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Abstract: OBJECTIVE: Deficiencies of vitamin A and iodine are common in many developing countries. Vitamin A deficiency (VAD) may adversely affect thyroid metabolism. The study aim was to investigate the effects of concurrent vitamin A and iodine deficiencies on the thyroid-pituitary axis in rats. DESIGN: Weanling rats (n = 56) were fed diets deficient in vitamin A (VAD group), iodine (ID group), vitamin A and iodine (VAD + ID group), or sufficient in both vitamin A and iodine (control) for 30 days in a pair-fed design. Serum retinol (SR), thyroid hormones (FT(4), TT(4), FT(3), and TT(3)), serum thyrotropin (TSH), pituitary TSHbeta mRNA expression levels, and thyroid weights were determined at the end of the depletion period. MAIN OUTCOME: Compared to the control and ID groups, SR concentrations were about 35% lower in the VAD and VAD + ID groups (p < 0.001), indicating moderate VA deficiency. Comparing the VAD and control groups, there were no significant differences in TSH, TSHbeta mRNA, thyroid weight, or thyroid hormone levels. Compared to the control group, serum TSH, TSHbeta mRNA, and thyroid weight were higher (p < 0.05), and FT4 and TT4 were lower (p < 0.001), in the VAD + ID and ID groups. Compared to the ID group, TSH, TSHbeta mRNA, and thyroid weight were higher (p < 0.01) and FT(4) and TT(4) were lower (p < 0.001) in the VAD + ID group. There were no significant differences in TT3 or FT3 concentrations among groups. CONCLUSION: Moderate VAD alone has no measurable effect on the pituitary-thyroid axis. Concurrent ID and VAD produce more severe primary hypothyroidism than ID alone.

Effect of Concurrent Vitamin A and Iodine Deficiencies on the Thyroid-Pituitary Axis in Rats

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Objective: Deficiencies of vitamin A and iodine are common in many developing countries. Vitamin A deficiency (VAD) may adversely affect thyroid metabolism. The study aim was to investigate the effects of concurrent vitamin A and iodine deficiencies on the thyroid–pituitary axis in rats. *Design:* Weanling rats ($n = 56$) were fed diets deficient in vitamin A (VAD group), iodine (ID group), vitamin A and iodine (VAD + ID group), or sufficient in both vitamin A and iodine (control) for 30 days in a pair-fed design. Serum retinol (SR), thyroid hormones (FT$_4$, TT$_4$, FT$_3$, and TT$_3$), serum thyrotropin (TSH), pituitary TSHβ mRNA expression levels, and thyroid weights were determined at the end of the depletion period. *Main outcome:* Compared to the control and ID groups, SR concentrations were about 35% lower in the VAD and VAD + ID groups ($p < 0.001$), indicating moderate VA deficiency. Comparing the VAD and control groups, there were no significant differences in TSH, TSHβ mRNA, thyroid weight, or thyroid hormone levels. Compared to the control group, serum TSH, TSHβ mRNA, and thyroid weight were higher ($p < 0.05$), and FT$_4$ and TT$_4$ were lower ($p < 0.001$), in the VAD + ID and ID groups. Compared to the ID group, TSH, TSHβ mRNA, and thyroid weight were higher ($p < 0.01$) and FT$_4$ and TT$_4$ were lower ($p < 0.001$) in the VAD + ID group. There were no significant differences in TT$_3$ or FT$_3$ concentrations among groups. *Conclusion:* Moderate VAD alone has no measurable effect on the pituitary–thyroid axis. Concurrent ID and VAD produce more severe primary hypothyroidism than ID alone.

Introduction

VITAMIN A DEFICIENCY (VAD) and iodine deficiency (ID) are major global public health problems, affecting more than 30% of the population worldwide (1). In many regions of the developing world, vulnerable groups, such as young women, infants, and children, suffer from both VAD and ID (2,3). In areas of endemic goiter, micronutrient status is an important determinant of iodine and thyroid metabolism (4). Deficiencies of selenium (5) and iron (2) can act in concert with iodine deficiency to impair thyroid metabolism and modify the response to prophylactic iodine (6,7).

In animals, VAD has multiple effects on thyroid metabolism. Severe VAD decreases thyroidal iodine uptake, impairs thyroglobulin (Tg) synthesis, and increases thyroid size (8,9). In the periphery, VAD increases free and total circulating thyroid hormone (10–13), and binding of transthyretin (TTR) to retinol-binding protein (RBP) decreases VA turnover and enhances VA delivery (14,15). Centrally, because retinoic acid suppresses transcription of the pituitary TSHβ gene through activation of the retinoid X receptor (RXR) (16–18),

VA status may modulate thyroxine (T$_4$) feedback of thyrotropin (TSH) secretion. VAD in rats increases pituitary TSHβ mRNA and TSH secretion; both return to normal after treatment with retinoic acid (19).

In a recent study in African children with ID and VAD, increasing VAD severity was a predictor of greater thyroid volume and higher concentrations of TSH and T$_4$ (3). This study suggested that VAD in goitrous children increases TSH stimulation and thyroid size, and reduces risk for hypothyroidism, an effect that could be due to modulation of the pituitary TSHβ gene by VA (3). Therefore, the aim of this study was to investigate the effects of concurrent VAD and ID on the thyroid–pituitary axis in rats.

Materials and Methods

Animals and diets

The Veterinary Office of the Canton of Zurich's Department of Health gave the ethical approval for the study. Male weanling Sprague–Dawley rats ($n = 56$; Charles River

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GmbH, Germany) at 21 ± 3 days of age were housed individually in random order in stainless steel cages with grated stainless steel floors. The rats were kept under controlled conditions at 21°C and 55% humidity with a 12-hour light:dark cycle. Rats consumed Millipore water (Milli-Q UF Plus, Millipore, Bedford, MA) *ad libitum*. The diets were prepared by Dyets Inc. (Bethlehem, PA) following the AIN-93G purified rodents guidelines (20), and were based on ʟ-amino acids to avoid iodine contamination from casein as a major protein source.

Study design

The depletion period lasted 30 days. The rats ($n = 8$ in each group) were randomly divided into seven groups: a control group fed a VA- and iodine-sufficient diet (control group), a group fed the VA-deficient diet (VAD group), a group fed the iodine-deficient diet (ID group), and a group fed the VA and iodine-deficient diet (VAD + ID group). Animals in the VAD, ID, and VAD + ID groups were matched to pair-fed animals fed the control diet ($n = 8$ in each of the three pair-fed groups). All animals were handled daily to reduce stress at blood collection and sacrifice. Food intake was recorded daily and the body weight was measured three times per week. Blood was collected by tail vain incision (21) and serum was stored at -20°C. After the animals were killed by decapitation, the thyroid and pituitary were immediately dissected and removed. Thyroid dissection was done using a dissecting microscope under supervision of an experienced animal technician. Thyroids were weighed immediately after dissection, and then wrapped in aluminum foil, shock frozen in liquid nitrogen, and stored at -60°C. The pituitaries were dissected within 60 seconds and stored in RNA*later* solution (RNeasy Protect Mini Kit, cat. no. 74104, Qiagen GmbH Hilden, Germany) at –60°C.

Laboratory analysis

Free and total thyroxine and triiodothyronine (FT$_4$, TT$_4$, FT$_3$, and TT$_3$) and serum TSH were measured using immuno-chemiluminescent assays (IMMULITE®, Buehlmann Laboratories AG, Switzerland). Serum retinol (SR) was measured with an optimized high-pressure liquid chromatography method (22,23). For measurement of TSHβ mRNA, total RNA extraction was done using the RNeasy Protect Mini Kit (Qiagen GmbH, Hilden, Germany), with samples stored after each working step at –80°C. To avoid DNA contamination, a DNA on column digestion was carried out during the total RNA extraction (RNase-free DNase set, cat. no. 79252, Qiagen GmbH Hilden, Germany). TSHβ mRNA expression levels were measured with SYBR-Green® one-step RT-qPCR (QuantiTect SYBR Green RT-PCR Kit, cat. no. 204243, Qiagen GmbH, Hilden, Germany) using an ABI Prism 7900 Thermocycler (Applied Biosystems, Foster City, CA). The specific primer pairs were purchased from Qiagen, Germany. TSHβ mRNA expression levels were normalized with a housekeeping gene (Hrpt). For each animal, five replicates for the target gene and three replicates for the housekeeping gene were performed.

Statistical analysis

Data processing and analysis were done using SPSS 13.0 (SPSS Chicago, IL) and Microsoft Excel 2002 (Seattle, WA).

Data not normally distributed (TSH) were log transformed before comparisons. Comparisons between the treatment groups were done with analysis of variance. If the interaction effect was significant, t tests were done and adjusted for multiple comparisons (Bonferroni). Significance was set at $p < 0.05$.

Results

Compared to the control group, there was no significant difference in any of the measured biochemical or anatomic variables in the pair-fed groups (data not shown). Food intake and body weights (grams) did not differ significantly in all treatment groups; body weights were as follows: control 267 ± 16, VAD 258 ± 17, ID 260 ± 14, VAD + ID 281 ± 25. As shown in Table 1, compared to the ID and control groups, SR concentrations were about 35% lower in the VAD and VAD + ID groups ($p < 0.001$), indicating moderate VA deficiency. There was no significant difference in SR comparing the VAD + ID and VAD groups.

Serum concentrations of TSH, TT$_3$, FT$_3$, TT$_4$, and FT$_4$, and thyroid weights among the groups are shown in Table 1. Levels of pituitary TSHβ mRNA among the groups are shown in Figure 1. Comparing VAD to control, there was no significant difference in TT$_3$, FT$_3$, TT$_4$, or FT$_4$. In ID and ID + VAD groups, FT$_4$ and TT$_4$ were lower compared to the control group ($p < 0.001$). FT$_4$ and TT$_4$ were lower in the ID + VAD group compared to the ID group ($p < 0.05$). There were no significant differences in TT$_3$ or FT$_3$ concentrations between the groups. Mean TSH, TSHβ mRNA, and thyroid weight were significantly greater in the VAD + ID and ID groups compared to the control group ($p < 0.05$). TSH, TSHβ mRNA, and thyroid weight were significantly greater in the VAD + ID group compared to the ID group ($p < 0.01$).

Discussion

Our findings demonstrate that combined deficiencies of VA and iodine have greater adverse effects on the pituitary–thyroid axis compared to single deficiencies of VA or iodine. The data from the pair-fed controls indicate that these changes were not due to reduced food intake. Compared to the ID group, TSH, TSHβ mRNA, and thyroid weight were ≈30% higher in the VAD + ID group. Compared to the ID group, FT$_4$ and TT$_4$ were reduced by >50% and >75%, respectively, in the VAD + ID group. Thus, concurrent VAD aggravates ID-induced primary hypothyroidism.

There are several potential mechanisms that could explain this effect. In iodine-sufficient animals, severe VAD alone causes thyroid hypertrophy (9,24), reduces thyroidal iodine uptake (25), impairs synthesis of Tg and coupling of iodotyrosine residues to form thyroid hormone (8), and decreases intrathyroidal T$_3$ and T$_4$ (8,9). In the periphery, severe VAD increases total and free T$_3$ and T$_4$ (8,10), reduces hepatic conversion of T$_4$ to T$_3$ (8,26), and decreases T$_3$ uptake and binding (11,12). Because we did not measure deiodinase expression and/or activity in the thyroid or the liver, this study does not provide data on these potential mechanisms.

Although we did not measure thyroid binding proteins or binding affinity in this study, VAD may affect thyroid metabolism through shared transport proteins. In humans, thyroid-binding globulin (TBG) carries the majority of T$_4$ and T$_3$ in plasma (ca. 70%), whereas TTR binds 10–15% (14). Rats lack TBG and show different binding ratios between thyroid

TABLE 1. MEAN VALUES ± SD FOR SERUM RETINOL, TSH, THYROID WEIGHT, AND THYROID HORMONE CONCENTRATIONS IN WEANLING RATS FED DIETS DEFICIENT IN VITAMIN A (VAD GROUP), IODINE (ID GROUP), VITAMIN A AND IODINE (VAD + ID GROUP), OR SUFFICIENT IN BOTH VITAMIN A AND IODINE (CONTROL) FOR 30 DAYS

	Control	VAD	ID	VAD + ID
Serum retinol (μg/dL)	52.71 ± 5.93	33.93 ± 3.61[a]	51.70 ± 4.73	31.61 ± 2.33[a]
TSH (mIU/mL)*	0.13 ± 0.35	0.14 ± 0.11	0.29 ± 0.11[b,d]	0.77 ± 0.30[b,d]
Thyroid weight (mg)	17.5 ± 2.2	14.2 ± 3.1	26.4 ± 5.9[b,d]	37.8 ± 9.4[b,d]
TT_4 (μg/dL)	6.56 ± 0.65	6.60 ± 0.62	3.98 ± 1.37[c,d]	1.16 ± 0.31[c,d]
FT_4 (ng/dL)	3.27 ± 0.46	3.35 ± 0.36	2.06 ± 0.60[c,d]	0.95 ± 0.44[c,d]
TT_3 (ng/dL)	135.36 ± 18.49	135.71 ± 20.69	124.36 ± 22.42	107.47 ± 16.93
FT_3 (pg/mL)	5.64 ± 0.67	5.80 ± 1.59	4.60 ± 1.12	5.10 ± 0.86

*Data were log transformed for statistical analysis.
[a]Significantly different from control and ID group ($p < 0.001$; ANOVA, *post-hoc* test: Bonferroni).
[b,c]Significantly different from control and VAD group ([b]$p < 0.05$, [c]$p < 0.01$; ANOVA, *post-hoc* test: Bonferroni).
[d]Significantly different between ID and VAD + ID group ($p < 0.01$; ANOVA, *post-hoc* test: Bonferroni).

transport proteins (27). In rats and humans, TTR is a primary indirect carrier of VA; RBP is secreted from the hepatocyte as a complex with TTR, and binding of RBP to TTR prevents renal clearance of RBP, thereby enhancing VA delivery (28,29). Although VAD decreases hepatic release of RBP, release of TTR and serum TTR concentrations are similar during vitamin A depletion and repletion in rats (30,31). However, animal studies have suggested binding capacity and affinity of TTR for thyroid hormone may be modified by interaction with RBP (28,32–34).

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 promotor DNA of the gene (16–18). Breen et al. (19) found that severe VAD in rats increased pituitary TSHβ mRNA levels twofold, and increased serum TT$_4$; both returned to normal after VA treatment. They con-

cluded that the increased TSHβ mRNA despite high serum TT$_4$ implied VAD had made the pituitary thyrotrope relatively insensitive to feedback control by thyroid hormone. In pair-fed rats with VAD, Morley et al. (10) also found an increase in hypothalamic thyrotropin-releasing hormone and pituitary TSH despite high circulating thyroid hormone. In contrast to these previous studies in severely VA-deficient rats, where higher TSH concentrations in the face of higher circulating TT$_4$ suggest central resistance to normal TSH suppression by thyroid hormone, our data suggest that moderate VAD alone does not adversely affect the pituitary–thyroid axis.

To our knowledge, this is the first study to demonstrate that VAD in the presence of ID enhances the overexpression of TSHβ that is characteristic of ID. Mean TSHβ mRNA was more than 100% higher in the VAD + ID group compared to the ID group. Increased expression of TSHβ was likely due to aggravation of primary hypothyroidism; that is, lower
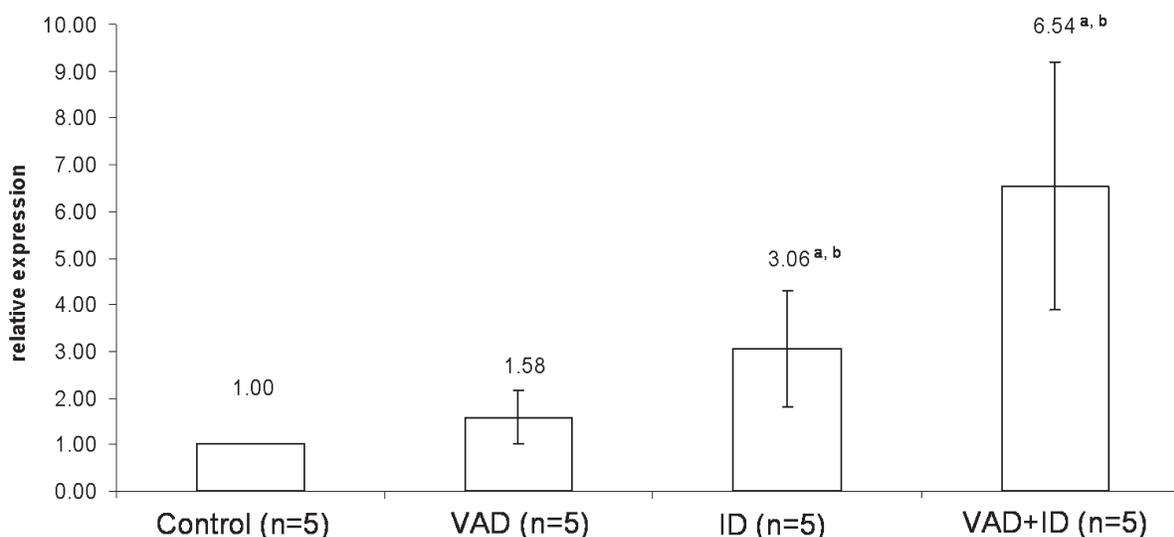

FIG. 1. Pituitary thyrotropin β mRNA concentrations in weanling rats fed diets deficient in vitamin A (VAD group), iodine (ID group), vitamin A and iodine (VAD + ID group), or sufficient in both vitamin A and iodine (control) for 30 days. a: Significantly different from control group ($p < 0.05$; ANOVA, *post-hoc* test: Bonferroni). b: Significantly different between ID and VAD + ID group ($p < 0.01$; ANOVA, *post-hoc* test: Bonferroni).

circulating T_4 levels in the VAD + ID group compared to the ID group stimulated greater TSHβ mRNA production. It is also possible that VAD, in the setting of ID, enhanced TSHβ mRNA expression through reduced suppression by the thyroid hormone–activated thyroid receptor and the retinoic acid (RA)-activated retinoid X receptor of the pituitary TSHβ gene. Haugen et al. (18), in an *in vitro* study, reported that both T_3 and RA independently suppress promotor activity. Li et al. (35,36) have suggested that the retinoid X receptor (RXR) component in a thyroid hormone receptor TR/RXR heterodimer is not a silent partner, but has important regulatory effects on the expression of the pituitary TSHβ gene.

One previous study examined the effect of concurrent VAD + ID in rats. Ingenbleek (8) provided rats either iodine-deficient (ID), vitamin A–deficient (VAD), or iodine and vitamin A–deficient (ID + VAD) diets for 60 days. Concentrations of FT_4 and TT_4 were greater in the VAD group, lower in the ID group, and intermediate in the VAD + ID group, compared to control. Serum TSH and TT_3 concentrations were increased in both the ID and the VAD + ID group. In the VAD and VAD + ID groups, synthesis of Tg and coupling of iodotyrosine residues to form thyroid hormone was impaired, and it was suggested that this was the primary cause of the adverse effects of VAD on thyroid metabolism (8). These data differ from our findings; in our study, VAD alone had no effect on thyroid metabolism, and there was no change in T_3 levels and a significant decrease in T_4 in the VAD + ID group. Differences in study design likely explain the varying findings between the two studies. The depletion period was twice as long in the study by Ingenbleek, and serum retinol levels fell to 10% of the control group, indicating severe VAD. Also, the rats were not pair-fed in that study (8), and significant decreases in body weight in the VAD and VAD + ID groups may have confounded the results.

The results of the present study differ somewhat from the findings of our previous study in African children with ID and VAD, where increasing VAD severity was a predictor of greater thyroid volume and higher concentrations of TSH and T_4 (3). Both studies found an increase in TSH concentration and thyroid size in concurrent ID and VAD, but in the present study, thyroid hormone concentrations were decreased. This difference could be due to several factors, including differences in study design, varying degrees of VAD, and/or confounding factors in the human, cross-sectional study, such as other nutritional deficiencies in the children.

Acknowledgments

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