

Vitamin A supplementation in iodine-deficient African children decreases thyrotropin stimulation of the thyroid and reduces the goiter rate¹⁻³

Michael B Zimmermann, Pieter L Jooste, Ngoako Solomon Mabapa, Serena Schoeman, Ralf Biebinger, Linda F Mushaphi, and Xikombiso Mbhenyane

ABSTRACT

Background: Vitamin A (VA) deficiency (VAD) and iodine deficiency (ID) often coexist in children in Africa. VAD may affect thyroid function and the response to iodine prophylaxis.

Objective: The aim was to investigate the effects of supplementation with iodine or VA alone, and in combination, in children with concurrent VAD and ID.

Design: A 6-mo randomized, double-blind, 2 × 2 intervention trial was conducted in 5–14 y-old South African children (*n* = 404), who, on average, had mild-to-moderate VAD and ID. At baseline and after 3 mo, children received 1) iodine (191 mg I as oral iodized oil) + placebo (IS group), 2) VA (200 000 IU VA as retinyl palmitate) + placebo (VAS group), 3) both iodine and VA (IS+VAS group), or 4) placebo. At baseline, 3 mo, and 6 mo, urinary iodine (UI), thyroid volume, thyrotropin (thyroid-stimulating hormone; TSH), total thyroxine (TT₄), thyroglobulin, serum retinol (SR), and retinol-binding protein (RBP) were measured.

Results: SR and RBP increased significantly with VA supplementation (*P* < 0.05). For UI, SR, and RBP, there were no significant treatment interactions between iodine and vitamin A. The 3-factor and all three 2-factor interactions were significant for thyroid volume, TSH, and thyroglobulin (*P* < 0.001), whereas none of these interactions were significant for TT₄. There was a clear effect of VAS without IS on TSH, thyroglobulin, and thyroid volume; all 3 variables decreased significantly (*P* < 0.05).

Conclusions: Iodine prophylaxis is effective in controlling ID in areas of poor vitamin A status. VA supplements are effective in treating VAD in areas of mild ID and have an additional benefit—through suppression of the pituitary *TSHβ* gene, VAS can decrease excess TSH stimulation of the thyroid and thereby reduce the risk of goiter and its sequelae. *Am J Clin Nutr* 2007;86:1040–4.

KEY WORDS Vitamin A, iodine, supplementation, deficiency, Africa, children

INTRODUCTION

More than one-third of the global population is affected by either vitamin A (VA) deficiency (VAD) or iodine deficiency (ID) (1–4). These deficiencies often coexist, and 32–50% of school-age children in rural West and North Africa have both VAD and goiter (5–7). High-dose oral VA supplementation (VAS) is a recommended strategy to control VAD in affected

populations (2), many of whom are also iodine deficient. Conversely, many VA-deficient children in the developing world are consuming iodized salt.

Thyroid metabolism and the response to iodine prophylaxis in areas of ID are influenced by multiple nutritional factors (8–13), including VA status (14, 15). VAD has multiple effects on thyroid function in animals (14): 1) in the thyroid, VAD decreases thyroidal iodine uptake and iodine incorporation into thyroglobulin and increases thyroid size (16–20); 2) in the periphery, VAD increases circulating thyroid hormone concentrations (21); and 3) in the pituitary, VA status modulates thyrotropin (thyroid-stimulating hormone; TSH) production by retinoid X receptor (RXR)-mediated expression of pituitary *TSHβ* mRNA (22–27), and VAD in rats increases pituitary *TSHβ* mRNA, TSH, and circulating thyroid hormone (21). In a recent study (28), rats with concurrent ID and VAD were supplemented with iodine and VA, alone and in combination. Primary hypothyroidism in animals with concurrent VAD and ID did not reduce the efficacy of VAS, nor did VAD reduce the efficacy of iodine to correct thyroid dysfunction due to ID. Moreover, VAS given alone without iodine supplementation reduced pituitary *TSHβ* mRNA expression, circulating TSH, and thyroid weight (28).

In a cross-sectional study of African children, VAD in children with severe ID was associated with an increase in TSH stimulation and thyroid size and a reduced risk of hypothyroidism (15). In the same population, an intervention trial compared the efficacy of iodized salt alone to iodized salt given with VAS and found greater decreases in TSH and thyroid volume in the

¹ From the Laboratory for Human Nutrition, Swiss Federal Institute of Technology, Zürich, Switzerland (MBZ and RB); the Division of Human Nutrition, Wageningen University, Wageningen, Netherlands (MBZ); the Medical Research Council, Cape Town, South Africa (PLJ and SS); and the University of Venda, Thohoyandou, South Africa (NSM, LFM, and XM).

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³ Reprints not available. Address correspondence to MB Zimmermann, Laboratory for Human Nutrition, ETH Zürich, LFV E19, Schmelzbergstrasse 7, CH-8092, Zürich, Switzerland. E-mail: michael.zimmermann@ilw.agrl.ethz.ch.

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IS+VAS group (15). However, in that study, there was no control group and VAS was given only with iodized salt. Therefore, the aim of the present study was to investigate the safety and efficacy of repletion with iodine or VA alone, and in combination, in school-age children with concurrent VAD and ID.

SUBJECTS AND METHODS

The study was conducted in Limpopo Province in South Africa. The subjects were 5–14-y-old children from rural primary schools. Power calculations indicated that a sample size of 85 children per group was required to yield $\geq 80\%$ power at 5% significance to detect a 20 nmol/L difference in mean total serum thyroxine concentration, which allowed for 10% loss to follow-up. Ethical approval for the study was obtained from ETH Zürich, the University of Venda, and the Provincial Department of Education in South Africa. Informed written consent was obtained from the parents, and oral assent was obtained from the participating children. All children in the schools were invited to participate; the only exclusion criteria were major chronic medical illnesses and recent consumption of iodine, VA supplements, or both. None of the consenting children were excluded on the basis of these criteria. At baseline, weight was measured with a TANITA Digital Scale 1631 (Itin Scale, Brooklyn, NY) and height with a rigid stadiometer. A spot urine sample was collected for measurement of urinary iodine concentration (UI). Whole blood was collected by venipuncture for the measurement of total thyroxine (TT₄), thyrotropin (TSH), thyroglobulin, serum retinol (SR), retinol-binding protein (RBP), and C-reactive protein (CRP) concentrations. Thyroid volume was measured with a portable Aloka SSD-500 Echocamera (Aloka, Mure, Japan) or a Toshiba Justvision 200 (Toshiba, Tokyo, Japan) with high-resolution 7.5-MHz linear transducers (29).

The 6-mo study was a double-blind trial that used a 2 × 2 factorial design. Children from the screening were randomly assigned to receive, at baseline and at 3 mo, one of the following: 1) iodine (191 mg I as oral iodized oil; Lipiodol, Guerbet, Paris) + placebo (sunflower oil) (IS group), 2) vitamin A (200 000 IU as retinyl palmitate; RpScherer, Aprilia, Italy) + placebo (VA group), 3) both iodine and VA (IS+VAS group), or 4) placebo. The capsules were swallowed with water under direct supervision by the investigators. Baseline measurements were repeated at 3 and 6 mo. At completion, all study children who had not received supplementation were given VAS, IS, or both.

Laboratory analyses

Serum and urine samples were portioned and frozen at -20°C until analyzed. UI was measured by using the Pino modification of the Sandell-Kolthoff reaction (30). At UI concentrations of 47 and 79 $\mu\text{g/L}$, the CVs of this assay in our laboratory are 10.3% and 12.7%, respectively. Whole-blood TSH and serum TT₄ were measured by using immunoassays (31); normal reference values are <3.7 mU/L for TSH and 65–165 nmol/L for TT₄. Whole blood was spotted onto filter paper, and thyroglobulin was measured by using an immunoassay; the normal reference value is 4–40 $\mu\text{g/L}$ thyroglobulin (32). SR was measured by HPLC (33) and RBP by an immunoassay (Immundiagnostik AG, Bensheim, Germany). VAD was defined as an SR concentration <0.70 $\mu\text{mol/L}$ (2), and an SR concentration <1.05 $\mu\text{mol/L}$ indicated low VA status (4). CRP was measured by nephelometry

TABLE 1

Baseline characteristics of children from primary schools in Limpopo Province, South Africa

Variable	Value (<i>n</i> = 404)
Age (y)	9.0 ± 2.3 ¹
Sex ratio (F/M)	1.6
Height-for-age <i>z</i> score	−0.56 (−3.18–3.96) ²
Weight-for-age <i>z</i> score	−0.59 (−2.54–2.53)
Serum retinol ($\mu\text{mol/L}$)	0.93 ± 0.22
Vitamin A deficiency [<i>n</i> (%)] ³	49 (12)
Low vitamin A status [<i>n</i> (%)] ⁴	256 (63)
Retinol-binding protein (mg/L)	20.7 ± 11.1
Urinary iodine ($\mu\text{g/L}$)	74 (4–838)
Thyrotropin (mU/L)	1.6 (0.5–14.1)
Thyroid volume (mL)	3.21 (1.04–13.43)
Goiter [<i>n</i> (%)]	110 (27)
Thyroglobulin ($\mu\text{g/L}$)	17.2 (5.6–79.7)
Total thyroxine (nmol/L)	98 ± 21
Hypothyroxinemia [<i>n</i> (%)]	18 (4.5)

¹ $\bar{x} \pm \text{SD}$ (all such values).

² Median; range in parentheses (all such values).

³ Defined as a serum retinol concentration <0.70 $\mu\text{mol/L}$.

⁴ Defined as a serum retinol concentration <1.05 $\mu\text{mol/L}$.

(TURBOX; Orion Diagnostica, Espoo, Finland); the normal reference value is a CRP concentration <10 mg/L.

Data and statistical analyses

Data processing and statistical analyses were done by using SPLUS (2000; Insightful Corporation, Seattle, WA), and PRISM (version 3; GraphPad, San Diego, CA). References for thyroid volume in school-age children according to sex and body surface area were used to define goiter (29). EPINFO (version 3.3.2; Centers for Disease Control and Prevention, Boston, MA) was used to calculate height-for-age *z* scores and weight-for-age *z* scores using World Health Organization (WHO) references. If data were not normally distributed, statistical analyses were done after log transformation. We studied the effects of the 2 treatments and of their interactions by analysis of covariance. If the interaction was significant, the differences were given for both groups, those also receiving the other treatment and those not receiving it. Because of the potential confounding effects of inflammation on SR and RBP, SR and RBP values from children with an elevated CRP (>10 mg/L) were not included in the data analyses. Significance was set at $P < 0.05$.

RESULTS

The results of the baseline screening are shown in **Table 1**. Overall, the children were reasonably well-nourished, as reflected by median height-for-age and weight-for-age *z* scores of -0.56 and -0.59 , respectively. However, 12% of the children had an SR concentration <0.7 $\mu\text{mol/L}$, which indicated that VAD in this region is a moderate public health problem according to the WHO (34). The median UI was 74 $\mu\text{g/L}$, which indicated mild ID; 31% of the children had a UI <50 $\mu\text{g/L}$, and 27% of the children were goitrous. Thyroid hormone concentrations were generally in the normal range; only 4.5% of the children were hypothyroxinemic. At baseline, 3 mo, and 6 mo, 3–6% of children had an elevated CRP value, but no differences in the

TABLE 2

Age, sex ratio, weight, and height in children who received, at baseline and at 3 mo, 1 of 4 treatments¹

Measure	Group			
	Placebo (n = 88)	IS (n = 100)	VAS (n = 115)	IS+VAS (n = 101)
Age (y)	9.2 ± 2.5 ²	9.1 ± 2.3	9.0 ± 2.4	8.9 ± 2.3
Sex ratio (F/M)	1.6	1.5	1.6	1.6
Weight (kg)	29.0 ± 8.9	27.6 ± 8.3	28.5 ± 7.4	29.3 ± 8.6
Height (m)	1.32 ± 0.14	1.30 ± 0.14	1.31 ± 0.13	1.33 ± 0.13

¹ IS, 191 mg I + placebo; VAS, 200 000 IU retinylpalmitate + placebo. There were no significant differences between groups.

² $\bar{x} \pm$ SD (all such values).

prevalence of elevated CRP values between the 4 groups was found at any time point (data not shown). At 6 mo, because subjects moved away from the study area or were absent on the days of data collection, the dropout rates in the placebo, IS, VAS, and IS+VAS groups were 7%, 8%, 5%, and 7%, respectively.

There were no significant differences in any of the baseline variables in **Table 2**, **Table 3**, or **Table 4** between the 4 groups after randomization. As shown in Table 3, both iodine and VA supplementation were effective: the iodine-by-time interaction was significant for UI, with an effect size of 117.9 $\mu\text{g/L}$ ($P < 0.0001$); the vitamin A-by-time interaction was significant for SR and RBP, with effect sizes of 0.029 $\mu\text{mol/L}$ and 9.47 mg/L, respectively ($P < 0.001$). However, for UI, SR, and RBP, there were no significant treatment interactions between iodine and vitamin A. Changes in the thyroid variables are shown in Table 4. The 3-factor and all three 2-factor interactions were significant for thyroid volume, TSH, and thyroglobulin ($P < 0.001$), whereas none of these interactions were significant for TT₄.

TABLE 3

Urinary iodine (UI), serum retinol (SR), and retinol-binding protein (RBP) concentrations in children who received, at baseline and at 3 mo, 1 of 4 treatments¹

Measure	Group				Iodine			Vitamin A		
	Placebo (n = 88)	IS (n = 100)	VAS (n = 115)	IS+VAS (n = 101)	Effect size	SE	P	Effect size	SE	P
UI ($\mu\text{g/L}$)										
Baseline	78 (21–299) ²	70 (17–609)	73 (7–742)	78 (4–838)						
3 mo	93 (4–265) ^a	727 (140–12288) ^{b,3}	94 (2–427) ^a	610 (83–9616) ^{b,3}						
6 mo	88 (13–455) ^a	149 (1–1044) ^{b,4}	97 (15–470) ^a	175 (4–1567) ^{b,4}	117.9	17.4	<0.0001	33.2	17.5	0.059
SR ($\mu\text{mol/L}$)										
Baseline	0.93 ⁷ ± 0.21 ⁵	0.91 ± 0.24	0.93 ± 0.23	0.89 ± 0.25						
3 mo	0.92 ± 0.24	0.88 ± 0.24	1.01 ± 0.22	1.11 ± 0.24						
6 mo	0.91 ± 0.21 ^a	0.91 ± 0.24 ^a	1.22 ± 0.21 ^{b,4}	1.19 ± 0.22 ^{b,4}	−0.02	0.02	NS	0.29	0.022	<0.0001
RBP (mg/L)										
Baseline	19.5 ± 10.1	21.8 ± 14.2	20.3 ± 11.3	20.1 ± 13.8						
3 mo	21.6 ± 10.1	22.8 ± 11.7	26.6 ± 11.4	28.1 ± 13.7						
6 mo	20.5 ± 10.1 ^a	21.3 ± 13.1 ^a	30.3 ± 11.8 ^{b,4}	29.6 ± 14.3 ^{b,4}	−0.89	0.49	NS	9.47	0.498	<0.0001

¹ IS, 191 mg I + placebo; VAS, 200 000 IU retinyl palmitate + placebo. ANCOVA with the baseline values as covariates, with the effects of iodine and vitamin A presented as estimated average differences between the subjects receiving a treatment (IS or VAS) and those not receiving it. The iodine-by-time interaction was significant for UI, and the vitamin A-by-time interaction was significant for SR and RBP. There were no significant treatment interactions between iodine and vitamin A. Values in a row with different superscript letters are significantly different, $P < 0.05$.

² Median; range in parentheses (all such values).

^{3,4} Significantly different from baseline: ³ $P < 0.0001$, ⁴ $P < 0.05$.

⁵ $\bar{x} \pm$ SD (all such values).

DISCUSSION

Our findings showed that mild ID does not impair the SR and RBP response to VAS in children with concurrent ID and VAD. Conversely, the data also indicate that mild VAD does not reduce the efficacy of IS to correct thyroid dysfunction in children with concurrent ID and VAD. These latter findings differ somewhat from those of several animal studies in which severe VAD impaired the pituitary-thyroid axis, even when the iodine supply was adequate. The adverse effects in these studies included reduced thyroidal iodine uptake (19), impaired synthesis of thyroglobulin and coupling of iodotyrosine residues to form thyroid hormone, and reduced hepatic conversion of T₄ to triiodothyronine (T₃) (20). However, a recent study in rats with only mild-to-moderate VAD and ID (28) reported findings similar to those in the present study; that is, provision of an iodine-sufficient diet entirely reversed the abnormalities of the pituitary-thyroid axis produced by VA and iodine depletion, regardless of VA status.

Our data indicate that VAS in children receiving IS had minimal effects on the thyroid axis compared with the effects of IS alone (Table 4). These findings contrast with those of several previous studies in iodine-sufficient animals, in which pharmacologic doses of VA did affect the pituitary-thyroid axis and decreased thyroid size, pituitary TSH content, and circulating TT₃ and TT₄ (16, 35). A similar effect was reported in lymphoma patients who developed hypothyroidism after treatment with a synthetic retinoid that specifically binds to the RXR (36). In contrast, a recent study in iodine-sufficient rats with mild-to-moderate VAD given high-dose VAS (≈ 50 mg/kg body wt), reported that VA treatment had no discernible effects on the pituitary-thyroid axis (28). Together with the findings of the present study, these data suggest that high-dose VAS in iodine-sufficient areas is unlikely to affect thyroid function.

The major new finding of this study was that VAS alone in iodine-deficient children with mild VAD reduces circulating

TABLE 4Thyroid volume (Tvol), whole-blood thyrotropin (TSH) and thyroglobulin (Tg), and serum total thyroxine (TT₄) in children who received, at baseline and after 3 mo, 1 of 4 treatments¹

Measure	Group			
	Placebo (n = 88)	IS (n = 100)	VAS (n = 115)	IS+VAS (n = 101)
Tvol (mL)				
Baseline	3.19 (1.10–9.03) ²	3.23 (1.12–13.43)	3.25 (1.04–10.28)	3.18 (1.22–8.33)
3 mo	3.26 (0.97–9.69)	3.09 (0.98–12.25)	2.97 (1.01–10.39)	3.10 (0.96–7.74)
6 mo	3.29 (1.08–10.08) ^a	2.34 (0.87–9.97) ^{b,3}	2.91 (0.88–8.96) ^{c,4}	2.50 (1.01–8.18) ^{b,3}
TSH (mU/L)				
Baseline	1.4 (0.9–4.3)	1.6 (0.6–14.1)	1.5 (0.5–5.0)	1.7 (0.6–8.8)
3 mo	1.6 (0.6–4.8) ^a	0.9 (0.4–4.7) ^{b,4}	1.0 (0.4–2.6) ^{b,4}	0.7 (0.4–3.8) ^{b,3}
6 mo	1.7 (0.7–4.1) ^a	0.6 (0.3–4.6) ^{b,3}	1.1 (0.5–2.9) ^{c,4}	0.5 (0.4–2.9) ^{b,3}
Tg (μg/L)				
Baseline	16.7 (7.7–79.7)	17.8 (6.1–73.4)	17.4 (5.8–60.9)	16.6 (5.6–50.6)
3 mo	17.6 (7.9–74.1) ^a	6.9 (1.4–31.7) ^{b,3}	12.9 (7.7–79.7) ^{c,4}	5.7 (0.7–28.9) ^{b,3}
6 mo	18.3 (6.3–69.2) ^a	4.3 (1.1–18.8) ^{b,5}	13.1 (7.7–79.7) ^{c,4}	4.0 (0.8–20.3) ^{b,5}
TT ₄ (nmol/L)				
Baseline	96 ± 19 ⁶	105 ± 23	97 ± 15	97 ± 18
3 mo	101 ± 15	105 ± 19	99 ± 17	102 ± 18
6 mo	99 ± 19	100 ± 17	97 ± 16	102 ± 18

¹ IS, 191 mg I + placebo; VAS, 200 000 IU retinyl palmitate + placebo. ANCOVA with the baseline values as covariates. The 3-factor and all three 2-factor interactions were significant for Tvol, TSH, and Tg, whereas none of these interactions were significant for TT₄. Values in a row with different superscript letters are significantly different, *P* < 0.05.

² Median; range in parentheses (all such values).

^{3–5} Significantly different from baseline: ³*P* < 0.01, ⁴*P* < 0.05, ⁵*P* < 0.001.

⁶ $\bar{x} \pm$ SD (all such values).

TSH, serum thyroglobulin, and thyroid size without significantly affecting thyroid hormone concentrations (Table 4). This finding is consistent with that of a previous report in hypothyroid rats, in which pharmacologic doses of VA reduced basal TSH secretion and the TSH response to thyroid-releasing hormones (27). This finding is also consistent with that of a recent study in iodine- and vitamin A-deficient rats (28), in which VAS reduced TSHβ mRNA expression, serum TSH concentrations, and thyroid weights. Control of TSH production by the pituitary depends on 2 main factors: the binding of the thyroid hormone receptor, which is activated by T₃ and T₄, and the binding of the RXR, which is activated by retinoic acid (23). Both receptors suppress the transcription of the pituitary *TSHβ* gene by occupying half-sites on the promoter DNA of the gene; thus, VA status modulates TSH production (23–25). In the present study, reduced TSH stimulation might have been expected to reduce thyroid hormone production, but circulating concentrations of TT₄ did not decrease. This implies that either the sensitivity of the thyroid to TSH improved with VA repletion or that the metabolism of circulating thyroid hormone was altered to maintain their concentrations. The findings of previous animal studies support both of these mechanisms. For example, Ingenbleek (20) reported that a combination of VAD and ID in rats impaired thyroid hormone synthesis by reducing iodine incorporation into thyroglobulin; these adverse effects were reversed by VA treatment. On the other hand, Morley et al (35) found that high-dose VAS in iodine-sufficient rats altered peripheral thyroid hormone metabolism and increased hepatic conversion of T₄ to T₃.

These results are important in the context of the findings from a previous cross-sectional study in African children (15), in which increasing VAD severity was a predictor of greater thyroid volume and higher concentrations of TSH, thyroglobulin, and

TT₄; in children with VAD, the odds ratio for goiter was 6.51, whereas the odds ratio for hypothyroidism in VAD was 0.06. A concern raised by that study (15) was that VAS given alone in areas of ID might reduce pituitary TSH secretion and thereby impair thyroid hormone production in the face of marginal iodine status. The findings of the present study do not support that contention; there was no significant decrease in thyroid hormone concentrations in the children receiving VAS, despite the reduction in circulating TSH.

In a previous study (15), the efficacy of iodized salt alone was compared with that of iodized salt given with VAS (200 000 IU at 0 and 5 mo) in a 10-mo trial. In contrast with the results in the present study, in which IS+VAS had no measurable effect on thyroid status compared with IS alone, in the study by Zimmermann et al (15), mean thyroglobulin, median TSH, and the goiter rate significantly decreased in the IS+VAS group compared with iodized salt alone. The varying results of these 2 studies may have been due to differences in the severity of ID, study length, the route of iodine repletion (iodized oil compared with salt), or a combination thereof.

It is possible that only in the case of severe ID, when TSH stimulation of the thyroid is very high, does the additive effect of concurrent VA repletion become apparent. Thus, the results in this population with moderate VAD and mild ID may not be generalizable to populations with more severe deficiencies.

The findings of the present study may at least partially explain the inverse correlation between VA status and goiter reported in previous cross-sectional studies in developing countries. In Senegalese adults, there was a strong negative correlation between increasing severity of goiter and SR and RBP concentrations (37, 38). In Ethiopian children, those with visible goiters

had significantly lower SR and RBP concentrations than did children without goiters or with smaller goiters (39). However, in these studies it was not possible to distinguish the effects of VAD from protein malnutrition, which also can reduce SR and RBP concentrations. Among adequately nourished 7–14-y-old Filipino children, the prevalence of goiter was 1.8% in children without VAD and 5.3% in those with VAD (40).

In summary, our data indicate that iodine prophylaxis will be effective in controlling ID, even in areas of poor VA status, and high-dose VAS is likely safe and effective in areas of mild ID. The findings suggest that high-dose VAS in a population can modify indicators of ID, such as thyroglobulin and goiter, independent of a change in iodine nutrition. In areas of endemic goiter, through suppression of transcription of the pituitary *TSHB* gene, VAS may decrease excess TSH stimulation of the thyroid and thereby reduce the risk of goiter and its sequelae.

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