The Effects of Vitamin A Deficiency and Vitamin A Supplementation on Thyroid Function in Goitrous Children

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In developing countries, children are at high risk for both the iodine deficiency disorders (IDD) and vitamin A deficiency (VAD). The study aim was to determine the effects of VAD and vitamin A (VA) supplementation on thyroid function in an area of endemic goiter. In a double-blind, randomized, 10-month trial, Moroccan children with IDD and VAD (n = 138) were given iodized salt and either VA (200,000 IU) or placebo at 0 and 5 months. At 0, 5, and 10 months, measurements of VA status and thyroid function were made. At baseline, increasing VAD severity was a predictor of greater thyroid volume and higher concentrations of TSH and thyroglobulin ($P <$ 0.001). In children with VAD, the odds ratio for goiter was 6.51 (95% confidence interval, 2.94, 14.41). VAD severity was also a strong predictor of higher concentrations of total T_4 ($P <$ 0.001); the odds ratio for hypothyroidism in VAD was 0.06 (95% confidence interval, 0.03, 0.14). During the intervention, mean thyroglobulin, median TSH, and the goiter rate significantly decreased in the VA-treated group compared with those in the placebo group ($P <$ 0.01). The findings indicate that VAD in severely IDD-affected children increases TSH stimulation and thyroid size and reduces the risk for hypothyroidism. This effect could be due to decreased VA-mediated suppression of the pituitary TSHβ gene. In IDD- and VAD-affected children receiving iodized salt, concurrent VA supplementation improves iodine efficacy. (*J Clin Endocrinol Metab* 89: 5441–5447, 2004)

MORE THAN 30% of the global population is affected by either vitamin A (VA) deficiency (VAD) or the iodine deficiency disorders (IDD) (1). The most vulnerable groups are women of reproductive age and young children (2, 3). IDD is the most common cause of preventable mental retardation worldwide, and severe iodine deficiency increases infant mortality (2). VAD is the leading cause of preventable blindness in children and increases morbidity and mortality from serious infections, including measles and diarrheal disease. In pregnant women, VAD causes night blindness and may increase the risk of maternal mortality (1, 3). These deficiencies often coexist in children in developing countries. In rural Côte d'Ivoire, 32–50% of school-age children suffer from both VAD and goiter (4). In northern Morocco, 41% of children have VAD, and 50% are goitrous (5, 6). In areas of endemic goiter, micronutrient status can be an important determinant of iodine and thyroid metabolism (7). Deficiencies of selenium (8) and iron (4) can act in concert with iodine deficiency to impair thyroid metabolism and modify the response to prophylactic iodine (9, 10).

In animals, VAD has multiple effects on thyroid metabolism. VAD decreases thyroidal iodine uptake, impairs thyroglobulin (Tg) synthesis, and increases thyroid size (11, 12). In the periphery, VAD increases free and total circulating thyroid hormone (13–16), and binding of transthyretin (TTR) to retinol-binding protein (RBP) decreases VA turnover and enhances VA delivery (17, 18). Centrally, because retinoic acid suppresses transcription of the pituitary TSHβ gene through activation of the retinoid X receptor (19–21), VA status may modulate T_4 feedback of TSH secretion. VAD in rats increases pituitary TSHβ mRNA and TSH secretion; both return to normal after treatment with retinoic acid (22).

Although VAD and IDD are common in many developing countries, there are few human data on their potential interaction in endemic regions. Several cross-sectional studies have found that VAD increases the risk for goiter. In Senegalese adults (23, 24) and Ethiopian children (25), there was a strong negative correlation between increasing severity of goiter and serum retinol (SR). Our aim in this study was to investigate the effects of VAD on thyroid metabolism in an area of severe IDD and compare the efficacy of iodized salt (IS) alone to IS given with VA supplementation in a randomized, double-blind trial in children.

Abbreviations: BSa, Body surface area; CRP, C-reactive protein; Hb, hemoglobin; IDD, iodine deficiency disorder; IS, iodized salt; RBP, retinol-binding protein; SF, serum ferritin; SR, serum retinol; TBG, thyroid-binding globulin; Tg, thyroglobulin; TT_4, total T_4; TTR, transthyretin; Tvol, thyroid volume; UI, urinary iodine concentration; VA, vitamin A; VAD, VA deficiency.

Subjects and Methods

The study was conducted in villages in the Rif Mountains of northern Morocco. The villages are approximately 600 m above sea level, with a temperate climate, and have a population of mixed Berber and Arab descent. This region is isolated from commercial routes, more than 95% of the population is rural, and most available food is produced locally on small farms (26). A small local cooperative supplies nearly all salt for the villages. It is produced in drying ponds using water from a salty

spring and contains less than 2 ppm iodine. Although Morocco legislated mandatory salt iodization in 1997, because of financial constraints, this cooperative has not yet begun iodization. The dietary staples are wheat bread, pulses, and olive oil (26). Calculated as retinol activity equivalents using a conversion factor of 12 μg β-carotene to 1 μg retinol for a mixed fruit and vegetable diet (27, 28), VA intakes (mean $\pm$ SD) are 206 $\pm$ 67 and 288 $\pm$ 71 μg retinol activity equivalents/d in children 6–8 and 9–13 yr, respectively. This is 48–52% of the recommended daily allowance for VA in these age groups (27).

For children in both the cross-sectional study and the intervention study described below, informed written consent (or, if the family was illiterate, informed oral consent) was obtained from the parents of the children, and informed oral assent was obtained from the children. The institutional review boards of the Swiss Federal Institute of Technology Zurich and the Moroccan Ministry of Health in Rabat gave ethical approval for the studies.

Cross-sectional study

The subjects were 6- to 14-yr-old children from three neighboring primary schools. All children in the three schools were invited to participate; all accepted and were enrolled (n = 298). At baseline, weight and height were measured, and a spot urine sample was collected for measurement of urinary iodine concentration (UI). Five milliliters of whole blood were collected by venipuncture for determination of SR, RBP, TTR, TSH, total T$_4$ (TT$_4$), thyroid-binding globulin (TBG), Tg, hemoglobin (Hb), serum ferritin (SF), and C-reactive protein (CRP) concentrations. Thyroid volume (Tvol) was measured using a portable Aloka SSD-500 Echocamera (Aloka, Mure, Japan) with a high-resolution 7.5-MHz linear transducer (29).

Efficacy study

All children from two schools in the cross-sectional study with either VAD or low VA status, defined as SR less than 1.05 μmol/liter (30 μg/dl) (3) were invited to participate in the 10-month study; 138 children were enrolled. The children were randomly divided by household into two groups. Both groups were given IS, *i.e.* salt fortified with 25 μg iodine/g. In addition, group 1 (IS group) received an oral placebo capsule (sunflower oil) at 0 and 5 months, while group 2 (IS+VA) was given an oral VA supplement (200,000 IU as retinyl palmitate; RpScherer, Aprilia, Italy) at 0 and 5 months (30). Both investigators and children were blind to group assignment.

To iodize the salt, iodine was added as reagent grade potassium iodate (KIO$_3$; Sigma-Aldrich Corp., Buchs, Switzerland) using an electric rotating drum mixer (ELTE 650; Engelsmann, Ludwigshafen, Germany). We chose the fortification level based on local salt intake data of 7–12 g/d in children, aged 6–14 yr (31), and anticipated a 25% loss of iodine during storage and cooking. Each participating family shared a monthly IS portion. For monitoring, 30-g aliquots (n = 6) of the salt were taken and measured for iodine content at each monthly mixing. Based on local census data indicating an average of 7.5 members/household, each household was provided with 2 kg salt at the beginning of each month for 10 months (30). The salt was dispensed directly to the head of the household from a central supply at the local health center. It was emphasized that the salt should be used for all cooking and food preparation as well as at the table. This message was reinforced at each of the monthly salt distributions. At 5 and 10 months, all baseline measurements were repeated. After completion of the study, all remaining children with VAD were treated with 200,000 IU VA.

Laboratory analyses

Serum and urine samples were aliquoted and frozen at −20 C until analyzed. UI and salt iodine content were measured using the Pino modification of the Sandell-Kolthoff reaction (32). At UI concentrations of 47 μg/liter (0.37 μmol/liter) and 79 μg/liter (0.62 μmol/liter), the coefficients of variation of this assay in our laboratory are 10.3% and 12.7%, respectively. The limit of detection is 2 μg/liter (0.016 μmol/liter); samples below this limit were assigned a value of 0. SR was measured by HPLC (33), and RBP by an ELISA (Immundiagnostik AG, Bensheim, Germany). VAD was defined as an SR less than 20 μg/dl (0.70 μmol/liter) (34). Because an SR less than 30 μg/dl (1.05 μmol/liter)

indicates low VA status (3), this criterion was also used. SR data were presented both as the proportion of children below these cut-offs and as distributions. Varying cut-offs for RBP have been proposed, but there is not yet a consensus value (35–37). Therefore, we presented our RBP data only as distributions. CRP and TTR were measured using nephelometry (TURBOX, Orion Diagnostica, Espoo, Finland). The RBP/TTR ratio has been proposed as an additional biochemical indicator of VAD (38). However, there is no agreement on a cut-off (39), so these data were presented as distributions. Dried blood spots on filter paper were analyzed for whole blood TSH (40) and serum TT$_4$ (DELFIA Neonatal T$_4$ Kit; PerkinElmer, Wallac, Turku, Finland). Normal reference values are: whole blood TSH, 0.2–3.7 mU/liter; and serum TT$_4$, 5.0–12.8 μg/dl (65–165 nmol/liter). Tg and TBG were measured on dried whole blood spots using a serum immunoassays (PerkinElmer) adapted for dried blood spots (41). Hb was measured using an AcT8 counter (Beckman Coulter, Krefeld, Germany), and SF was measured using an immunoassay (RAMCO, Houston, TX). Thyroid volume was calculated using the method of Brunn *et al.* (42). M.B.Z. performed all ultrasound measurements during the study. To estimate intraobserver variability, duplicate thyroid volume measurements were performed in 20 children at the 0 and 10 month visits; the mean ($\pm$SD) variability was 3.9% ($\pm$2.2%). New World Health Organization normative values for thyroid volume in school-age children according to sex and body surface area (BSa) were used to define goiter (29).

Statistical analysis

Data processing and statistics were performed using SPLUS (2000; Insightful Corp., Seattle, WA), PRISM (version 3, GraphPad, San Diego, CA), and Excel (XP 2002; Microsoft Corp., Seattle, WA). When data were not normally distributed, statistical analysis was performed after log transformation. At baseline, stepwise linear regression models were calculated with TT$_4$, log(TSH), log(Tg), and log(Tvol) as dependent variables, and height, weight, BSa, Hb, SF, CRP, SR, RBP, TTR, and TBG as independent variables. Logistic regression was used to test for associations between SR and the binary variables of low TT$_4$ (<5.0 μg/dl; 65 nmol/liter), elevated TSH (>3.7 mU/liter), and goiter (increased thyroid volume by ultrasound). A two-factor, repeated measures ANOVA was performed to compare effects of group × time for CRP, SR, RBP, RBP/TTR, UI, TT$_4$, TSH, TBG, TTR, Tg, and Tvol. If the interaction effect was significant, *t* tests between groups and paired *t* tests within groups were performed and adjusted for multiple comparisons (Bonferroni correction). Logistic regression was performed to compare effects of time × group for the binary variables of VAD, low VA status, and goiter. Proportions were compared using the χ^2 test. Significance was set at *P* < 0.05.

Results

Cross-sectional study

The characteristics of the children are shown in Table 1. The children were severely iodine deficient: the median UI was 10 μg/liter (0.079 μmol/liter); 71% of the children had a UI less than 20 μg/liter (0.158 μmol/liter), and the prevalence of goiter was 89% (1). The prevalence of low SR was 17%, indicating moderate VAD (34); an additional 50% of children had low VA status. No child exhibited clinical eye signs of VAD. The protein-energy nutrition of the children was generally adequate, as indicated by normal TTR concentrations (43) and a low prevalence of stunting (44) (data not shown). Mean $\pm$ SD weight and height were 29.7 $\pm$ 8.3 kg and 1.34 $\pm$ 0.13 m, respectively. The median (range) serum CRP was 1 (0–61) mg/liter, and only 4% of children had elevated CRP values. Iron deficiency was common; the mean $\pm$ SD Hb was 115 $\pm$ 10 g/liter, and the median (range) SF was 14 (5–204) μg/liter. Hb and SF were therefore included in the regression models, because iron status can modify thyroid metabolism in areas of endemic goiter (8–10).

TABLE 1. Characteristics of the 6- to 14-yr-old children in the cross-sectional study

Characteristic	(n = 298)
Age (yr)[a]	10.5 ± 2.2
Sex (male/female)[b]	162/136
Serum retinol (μmol/liter)	0.95 ± 0.25
Serum RBP (mg/liter)	22.7 ± 12.2
Serum TTR (mg/liter)	220 ± 66
RBP/TTR[c]	0.27 ± 0.12
Prevalence of VAD[d]	50 [17]
Prevalence of low VA status	149 [50]
Whole blood TSH (mU/liter)[e]	1.5 (0.3–120.0)
Serum T_4 (nmol/liter)	96 ± 21
Serum TG (ng/ml)	56 (1–788)
Serum TBG (mg/liter)	26.6 ± 6.8
Tvol (ml)	7.0 (2.3, 18.7)
Prevalence of goiter	265 [89]
UI (μg/liter)	10 (0–198)

To convert serum retinol values to micrograms per deciliter, divide by 0.349. To convert T_4 values to micrograms per deciliter, divide by 12.9. To convert iodine values to micromoles per liter, divide by 126.9.
[a] Mean ± SD.
[b] Number.
[c] Ratio.
[d] Number [percentage].
[e] Median (range).

SR was negatively correlated with log(TSH) (-0.84; $P <$ 0.0001), log(Tvol) (-0.78; $P <$ 0.0001), log(Tg) (-0.84; $P <$ 0.0001), and TT_4 (-0.77; $P <$ 0.0001; Fig. 1). There was a strong correlation between SR and RBP (0.8; $P <$ 0.001). RBP was negatively correlated with log(TSH) (-0.61; $P <$ 0.0001), log(Tvol) (-0.62; $P <$ 0.0001), log(Tg) (-0.71; $P <$ 0.0001), and TT_4 (-0.62; $P <$ 0.0001). In the stepwise linear regression, addition of the other independent variables (height, weight, BSa, Hb, SF, CRP, RBP, TTR, and TBG) did not improve the predictions once SR had been included. By logistic regression, SR was a significant predictor of a low TT_4, an elevated TSH, and goiter ($P <$ 0.0001). The frequency of these abnormalities by VA status is shown in Table 2. In the combined VAD and low VA status groups, the odds ratios (95% confidence interval) for hypothyroidism and goiter were 0.06 (0.03, 0.14) and 6.51 (2.94, 14.41), respectively.

Efficacy trial

There were no significant differences in baseline characteristics between the two groups (Table 3). Of the 138 children who began the study, 136 completed it; two children in the IS group moved away. The mean ± SD iodine concentration in the salt aliquots taken directly after mixing was 22.9 ± 3.0 mg/g salt, respectively. The prevalence of an
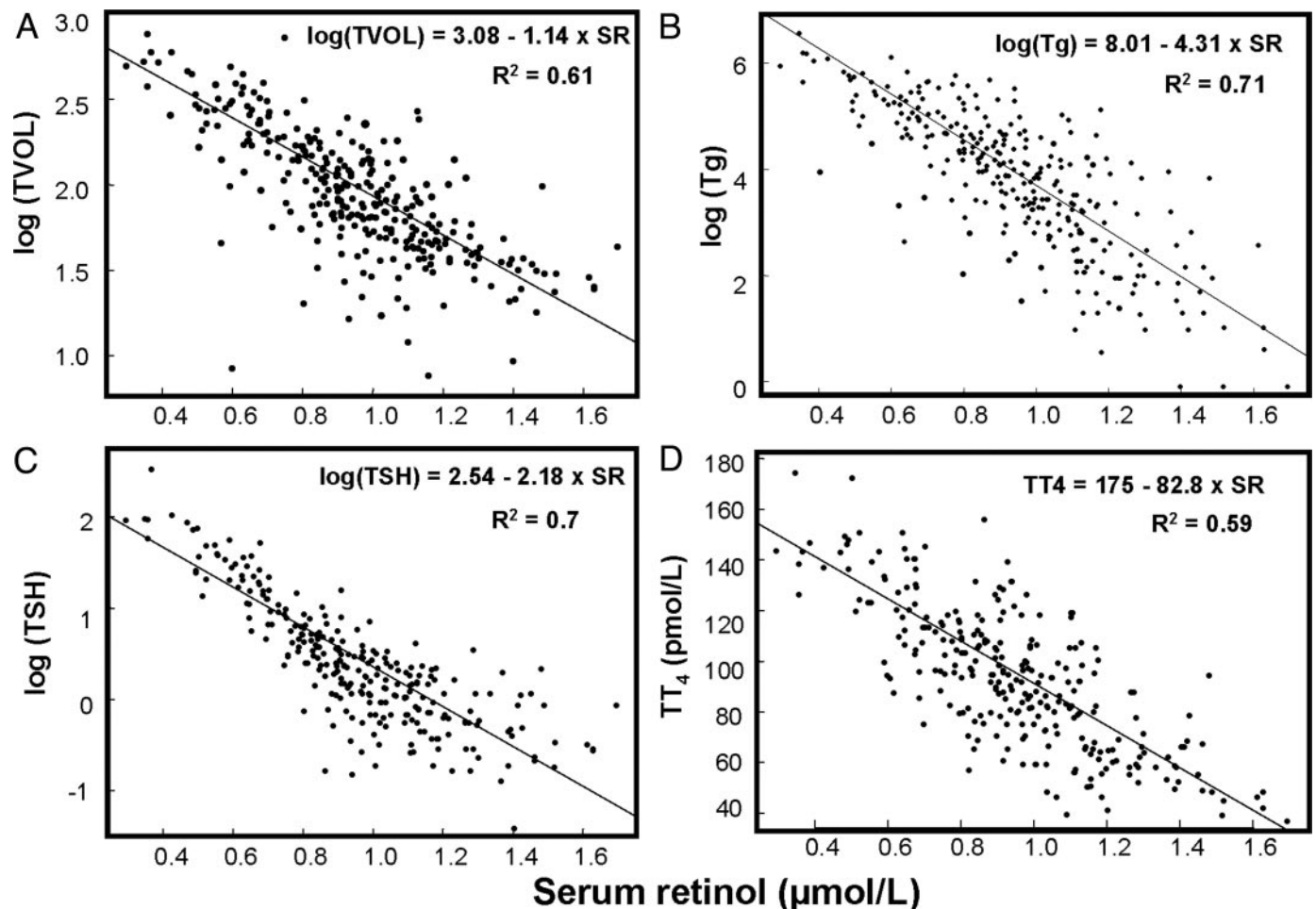

FIG. 1. Associations between the serum retinol concentration and log(Tvol) (A; by ultrasound), log(serum Tg) (B), log(whole blood TSH) (C), and serum TT_4 concentration (D) in 6- to 14-yr-old Moroccan children (n = 298).

elevated CRP (>10 mg/liter) was 4–6% in all groups at all time points, and there was no significant difference in mean CRP or prevalence of an elevated CRP between groups (data not shown).

The efficacy of the VA supplementation is shown in Table 4. In the IS+VA group at 10 months, mean SR, RBP, and the RBP/TTR ratio increased significantly ($P < 0.02$), and the number of children with VAD and low VA status was significantly reduced ($P < 0.01$). Tables 5 and 6 show the change in iodine status and thyroid parameters during the trial. There were no significant differences in median UI between

the groups throughout the study. In both groups, median UI increased significantly ($P < 0.0001$) from levels indicating severe IDD at baseline to at or near iodine sufficiency (>100 μg/liter; 0.79 μmol/liter) at 10 months (6). The median TSH and Tg levels decreased significantly ($P < 0.01$) in the IS+VA group compared with the IS group. There was no significant change in mean TT_4, TTR, and TBG in either group. At 10 months, there was a significant decrease in mean Tvol ($P < 0.05$) and goiter rate ($P < 0.01$) in the IS+VA group compared with the IS group.

Discussion

The findings from the cross-sectional study suggest that mild to moderate VAD in IDD-affected children increases the risk for goiter, but decreases the risk for hypothyroidism. Hypothyroidism, not goiter, is the primary cause of the adverse health affects associated with iodine deficiency (45). Diffuse goiter in children in endemic areas is an adaptive response that helps maintain adequate thyroid hormone status (45).

The data from the intervention indicate that VA status may also modify the response to iodine repletion. In the trial, there was a significant decrease in median TSH, Tvol, mean Tg, and goiter rate in the IS+VA group compared with the IS group. In areas of endemic goiter, the major determinant of serum Tg and Tvol is TSH stimulation of the thyroid (42, 45). Our findings suggest TSH hyperstimulation, indicated by increased TSH, Tg, and Tvol, was reduced by VA treatment. Although there was no change in TT_4 in either group, only children with VAD or low VA status were included in the trial, and VAD was a strong baseline predictor of increased TT_4 concentration. Mean TT_4 was already well within the normal range at baseline, and only 5% of children began the trial with low TT_4. Overall, these data suggest that VA supplementation improves the efficacy of IS to control goiter in children with moderate VAD.

There are several potential mechanisms that could explain these findings. VAD has multiple effects on the pituitary-thyroid axis and on peripheral thyroid hormone metabolism. In animals, VAD causes thyroid hypertrophy (11, 46), reduces thyroidal iodine uptake (47), impairs synthesis of Tg and coupling of iodotyrosine residues to form thyroid hormone (12), and decreases intrathyroidal T_3 and T_4 (11, 12). In

TABLE 2. Frequency of low TT_4 concentrations, elevated TSH concentrations, and goiter in 6- to 14-yr-old Moroccan children (n = 298) by VA status

	VA deficient (SR, <0.70 μmol/liter)	Low VA status (SR, ≥0.70 and <1.05 μmol/liter)	VA sufficient (SR, ≥1.05 μmol/liter)
TT_4[a]			
<65 nmol/liter	0	7	38
≥65 nmol/liter	50	142	61
TSH[a]			
>3.7 mU/liter	40	1	0
≤3.7 mU/liter	10	148	99
Goiter[a]			
Present	49	141	75
Absent	1	8	24

Values given are the number of children. To convert serum retinol values to micrograms per deciliter, divide by 0.349. To convert T_4 values to micrograms per deciliter, divide by 12.9.
[a] Significant difference among VA groups, $P < 0.001$ (by χ^2 test).

TABLE 3. Characteristics of the children in the IS and the IS + VA groups at baseline

Characteristic	IS (n = 71)	IS + VA (n = 67)
Age (yr)[a]	10.4 ± 2.2	10.1 ± 1.9
Sex (F/M)[b]	38/33	34/33
Weight (kg)	29.7 ± 9.5	29.1 ± 9.2
Height (m)	1.33 ± 0.11	1.32 ± 0.14
Hb (g/liter)	112 ± 9	114 ± 12
SF (μg/liter)[c]	16 (4, 79)	14 (6, 84)
Serum CRP (mg/liter)	1 (0–54)	1 (0–61)

There were no significant differences between groups. F, Female; M, male.
[a] Mean ± SD.
[b] Number of children.
[c] Median (range).

TABLE 4. SR, RBP, RBP/TTR ratio, and prevalences of VAD and low VA status in the IS and IS + VA supplementation groups over 10 months

Time (months)	Retinol (μmol/liter)[a] IS	IS + VA	RBP (mg/liter)[a] IS	IS + VA	RBP/TTR ratio[a] IS	IS + VA	VAD (no. [%])[b] IS	IS + VA	Low VA status (no. [%])[b] IS	IS + VA
0	0.80 ± 0.15[c]	0.83 ± 0.16	17.4 ± 9.1	18.1 ± 8.3	0.20 ± 0.12	0.21 ± 0.11	13 [18][d]	16 [24]	58 [81]	51 [76]
5	0.83 ± 0.14	0.97 ± 0.11[e]	19.2 ± 10.1	24.5 ± 10.0[f]	0.22 ± 0.14	0.29 ± 0.13[f,g]	11 [15]	3 [4]	51 [72]	40 [60]
10	0.79 ± 0.11	1.09 ± 0.13[e]	20.6 ± 8.8	30.2 ± 11.2[g,h]	0.24 ± 0.10	0.36 ± 0.12[h,i]	14 [20]	3 [4]	53 [75]	28 [42]

To convert serum retinol values to micrograms per deciliter, divide by 0.349.
[a] Significant treatment × time interaction, $P < 0.001$ (by ANOVA).
[b] Significant time × treatment effect, $P < 0.01$ (by logistic regression).
[c] Mean ± SD.
[d] Number [percentage].
[e] $P < 0.01$ *vs.* baseline. $P < 0.01$ *vs.* IS.
[f] $P < 0.05$ *vs.* baseline.
[g] $P < 0.05$ *vs.* IS.
[h] $P < 0.02$ *vs.* baseline.
[i] $P < 0.02$ *vs.* IS.

TABLE 5. Whole blood TSH, serum TT$_4$, Tg, and UI in the IS and IS + VA groups over 10 months

Time (months)	TSH (mU/liter)[a]		T$_4$ (nmol/liter)		Tg (ng/ml)[a]		UI (µg/liter)[b]	
	IS	IS + VA	IS	IS + VA	IS	IS + VA	IS	IS + VA
0	2.1 (0.7–118.0)[c]	2.3 (0.3–120.0)	111 ± 21[d]	109 ± 23	63 (3–742)	69 (2–788)	12 (2–69)	11 (2–181)
5	1.7 (0.3–4.4)	1.2 (0.3–4.3)[e,f]	121 ± 24	118 ± 26	29 (4–208)[e]	12 (2–84)[g,h]	74 (2–239)[g]	69 (6–319)[g]
10	1.6 (0.3–3.0)[i]	0.9 (0.3–2.1)[g,h]	119 ± 22	116 ± 22	31 (6–95)[e]	7 (2–31)[g,h]	104 (22–1104)[g]	99 (21–1124)[g]

To convert T$_4$ values to micrograms per deciliter, divide by 12.9. To convert iodine values to micromoles per liter, divide by 126.9.
[a] Significant treatment × time interaction, $P < 0.0001$ (by ANOVA).
[b] Significant time interaction, $P < 0.0001$ (by ANOVA).
[c] Median (range).
[d] Mean ± SD.
[e] $P < 0.02$ *vs.* baseline.
[f] $P < 0.05$ *vs.* IS.
[g] $P < 0.01$ *vs.* baseline.
[h] $P < 0.01$ *vs.* IS.
[i] $P < 0.05$ *vs.* baseline.

TABLE 6. Serum TTR, TBG, Tvol by ultrasound, and prevalence of goiter in the IS and IS + VA groups over 10 months

Time (months)	TTR (g/liter)		TBG (mg/liter)		Tvol (ml)[a]		Prevalence of goiter [no. (%)][b]	
	IS	IS + VA	IS	IS + VA	IS	IS + VA	IS	IS + VA
0	224 ± 68[c]	210 ± 64	26.4 ± 6.6	25.9 ± 7.0	7.4 (1.9, 18.7)[d]	7.2 (2.3, 16.6)	67 [94][e]	62 [92]
5	229 ± 58	218 ± 61	29.9 ± 5.8	28.7 ± 6.9	6.7 (2.1, 13.2)	5.9 (2.0, 12.5)[f,g]	52 [73]	45 [67]
10	220 ± 63	219 ± 68	25.2 ± 6.5	24.7 ± 6.0	6.2 (2.1, 11.9)[f]	5.3 (2.2, 12.4)[g,h]	45 [64]	34 [52]

[a] Significant treatment × time interaction, $P < 0.001$ (by ANOVA).
[b] Significant treatment × time effect, $P < 0.01$ (by logistic regression).
[c] Mean ± SD.
[d] Median (range).
[e] Percentage [number].
[f] $P < 0.05$ *vs.* baseline.
[g] $P < 0.05$ *vs.* IS.
[h] $P < 0.01$ *vs.* baseline.

the periphery, VAD increases total and free T$_4$ and T$_3$ (12, 13), reduces hepatic conversion of T$_4$ to T$_3$ (12, 48), and decreases T$_3$ uptake and binding (14, 15). Ingenbleek (12) fed rats iodine-deficient, VAD, or iodine-deficient and VAD diets, and reported that the iodine-deficient and VAD diet produced greater impairments in thyroid metabolism than either ID or VAD alone. Morley *et al.* (16) gave pharmacological doses of retinyl palmitate to rats and showed a decrease in thyroid gland size and serum TT$_4$ and TT$_3$ and an increase in thyroidal iodine uptake and hepatic conversion of T$_4$ to T$_3$. These animal data are consistent with our findings of increased TSH stimulation of the thyroid, greater Tvol, and higher circulating TT$_4$ in children with moderate VAD.

The effect of VAD on thyroid metabolism may be mediated at least partly through shared transport proteins. TTR binds 10–15% of T$_4$ and T$_3$ in plasma (49) and is also the primary indirect carrier of VA in the plasma through its interaction with RBP (18). RBP is secreted from the hepatocyte as a complex with TTR, and binding of RBP to TTR prevents glomerular filtration and renal clearance of RBP, thereby enhancing VA delivery (50). Although VAD decreases hepatic release of RBP, the release of TTR and serum TTR concentrations are similar during VA depletion and repletion in rats (51, 52). Consistent with these animal data, we found that VA status both at baseline and during supplementation did not influence serum TTR or TBG concentrations. Although we did not measure free T$_4$ concentrations, serum TTR and TBG concentrations were in the normal range, and there were no differences in TTR and TBG between groups

at any time, suggesting that TT$_4$ was a good indicator of thyroid hormone status. Because serum TTR and TBG concentrations were unaffected by VAD and VA repletion, it is unlikely that the effects of VAD on thyroid metabolism in this study were mediated through modification of thyroid hormone transport. However, animal studies have suggested that the binding capacity and affinity of TTR for thyroid hormone may be modified by interaction with RBP (18, 53–55), and this was not measured in the present study.

VAD may also affect thyroid metabolism through a central mechanism. Both the thyroid hormone-activated thyroid receptor and the retinoic acid-activated retinoid X receptor suppress transcription of the pituitary TSHβ gene by occupying half-sites on the promoter DNA of the gene (19–21). Breen *et al.* (22) found that VAD in rats increased pituitary TSHβ mRNA levels 2-fold and increased serum TT$_4$; both returned to normal after treatment with VA. They concluded that the increased TSHβ mRNA despite high serum TT$_4$ implied that VAD had made the pituitary thyrotrope relatively insensitive to feedback control by thyroid hormone. In pair-fed rats with VAD, Morley *et al.* (13) also found an increase in hypothalamic TRH and pituitary TSH despite high circulating T$_3$ and T$_4$ levels. We found evidence of a similar effect in the present study; in the children with VAD, the higher TSH concentrations in the face of higher circulating TT$_4$ suggest central resistance to normal TSH suppression by thyroid hormone.

Several cross-sectional studies have investigated the relationship between VAD and thyroid function or goiter. In

Senegalese adults, there was a strong negative correlation between increasing severity of goiter and SR, RBP, and TTR concentrations (23, 24). In Ethiopian children, those with visible goiters (grade IB or II) had significantly lower SR and RBP than children without or with grade IA goiter (25). In Ethiopian children with clinical signs of severe VAD, serum TSH was normal, and TT_3 (but not TT_4) was significantly correlated with SR and TTR (56). A limitation of these studies is that it was not possible to clearly distinguish the effects of VAD from those of protein malnutrition (57); protein malnutrition can decrease SR, RBP, and TTR independently of VA status. We measured TTR, a sensitive indicator of protein nutrition (57), at baseline and during the intervention. Nearly all children had a normal TTR concentration, and TTR did not change during the intervention trial, suggesting that protein status did not confound our results.

A question raised by these findings is the safety of VA repletion without concurrent iodine repletion in children with coexisting VAD and severe IDD. Our data suggest that moderate VAD in severely iodine-deficient children may reduce the risk for hypothyroidism. The data are consistent with the possibility that VAD may decrease activation of the pituitary retinoid receptor, thereby increasing transcription of the TSHβ gene and increasing TSH secretion (19–21). Increased TSH stimulation of the thyroid increases thyroid size, but maintains circulating thyroid hormone, protecting against hypothyroidism. Additional studies are clearly needed to resolve this question. Until then, a prudent course would be to provide oral iodized oil along with VA supplements to children in areas of severe endemic goiter that do not yet have IS. These data argue strongly for joint iodine and VA fortification and/or supplementation in areas of combined deficiency.

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