Thyroid function in rats with iodine deficiency is not further impaired by concurrent, marginal zinc deficiency

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The hypothesis tested was that Zn deficiency aggravates impaired thyroid function as induced by I deficiency. In two separate experiments male rats were fed on diets either deficient in Zn or in I, or deficient in both. An identical, restricted amount of food was given to each rat so that body-weight gain of the experimental groups was comparable. Zn deficiency was evidenced by reduced tibial Zn concentrations. I deficiency was evidenced by goitre, reduced urinary I excretion, reduced plasma thyroxine concentrations and reduced absolute amounts and concentrations of thyroxine in the thyroid. Zn deficiency had no effect on the raised thyroid weight as induced by I deficiency. Zn restriction from 184 μmol Zn/kg diet to 31 μmol Zn/kg diet, but not to 92 μmol Zn/kg diet, significantly lowered plasma thyroxine concentration. There were no interrelated effects of Zn and I deficiencies on thyroid hormone levels. These results indicate that marginal Zn deficiency does not influence thyroid hormone metabolism in I deficiency.

Rats: Zinc: Iodine: Thyroid

I deficiency is the major cause of disorders such as goitre and cretinism (Hetzel, 1988). Simultaneous deficiency of either retinol (Morley *et al.* 1978; Oba & Kimura, 1980; Nockels *et al.* 1984) or Se (Vanderpas *et al.* 1990) may aggravate the symptoms of I deficiency. Perhaps this also occurs in concurrent Zn deficiency. Sandstead *et al.* (1967) reported an unexpectedly high incidence of goitre in children with dwarfism caused by Zn deficiency. Furthermore, Wada & King (1986) found in young men a significant lowering of free serum thyroxine (T_4) levels after a period of low Zn intake. In rats Zn deficiency causes reduced triiodothyronine (T_3) levels in serum (Morley *et al.* 1980) and raised activities of hepatic thyroxine 5′ monodeiodinase (*EC* 3.8.1.4; Oliver *et al.* 1987).

There may be an interaction of I and Zn deficiencies with regard to thyroid function. The present study with rats was undertaken to test this hypothesis. We have attempted to use a model suitable for elucidating an interaction between Zn and I intakes in humans in areas where marginal Zn and/or I intake is a problem. Thus, we have induced in rats marginal Zn and I deficiencies without clinical signs.

MATERIALS AND METHODS

Animals and housing

Two separate experiments were performed. The experimental protocols were approved and their execution supervised by the animal welfare officer of the Wageningen Agricultural University. Male, weanling Wistar (Cpb:WU) rats, aged 21 d, were used in both

experiments. They were purchased from Harlan/Cpb, Zeist, The Netherlands. On arrival the rats were housed in groups of five animals in cages with wire-mesh bases (600 × 420 × 190 mm). After 14 d they were individually housed in metabolism cages (3·14 m^2 × 120 mm) with polycarbonate tops and stainless-steel wire-mesh bases. The cages were placed in a room with controlled ventilation (twenty air changes/h), relative humidity (45–65%), room temperature (20–22°) and lighting (light on: 06.00–18.00 h).

Diets and feeding

From arrival, all animals were fed *ad lib.* on the purified control diet for another 14 d. This diet was Zn and I sufficient (184 μmol added Zn/kg; 1·2 μmol added I/kg). The composition of the diet was as follows (g/kg): ovalbumin 151, maize oil 25, coconut fat 25, glucose 709·4, cellulose 30, $CaCO_3$ 12·4, $NaH_2PO_4.2H_2O$ 15·1, $MgCO_3$ 1·4, KCl 1·0, $KHCO_3$ 7·7, KIO_3 0·25 mg, $ZnSO_4.H_2O$ 33 mg, I and Zn-free mineral premix 10, vitamin premix 12. The composition of the mineral and vitamin premix has been reported elsewhere (Grooten *et al.* 1991). After the pre-experimental period (day 0 of the experiment), the rats were divided into four groups of twelve rats each and one group of six rats. In both Expts 1 and 2 one group of twelve rats and the group of six rats remained on the control diet. The other groups received a Zn-sufficient I-deficient diet, a Zn-deficient I-sufficient diet or a Zn-deficient I-deficient diet. To prepare the I-deficient diets, KIO_3 was omitted from the control diet. To prepare the Zn-deficient diets, part of the added $ZnSO_4.H_2O$ was removed. In Expt 1 the Zn-deficient diets contained 31 μmol added Zn/kg instead of 184 μmol Zn/kg. In Expt 2 the Zn-deficient diets contained 92 μmol added Zn/kg. Analysis showed that the control diet contained 200 and 182 μmol Zn/kg in Expts 1 and 2. The analysed Zn concentrations of the other diets were as follows: Zn-sufficient I-deficient diets, 194 and 214 μmol/kg in Expts 1 and 2; Zn-deficient I-sufficient diets, 43 and 89 μmol/kg in Expts 1 and 2; Zn-deficient I-deficient diets, 43 and 90 μmol/kg in Expts 1 and 2.

Because Zn deficiency is known to cause anorexia (Swenerton & Hurley, 1967; Chesters & Quarterman, 1970), the four experimental groups in each experiment were given an identical, restricted amount of food so that body-weight gain would be comparable. The group of six rats was given the control diet *ad lib.* The experimental groups received 60% of *ad lib.* intake. Food was administered daily. The diets were in powdered form and stored at 4° until required for feeding. Separate batches of diet were made for the two experiments. Twice-distilled water was freely available from polycarbonate bottles with stainless-steel mouthpieces.

Experimental procedures

Each experiment lasted 42 d. During the first 3 weeks the rats were weighed once weekly and during the last 3 weeks twice weekly. Food intake was recorded every 2 d. Urine and faeces were collected quantitatively from each rat during the last 3 d of week 6 of each experiment. In Expt 1 urine was also collected for 3 d in weeks 2 and 4.

In Expt 2 a clinical examination of the rats was carried out by two independent assessors who were unaware of treatment modality. Rats were examined in random order. An apparently healthy rat fed on a commercial, pelleted diet (RMH-B®, Hope Farms, Woerden, The Netherlands) served as reference. The following variables were scored: discharge from nose and eyes, condition of hair-coat, condition of skin on trunk, tail and paws, colour of tail and teeth divergence. The variables were scored on a scale from 0 (similar to reference rat) to 3 (extremely different from reference rat). One assessor examined the rats on day 40 and the other on day 41. This method of clinical examination has been detailed elsewhere (Beynen *et al.* 1987).

At the end of each experiment blood was taken by orbital puncture, while animals were under diethyl ether anaesthesia, and collected in heparinized tubes. The anaesthetized rats were subsequently killed by CO_2 inhalation. Thyroid gland, tibia and testes were carefully removed. The glands were immediately weighed. Plasma, thyroid gland and tibia samples were frozen at $-18°$ until analysis.

Plasma samples were analysed for T_4 and T_3 by radioimmunoassay (RIA) without previous extraction according to the method of Larsen (1972) with minor modifications as described by Van Hardeveld & Kassenaar (1976). The thyroid was homogenized and T_4 and T_3 were extracted according to the method for thyroglobulin hydrolysis as described by Rolland *et al.* (1970), and determined as described previously. Thyroid-stimulating hormone (TSH) was measured by the specific RIA for rat TSH as developed by the National Institute of Diabetes and Digestive and Kidney Diseases (National Institutes of Health, Bethesda, MD, USA). Reference preparation RP-2 was used as TSH standard.

Diet samples, tibiae and faeces were dry ashed at 500° for 17 h. The ash was dissolved in 6 mol HCl/l and Zn was determined by flame atomic absorption spectrophotometry with the use of a Varian AA-475 (Varian Techtron, Springvale, Australia).

I and creatinine in urine were determined as described by Kolthoff & Sandell (1936) and Folin (1914).

Statistics

For each experiment the data from the four groups fed on a restricted basis were subjected to two-way analysis of variance (ANOVA) to disclose I and Zn effects and their interaction. $P < 0{\cdot}05$ was pre-set as the criterion of statistical significance. Differences between two groups with one dietary variable (CONTRAST) were evaluated with Student's t test. To take into account the increasing risk of a type I error due to multiple comparisons, Bonferroni's adaptation of the P value was applied. All analyses were done using the SPSS PC+ computer program (SPSSx Inc., 1986).

RESULTS

Weight gain

Initial body weights (day 0 of the experiment) in Expts 1 and 2 were 125·1 (SE 1·3) and 131·4 (SE 1·4) g (n 54). Feed intake of the control groups fed *ad lib.* was 18·9 (SE 0·5) and 19·1 (SE 0·6) g/d (n 6) in Expts 1 and 2. Final body weights of the experimental groups in Expts 1 and 2 are shown in Table 1. Restricting feed intake to 60% of *ad lib.* intake had reduced body weight by about 40% at the end of the two experiments. In Expt 1, restricted feeding for 42 d of the Zn-deficient diets with 31 μmol added Zn/kg diet lowered group mean body weight by 6% when compared with restricted feeding of either the control or I-deficient diet. In the course of Expt 2, when the Zn-deficient diets contained 92 μmol added Zn/kg, body-weight gain of the four groups fed restrictedly were superimposable (Table 1); no clinical abnormalities were detected.

Zinc status

The Zn-deficient diets produced significantly reduced tibial Zn concentrations, the effect being greater in Expt 1 than in Expt 2 (Table 1). Relative testes weight was lower in Zn-deficient than in Zn-sufficient rats in Expt 1. This was not seen in Expt 2 when the Zn-deficient diets contained more Zn than in Expt 1. The amount of I in the diet had no effect on the indicators of Zn status. *Ad lib. v.* restricted feeding of the control diet did not affect the tibial Zn concentration and lowered relative testes weight (Table 1).

Table 1. *Body weight and indicators of zinc status in rats fed on the experimental diets*

(Results are means with pooled standard errors for twelve animals per group for rats fed restrictedly and for six animals per group for rats fed *ad lib.*)

Feeding regimen...	Restricted					*Ad lib.* control		Statistical analyses	
Diet*...	Control	I-deficient	Zn-deficient	I+Zn-deficient	Pooled SE	Mean	SE	Effects of I *v.* Zn *v.* I+Zn deficiency (ANOVA)†	CONTRAST
Body wt (g)									
Expt: 1	206·1	205·7	193·9	194·7	2·7	334·7	9·8	Zn	c, d
2	217·5	218·3	220·2	218·8	2·8	356·0	10·2	—	—
Tibial Zn (μmol/g dry wt)									
Expt: 1	3·20	3·21	1·43	1·51	0·04	3·15	0·08	Zn	c, d
2	3·21	3·18	2·18	2·19	0·05	3·26	0·06	Zn	c, d
Testes wt (g/kg body wt)									
Expt: 1	14·4	14·8	9·4	8·3	0·5	9·3	0·2	Zn	c, d, e
2	14·8	14·6	14·4	15·0	0·4	9·6	0·4	—	e

The following comparisons were found to be significantly different by CONTRAST: c, Zn deficient *v.* Zn sufficient when I was sufficient ($P < 0·017$); d, Zn deficient *v.* Zn sufficient when I was deficient ($P < 0·025$); e, *ad lib. v.* restricted feeding when I and Zn were sufficient ($P < 0·017$).

* The control diets in the two experiments contained 1·2 μmol added I and 184 μmol added Zn/kg. The I-deficient diets did not contain added I. The Zn-deficient diets contained either 31 μmol (Expt 1) or 92 μmol (Expt 2) added Zn/kg.

† The ANOVA effect of Zn deficiency was significant ($P < 0·05$) as indicated.

Table 2. *Indicators of iodine status in rats fed on the experimental diets*

(Results are means with pooled standard errors for twelve animals per group for rats fed restrictedly and for six animals per group for rats fed *ad lib.*)

Feeding regimen...	Restricted					*Ad lib.* control		Statistical analyses: Effects of I *v.* Zn *v.* I+Zn deficiency (ANOVA)†	Statistical analyses: CONTRAST
Diet*...	Control	I-deficient	Zn-deficient	I+Zn-deficient	Pooled SE	Mean	SE		
Urinary I (μmol I/mol creatinine)									
Expt: 1	317·6	87·3	323·0	98·1	16·4	288·2	22·0	I	a, b
2	310·2	67·1	345·4	60·6	16·3	231·7	23·9	I	a, b
Thyroid wt (mg)									
Expt: 1	16·1	19·5	14·2	19·5	0·8	22·9	0·6	I	a, b, e
2	18·4	22·9	16·2	22·8	1·0	24·3	0·5	I	a, b, e
Plasma T_4 (nmol/l)									
Expt: 1	86·5	62·4	68·3	56·4	4·3	86·8	5·7	I, Zn	a, c
2	68·6	60·0	71·1	52·8	3·7	81·5	4·4	I	b
Plasma T_3 (nmol/l)									
Expt: 1	1·28	1·30	1·16	1·27	0·08	1·50	0·12	—	—
2	1·06	1·31	1·10	1·22	0·07	1·38	0·12	I	—
Thyroid T_4 (nmol T_4/mg thyroid)									
Expt: 1	15·9	9·2	17·8	8·0	1·2	15·3	1·6	I	a, b
2	19·0	9·6	20·1	8·1	1·0	17·9	1·4	I	a, b
TSH (nmol/l)									
Expt: 1	0·33	0·45	0·39	0·66	0·07	0·43	0·05	I	—
2	0·24	0·68	0·34	0·67	0·09	0·48	0·02	I	a, b, e

T_4, thyroxine; T_3, triiodothyronine; TSH, thyroid-stimulating hormone.

The following comparisons were found to be significantly different by CONTRAST: a, I-deficient *v.* I-sufficient when Zn was sufficient ($P < 0·017$); b, I-deficient *v.* I-sufficient when Zn was deficient ($P < 0·025$); c, Zn-deficient *v.* Zn-sufficient when I was sufficient ($P < 0·017$); e, *ad lib.* *v.* restricted feeding when I and Zn were sufficient ($P < 0·017$).

* The control diets in the two experiments contained 1·2 μmol added I and 184 μmol added Zn/kg. The I-deficient diets did not contain added I. The Zn-deficient diets contained either 31 μmol (Expt 1) or 92 μmol (Expt 2) added Zn/kg.

† The ANOVA effects of I and Zn deficiency were significant as indicated.

Iodine status

The diets without added KIO_3 clearly lowered the excretion of I in urine (Table 2); this effect tended to be somewhat more marked in Expt 2 than in Expt 1. Zn deficiency did not affect I:creatinine in urine. In Expt 1 the ratios were also determined after 2 and 4 weeks, the values being 128·8 (SE 6·6) and 96·7 (SE 5·0) μmol I/mol creatinine for the I-deficient rats (n 24). I deficiency significantly raised group mean thyroid weight in both experiments by on average 30%. Relative thyroid weight, also, was elevated significantly by I restriction (results not shown). There was no interaction of I and Zn deficiency with regard to thyroid weight.

Ad lib. v. restricted feeding of the control diet did not alter I:creatinine in urine. In rats fed *ad lib.*, absolute group mean thyroid weight was somewhat higher (Table 2) and relative weight lower (not shown). The latter was due to the higher amount of body fat in rats fed *ad lib.* instead of restrictedly; the difference in body fat was observed clearly at autopsy.

The I-deficient diets consistently lowered T_4 concentrations in plasma and thyroid (Table 2). I deficiency also significantly reduced the amount of thyroid T_4 when expressed per whole thyroid (results not shown). In Expt 1 Zn deficiency also reduced plasma T_4; this was not seen in Expt 2 when Zn deficiency was less pronounced. Zn deficiency did not influence thyroid T_4 concentration. T_3 concentration in plasma was significantly raised by I deficiency in Expt 2. In Expt 1 this I effect was not found. Concerning T_4 and T_3 concentrations in plasma, there were no interactions between I and Zn deficiency. TSH levels were significantly elevated in I-deficient rats. The amount of Zn in the diet did not affect plasma TSH concentrations.

The rats given the restricted amount of the control diet had thyroid T_4 concentrations similar to that of their counterparts fed *ad lib.* In Expt 2, but not in Expt 1, T_4 concentrations in plasma tended to be lower in rats fed on a restricted basis. Rats fed on restricted amounts of the control diet had lower group mean T_3 concentrations than those fed *ad lib.* Restricted feeding lowered group mean TSH levels significantly in Expt 2. This was not seen in Expt 1 (Table 2).

DISCUSSION

The lowered tibial Zn levels in rats fed on the low-Zn diets indicate that the animals were indeed Zn deficient. As would be expected, in Expt 1 using diets containing 31 μmol added Zn/kg, tibial Zn concentrations were lower than those in Expt 2 using diets with 92 μmol added Zn/kg. The control group fed on restricted amounts of feed had tibial Zn concentrations similar to those of the control group fed *ad lib.* This suggests that restricted feeding *per se* had no impact on Zn status of the rats. The observed lowering effect of Zn deficiency on testes weight in Expt 1 has been reported earlier (Hurley, 1969; Giugliano & Millward, 1984). In Expt 2 this was not seen, indicating that 92 μmol added Zn/kg diet is sufficient for testes development despite the fact that it lowered tibial Zn concentration. In Expt 2 the rats were examined clinically, but no abnormalities could be detected. This also indicates that the rats fed on the diets with 92 μmol added Zn/kg in Expt 2 had a marginal Zn status without clinical signs.

In Expt 1, using diets with only 31 μmol added Zn/kg, the Zn-deficient animals had significantly lower body weights than their counterparts that were also fed restrictedly (Table 1). Thus, in Expt 1, non-specific Zn effects related to growth retardation cannot be excluded. Therefore, we repeated the experiment with low-Zn diets containing 92 μmol added Zn/kg instead of 31 μmol added Zn/kg.

To monitor I status of the rats, urinary I:creatinine ratios were determined. After 6 weeks the ratio was lowered by about 75% in the rats fed on diets without added I. In Expt

2 the reduction was somewhat greater than that in Expt 1. Based on the time-course of the I:creatinine ratio in urine, it can be concluded that the I-deficient status was reached gradually. Restricted feeding *per se* did not influence urinary I:creatinine. The control groups fed either *ad lib.* or restrictedly had similar ratios. Thus, the rats fed on restricted amounts of the diets supplemented with 1·2 μmol I/kg can be considered I sufficient. I intake of rats fed *ad lib.* was on average 0·02 μmol I/d, and that of rats fed restrictedly was 0·01 μmol/d. Apparently, this difference in I intake did not affect I status.

I deficiency raised thyroid weight and reduced thyroid T_4 in both experiments which was accompanied by lower plasma T_4 levels. This can be explained by a lowered T_4 synthesis and/or increased T_3 synthesis. Monodeiodination of T_4 to T_3 might also be enhanced (Laurberg, 1984). As would be anticipated (Schröder-Van der Elst, 1991), I deficiency raised plasma TSH concentrations.

Restricted feeding *v. ad lib.* feeding raised relative thyroid weight, which relates to the lower degree of adiposity in restrictedly-fed animals. Restricted feeding had no significant effect on plasma concentrations of T_4 and T_3. However, plasma T_4 may be lowered by more extreme restriction of feed intake (Schröder-Van der Elst & Van der Heide, 1992). Restricted feeding did not alter thyroid T_4 concentrations. In Expt 2 plasma TSH concentrations were significantly lower in animals fed on restricted amounts of the control diet than in their counterparts given the control diet *ad lib.* This was not seen in Expt 1. Thus, in essence, the animals fed on a restricted basis did not differ much from those fed *ad lib.* concerning thyroid function.

In Expt 1 Zn deficiency did not affect thyroid T_4 content, but significantly lowered plasma T_4. Possibly, T_4 release from the thyroid is impaired by Zn deficiency or T_4 monodeiodination into T_3 is enhanced. The latter possibility is supported by results of Oliver *et al.* (1987) who found an elevated activity of hepatic 5′-monodeiodination in Zn-deficient rats. The suggestion by these authors that this leads to a higher level of T_3 in plasma is not confirmed by our findings. Moreover, Morley *et al.* (1980) reported that Zn deficiency in rats lowers plasma T_3. Such a tendency was seen in Expt 1, but only when the diet was I sufficient. In Expt 2 there were no effects of Zn deficiency on plasma T_4, which most probably relates to the lesser degree of Zn deficiency. In both experiments there was no interactive effect of I and Zn deficiency on thyroid T_4 concentration and plasma T_4 and T_3 concentrations.

Inconsistent effects of Zn deficiency on plasma TSH levels have been found by various workers (Root *et al.* 1979; Morley *et al.* 1980; Oliver *et al.* 1987). We observed raised group mean TSH levels in both experiments. Since plasma T_4 in the Zn-deficient group in Expt 1 was lowered, the rise in TSH might be a feedback response to stimulate thyroid hormone production. In Expt 1 there tended to be an interaction between I and Zn deficiencies with respect to plasma TSH: combined I and Zn deficiencies raised group mean plasma TSH in a synergistic manner. However, this was not seen with the lower degree of Zn deficiency in Expt 2.

In summary, under the present experimental conditions I and Zn deficiencies had predictable effects on thyroid function and Zn status respectively. However, there appeared to be no interaction between Zn and I deficiencies with regard to thyroid function as assessed using the measures described.

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REFERENCES

Beynen, A. C., Baumans, V., Bertens, A. P. M. G., Havenaar, R., Hesp, A. P. M. & Van Zutphen, L. F. M. (1987). Assessment of discomfort in gallstone-bearing mice: a practical example of the problems encountered in an attempt to recognize discomfort in laboratory animals. *Laboratory Animals* **21**, 35–42.

Chesters, J. K. & Quarterman, J. (1970). Effects of zinc deficiency on food intake and feeding patterns of rats. *British Journal of Nutrition* **24**, 1061–1069.

Folin, O. (1914). On the determination of creatinine and creatine in urine. *Journal of Biological Chemistry* **17**, 469–473.

Giugliano, R. & Millward, D. J. (1984). Growth and zinc homoeostasis in the severely zinc deficient rat. *British Journal of Nutrition* **52**, 545–560.

Grooten, H. N. A., Ritskes-Hoitinga, J., Mathot, J. N. J. J., Lemmens, A. G. & Beynen, A. C. (1991). Dietary fluoride prevents phosphorus-induced nephrocalcinosis in female rats. *Biological Trace Element Research* **29**, 147–155.

Hetzel, B. S. (1988). *The Prevention and Control of Iodine Deficiency Disorders. United Nations Administrative Committee on Coordination, Subcommittee on Nutrition. State-of-the-art Series. Nutrition Policy Discussion Paper* no. 3. Geneva: United Nations.

Hurley, L. S. (1969). Zinc deficiency in the developing rat. *American Journal of Clinical Nutrition* **22**, 1332–1339.

Kolthoff, I. M. & Sandell, E. B. (1936). *Textbook of Quantitative Inorganic Analysis*. New York: MacMillan Co.

Larsen, P. R. (1972). Direct immunoassay of triiodothyronine in human serum. *Journal of Clinical Investigation* **51**, 1939–1949.

Laurberg, P. (1984). Mechanisms governing the relative proportions of thyroxine and 3,5,3′-triiodothyronine in thyroid secretion. *Metabolism* **33**, 379–390.

Morley, J. E., Damassa, D. A., Gordon, J., Eugene Pekary, A. & Hershman, J. M. (1978). Thyroid function and vitamin A deficiency. *Life Sciences* **22**, 1901–1906.

Morley, J. E., Gordon, J. & Hershman, J. M. (1980). Zinc deficiency, chronic starvation, and hypothalamic-pituitary-thyroid function. *American Journal of Clinical Nutrition* **33**, 1767–1770.

Nockels, C. F., Ewing, D. L., Phetteplace, H., Ritacco, K. A. & Kendall, N. M. (1984). Hypothyroidism: an early sign of vitamin A deficiency in chickens. *Journal of Nutrition* **114**, 1733–1736.

Oba, K. & Kimura, S. (1980). Effects of vitamin A deficiency on thyroid function and serum thyroxine levels. *Journal of Nutritional Science and Vitaminology* **26**, 327–334.

Oliver, J. W., Sachan, D. S., Su, P. & Applehans, F. M. (1987). Effects of zinc deficiency on thyroid function. *Drug Nutrient Interactions* **5**, 113–124.

Rolland, M., Aquaron, R. & Lissitzky, S. (1970). Thyroglobulin iodoamino acids estimation after digestion with pronase and leucylaminopeptidase. *Analytical Biochemistry* **33**, 307–317.

Root, A. W., Duckett, G., Sweetland, M. & Reiter, E. O. (1979). Effects of zinc deficiency upon pituitary function in sexually mature and immature rats. *Journal of Nutrition* **109**, 958–964.

Sandstead, H. H., Prasad, A. S., Schulert, A. R., Farid, Z., Miale, A., Basilly, S. & Darby, W. J. (1967). Human zinc deficiency, endocrine manifestations and response to treatment. *American Journal of Clinical Nutrition* **20**, 422–442.

Schröder-Van der Elst, J. P. (1991). Thyroid hormone secretion and metabolism in the rat: influence of pathophysiological conditions. PhD Thesis, Leiden, The Netherlands.

Schröder-Van der Elst, J. P. & Van der Heide, D. (1992). Thyroid hormone metabolism in the streptozotocin-induced diabetic rat: are the changes attributable to insulin or caloric shortage? *Diabetes* **41**, 147–152.

SPSS Inc. (1986). *SPSSx User's Guide*, 2nd ed. Chicago, IL: McGraw-Hill Book Co.

Swenerton, H. & Hurley, L. S. (1967). Severe zinc deficiency in male and female rats. *Journal of Nutrition* **95**, 8–18.

Vanderpas, J. B., Contempré, B., Duale, N. L., Goossens, W., Bebe, N., Thorpe, R., Ntambue, K., Dumont, J., Thilly, C. H. & Diplock, A. T. (1990). Iodine and selenium deficiency associated with cretinism in northern Zaire. *American Journal of Clinical Nutrition* **52**, 1087–1093.

Van Hardeveld, C. & Kassenaar, A. A. H. (1976). Concentration of thyroid hormone in skeletal muscle. *Acta Endocrinologica* **83**, 305–312.

Wada, L. & King, J. C. (1986). Effect of low zinc intakes on basal metabolic rate, thyroid hormones and protein utilization in adult men. *Journal of Nutrition* **116**, 1045–1053.

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