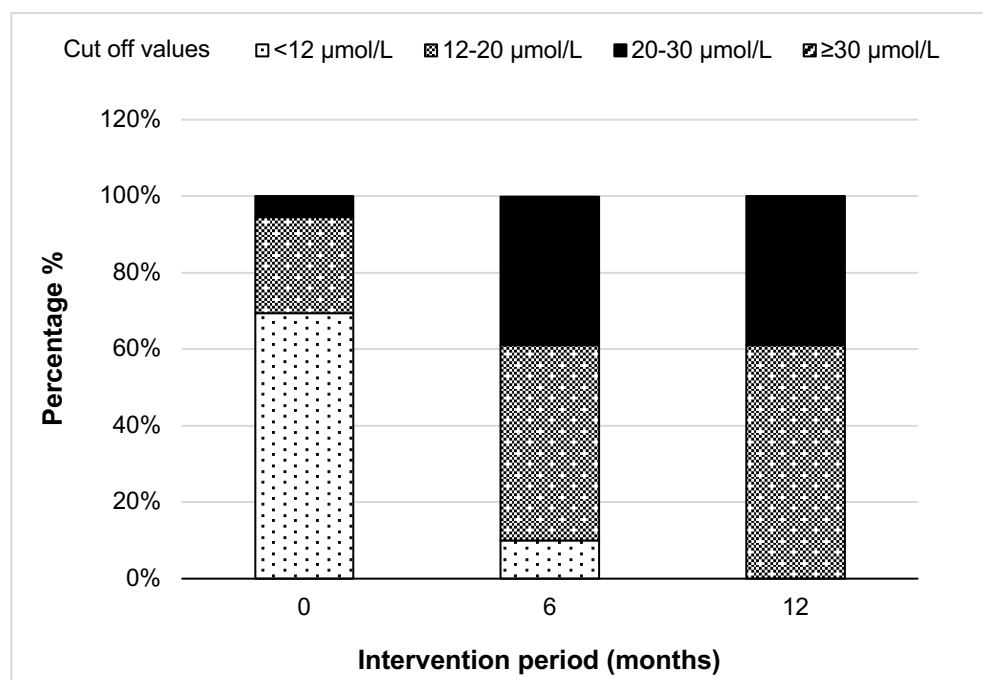

Figure 4: Vitamin E deficiency levels according to alpha-tocopherol cut-off values

The data indicates that the fortified biscuits had a significant effect in alleviating the deficiency during and even after the intervention. This shows that the vitamin E contained in the biscuit had a significant long-term effect on α-tocopherol levels. The results further suggest that after the intervention ended, α-tocopherol accumulated in the body and continued to provide the metabolic needs. It is also noteworthy that improvement of plasma vitamin E levels were observed by an estimated additional intake of only 4 mg vitamin E per day providing 27% of the Nutrient Reference Value.

Inflammatory markers
As CRP and IL-6 did not differ significantly between groups, results were pooled for statistical analysis (Figure 5 a and b).
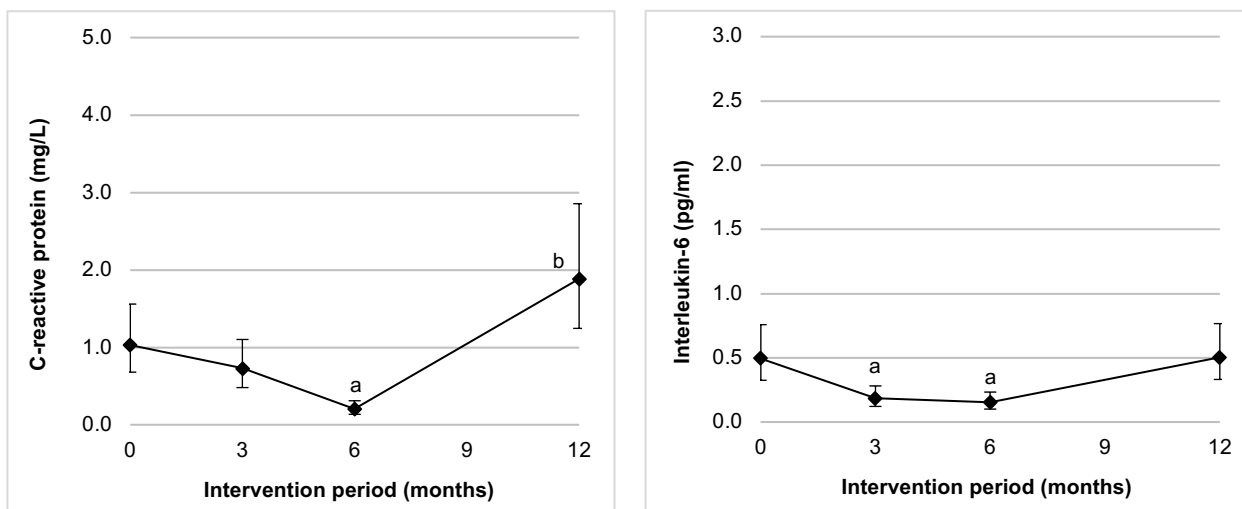
Figures 5a and 5b: Geometric means and confidence intervals for CRP and IL-6

a = Significantly different from baseline at p ≤ 0.05
b = Significantly different from 6 months at p ≤ 0.05

Plasma levels of CRP declined during intervention to reach significantly (p ≤ 0.05) lower levels at 6 months (0.20 mg/L; 95% CI: 0.14 – 0.31) when compared to baseline 1.03 mg/L (95% CI: 0.68 – 1.55). At 12 months, CRP increased (1.89 mg/L; 95% CI: 1.25 – 2.84) significantly (p ≤ 0.05) compared to levels observed after 6 months. IL-6 activity decreased significantly (p ≤ 0.05) from baseline (0.50 pg/ml; 95% CI: 0.34 – 0.75) at 3 months (0.18 pg/ml; 95% CI: 0.12 – 0.28) and 6 months (0.15 pg/ml; 95% CI: 0.10 – 0.23). At 12 months no significant differences were observed in comparison to baseline.

For the improvement in CRP and IL-6 levels during the supplementation period it is postulated that deficient α-tocopherol levels are only partially responsible. Vitamin E has many functions in the body and is best known for its role as an antioxidant and the normal functioning of immune cells [17]. It is also known that even marginal vitamin E deficiencies impair the immune response [46]. It is therefore possible that the decline in CRP and IL-6 could have been at least partly due to the known effect of vitamin E on immune function.

The increase in inflammatory markers after termination of intervention, suggest that the A and B biscuits contained elements that affected the level of inflammatory markers. When the intervention ended, these elements were lacking and subsequently inflammatory marker levels increased again. The most obvious candidates in this process appear to be vitamin A and other trace elements

included in both biscuits. Low intakes of micronutrients have been shown to suppress immune function by affecting the innate T- cell mediate immune response and antibody response, which in turn, increases the susceptibility to infections [47]. It must however be pointed out that the changes observed in the inflammatory markers occurred in levels within relatively low to normal ranges for CRP and IL-6.

Limitations
Firstly, the effects of using fortified flour for baking the biscuits must be considered. Fortification of all bread flour in South Africa is mandatory by law and non-fortified flour was not available. Hence it was not possible to isolate the effect of the carotenoids or the vitamin A fortified flour on the children's vitamin A status. It is recommended that in follow-up studies non-fortified flour should be used. Secondly, the sample size of the study group was relatively small due to a limited number of staff available at schools to assist with biscuit intake compliance as well as some mothers not willing to consent to blood sampling of the children. The relatively small sample size limits the generalisation of the results to larger communities with similar socio-economic conditions. Results for the current study can therefore only be applied to the specific population. Lastly, the children's dietary intake data from mothers/caregivers were not included for the reasons that: i) a large number of mothers were not aware which and how much food their children were consuming during the school day and ii) it was not always the mother or caretaker who took the child to school during dietary data collection which complicated accurate reporting. Low intakes of fruit and vegetables could therefore not be validated by dietary intake data.

CONCLUSION

Results from this study showed that consumption of a biscuit baked with RPO can play a significant role in alleviating vitamin E deficiency and can significantly increase plasma α- and β-carotene levels in pre-school children. Although these children appeared to be nutritionally in a good state, based on their weight and heights, vitamin E and omega-3 fatty acid dietary deficiencies as well as low plasma levels of carotenoids were still evident. This is indicative of poor dietary intakes of fruit, vegetables, fish and food sources rich in vitamin E and can potentially have far-reaching cognitive and future health implications. Additionally, notwithstanding the fact that bread flour is fortified in South Africa, this group of children displayed a moderate vitamin A deficiency at baseline and post intervention. This might be ascribed to very low intakes or lack of access to fortified foods. The dietary deficiency of micronutrients was eradicated with the feeding of the RPO biscuit but returned upon termination of the intervention. This underlines

the importance of continuation of dietary interventions in groups vulnerable to micronutrient malnutrition. Moreover, chronic vitamin E deficiency is characterised by poor cognitive performance. Although cognitive function was not measured in these children, it would be reasonable to hypothesize that against existing evidence, the cognitive function of those children with a low plasma vitamin E level was affected negatively and that supplementation with a fortified biscuit would have improved their cognitive function. Further studies are therefore needed in this regard. Lastly, despite the mandatory fortification of staples in South Africa, national evidence shows that nutritional deficiencies are still evident among children. It is therefore suggested that current fortification guidelines are revised as well to increase the access of vulnerable groups to fortified foods.

Declarations of interest: none

In memoriam
This article is dedicated to the memory of Dr Ambrose Jacob Spinnler Benade. He was a true scholar and was involved in nutrition research for more than 40 years. His numerous and influential contributions in the field of red palm oil and fatty acid research over the years are invaluable.

Table 1: Anthropometric data

	Baseline (means±SD)
n	47
Age (years)	4.2±0.8
Weight (kg)	16.3±3.0
Height (cm)	101.1±7.6
HAZ (-2SD)	6.4%
WAZ (-2SD)	1.6%

Table 2: Baseline blood measurements

Parameter	N	Plasma level	Universal cut-off points	Prevalence
Retinol (µmol/L) [5]	47	0.93 ± 0.18	<0.7	10%
α-carotene (µmol/L)	47	0.060 ± 0.040	-	-
β-carotene (µmol/L)	47	0.184 ± 0.081	-	-
α-tocopherol (µmol/L) [45]	47	11.00 ± 4.41	<12	69%
			12-20	25%
			≥30	0%
CRP (mg/L)	47	4.18 ± 8.50	>3	29%
IL-6 (pg/ml)	47	2.96 ± 6.11	>5	24%
Omega-3 index (%) [39]	47	6.2 ±1.2	<6	44%
			<8	98%
			<10	100%

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