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Low-iodine Diet for Producing Iodine Deficiency in Rats

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Abstract

A low-iodine diet has been prepared for rats, using locally available low-iodine ingredients. On analysis it has been shown to consistently contain 15–20 ng iodine/g. When fed to growing female rats, this diet produced severe iodine deficiency while not significantly affecting growth or reproduction. The deficiency was manifested by a fall in daily urinary iodine excretion (to less than 1 μg/day) and a seven-fold increase in thyroid uptake (^{131}I) observable within 3 months. Levels of plasma thyroxine (T_4) and thyroid stimulating hormone (TSH) continued to change for 4–5 months, T_4 falling from 69·9 to 7·5 nmol/l and TSH increasing seven-fold from a control value of 364 to 2406 ng/ml. Goitre was present in all iodine-deficient rats and iodine content in the thyroid was 10% of the control value.

Introduction

To study the influence of severe iodine deficiency upon neurological and somatic development in small laboratory rodents, it is desirable that a reliably low iodine experimental diet be available, which will promote optimal growth and development while maintaining extreme iodine deficiency. A number of investigations have been undertaken using the diet originally developed by Remington (1937) for the rat. However, this diet has been shown to vary considerably in its iodine concentration from batch to batch (Riesco *et al.* 1976), is expensive, and does not support optimal growth.

The purpose of this study has been to seek out suitable ingredients and develop a rodent diet which overcomes these deficiencies. Rats exhibit a rapid change in thyroid function with restriction of iodine intake, and have therefore been used to assess the suitability of the prepared diet (Studer and Greer 1965, 1968; Inoue and Taurog 1968; Ekpechi and Van Middlesworth 1973).

Materials and Methods

Animals

The rats used in this study were adult hooded Wistar females which were fed the diets for 4 months during which their weights increased from weaning (112 g) to maturity (230 g). They were initially divided into two groups, the control group receiving the low-iodine diet with an iodine supplement and the other receiving the low-iodine diet only. The iodine for the control group was added as stable potassium iodate in the proportion 1·7 mg per kilogram of feed. The rats were kept in chrome-plated wire cages and had access *ad libitum* to deionized distilled water (0·78 ng iodine/ml) and feed. There was no access to faeces or urine.

Diet

The study involved the examination of a large number of potential ingredients from around Australia with a view to supplying the protein, carbohydrate and roughage needs of guinea pigs

Table 1. Iodine concentration of potential ingredients for low-iodine diet

Ingredients were analysed as received

Type (brand)	Source	Iodine concn (ng/g)
Seeds		
Maize	Qld	7–12
	N.S.W.	15–22
Oats	S.A.	13, 30
	Tas.	39
Rye	S.A.	31
Peas	N.S.W.	60
	S.A.	7–44
Milo	Qld	13
Panicum	Qld	18
Japanese millet	Qld	100–140
White millet	Qld	11
Mung beans	Qld	16–44
Mung bean shoots		10–25
Lupins	S.A.	100
Haricot beans		38
Canneli beans		74
Blackeye beans		28
Brown cooking beans		23
Processed products and byproducts		
Pea pollard	S.A.	13, 28
Oat hulls	S.A.	125
Rice hulls	N.S.W.	500
Rolled oats	S.A.	120
Maize cobs	Qld	8
Wheat gluten	S.A.	93
Plain flour	(Lion)	13, 60
Cornflour	(Nurses)	230
Soy flour	(Sanitarium)	120
Maize oil	(Nuttelex)	14
Dog biscuits	(Menz)	40, 63, 300
Torula yeast	(St Regis)	15
Sucrose	(CSR)	11
Roughage		
Wood turnings (Oregon)		65, 72
Acacia gum		140
Paper pulp		16, 44
Paper		125, 400
Oaten hay	S.A.	100, 223
Wheat hay	S.A.	230
Barley hay	S.A.	300
Lucerne hay	S.A.	73, 500
Phalaris	S.A.	150
White clover	S.A.	150
Bananas	Qld	375

and rats. From Table 1 suitable ingredients were selected, large quantities procured, and diets prepared regularly to supply the rats' needs for approximately 1 month at a time. The quantities of

ingredients, their origin and iodine content are shown in Table 2. The components were mixed in the dry state, then moistened by the addition of 10% (w/w) deionized water, and, after further mixing, the formed diet was passed through a mincer (Nolex 14) with extrusion holes of 5 mm diameter. The extruded diet was dried at 70°C and fed in this pelleted form to the rats.

Table 2. Composition of low-iodine diet

Ingredient	Percentage in diet	Origin	Iodine concn (ng/g)
Maize (crushed)	50	Dalby, Qld	7
Peas (dried, crushed)	30	O'Halloran Hill, S.A.	11
Torula yeast (dried)	10	St Regis, Wisconsin, U.S.A.	15–18
Corn cobs (ground) or pea pollard	6	Gatton, Qld Anchor Products, S.A.	8 28
Maize oil	1·5	Nuttelex Products, Vic.	14
$CaCO_3$	1·5	Johns & Sturge, Birmingham, U.K.	4
NaCl	0·5	BDH 'Analar'	15
Trace elements and vitamins	[A]		
Total diet[B]			15–20
Control diet[C]			1000

[A] As for standard diet for rat. [B] On analysis. [C] Iodine added as KIO_3.

Measurement of Iodine in Diets, Thyroid Tissue and Urine

An alkaline ashing procedure (Foss *et al.* 1960), suitably modified to determine small amounts of iodine in biological material as described by Potter *et al.* (1980), was used for the estimation of iodine in the diet and thyroids. Urine was collected from the rats over a period of 24 h by housing them individually in metabolism cages. The urine was collected in glass flasks containing 8 M acetic acid to maintain acidity. Aliquots were refrigerated at 4°C until urine iodine could be assayed using a Technicon Auto-Analyser II (Garry *et al.* 1973) with modifications already described (Potter *et al.* 1980).

Plasma Hormone Assays

Thyroxine (T_4), triiodothyronine (T_3) and thyroid stimulating hormone (TSH) were assayed by radioimmunoassay (RIA) in plasma obtained by centrifugation of heparinized blood and then stored frozen. Unextracted plasma used in the RIA was treated with 8-anilino-1-naphthalene sulfonic acid (ANS) to inhibit binding to plasma proteins. The assay methods for T_3 and T_4 with modifications have been described (Potter *et al.* 1980). Rat TSH standard (NIAMD-RP-1), purified rat TSH for iodination (NIAMD rat TSH-1-3), and rabbit–anti-rat TSH antisera (NIAMD anti-rat TSH-S-3) for the assay of TSH were obtained in lyophilized form from the National Institutes of Health., Washington, D.C. The assay had a sensitivity and minimum detectable limit of 20 ng/ml.

Thyroid Glands

Thyroid glands obtained from rats killed at the end of the experiment were fixed in 10% neutral buffered formalin. After embedding the tissue in paraffin, sections 6 μm thick were prepared and stained with haematoxylin and eosin for histological examination. Thyroid iodine uptake in rats receiving the diet was assessed at 2-weekly intervals for 3 months. Each rat was given an intraperitoneal injection of 1 μCi ^{131}I (as NaI in normal saline) and 4 h later the iodine uptake was measured using a gamma probe with a lead collimator and an Ekco counter/scaler to monitor counts over the rat's thyroid region. This was compared with, and expressed as a ratio of, a standard dose measured in the same geometric relationship to the probe.

Results

Rat Growth

Twelve female rats were fed the iodine-deficient diet and 12 control rats received an iodine supplement in the diet. They readily accepted the diet, and their growth over 4 months to mature body weight is shown in Fig. 1. Although there was a slight, but not significant, reduction in growth of the iodine-deficient rats, there was no other evidence of abnormality in them. These growth rates compared favourably with those of rats on commercial diet in our breeding colony, and with those reported for rats receiving a normal diet (Anon. 1972).

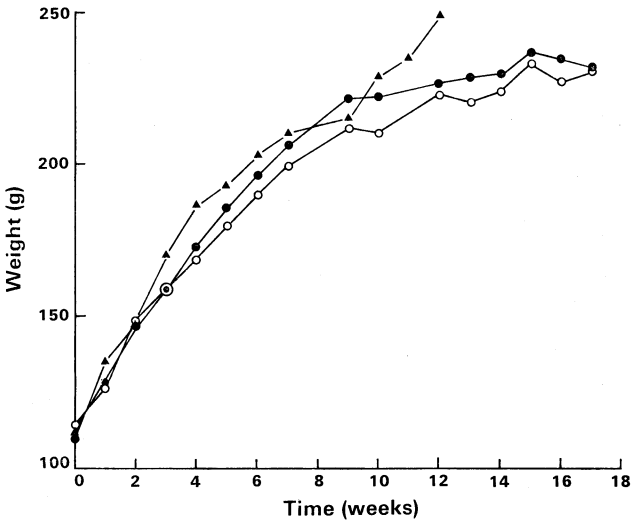

Fig. 1. Growth from postweaning to mature breeding weight of female rats fed on commercial (▲), low-iodine (○) and iodine-supplemented low-iodine (●) diets. Rats fed the commercial diet were mated at the ninth week, causing a rapid increase in body weight from then on.

Urinary Excretion of Iodine

The monitoring of urinary iodine excretion of rats showed a consistently lower iodine excretion (mean ± s.e.m.) of $0 \cdot 78 \pm 0 \cdot 04$ μg I/24 h at 2 months and $0 \cdot 77 \pm 0 \cdot 09$ μg I/24 h at 3 months in the deficient rats, compared with a constant value ($3 \cdot 36 \pm 0 \cdot 45$ μg/24 h) in the control rats. Dietary intake was approximately 0·2–0·4 μg iodine per day based on a daily intake of 15–20 g. The control diet on the other hand supplied 15–20 μg iodine daily.

Thyroid Iodine and Goitre

The avidity of the thyroid of the iodine-deficient rats was assessed every 14 days for 3 months by measuring the uptake of ^{131}I by the thyroid 4 h after injection intraperitoneally. Uptake increased rapidly to a plateau of 72% from 28 days onwards. The thyroid of the control rats remained at a constant level of about 10% throughout the 3 months of observations. The weight and iodine content of the thyroid gland were assessed in rats after the offspring from their first pregnancy were removed

(42 days after mating). Goitre was evident in the iodine-deficient rats, the mean (± s.e.m.) weight of the thyroid gland (27·3 ± 0·9 mg) being double that of the control rats (13·9 ± 2·8 mg). At the same time, the mean total iodine content of the thyroid glands of the deficient rats (1·53 ± 1·10 μg) was considerably lower than that of the control rats (10·53 ± 2·11 μg).

Plasma Hormones

Thyroxine (T_4) in the plasma of the iodine-deficient rats continued to fall to a base level at 5 months, while TSH rose at the same time to a level seven times the control value (Table 3). Shortage of plasma prevented T_3 being assayed in all samples. However, in the plasmas analysed at 20 weeks (n = 10) there was no significant shift in T_3 concentration from a mean (± s.e.m.) control value of 1·62 ± 0·06 nmol/l. It appeared therefore that a significant degree of iodine deficiency was achieved after 5 months on the diet.

Table 3. Concentration of T_4 and TSH in control and iodine-deficient rats over 5 months

Value given is mean ± s.e.m. Sample size is given in parentheses

Week	Control		Iodine-deficient	
	T_4 (nmol/l)	TSH (ng/ml)	T_4 (nmol/l)	TSH (ng/ml)
6	68·1 ± 2·5 (5)	305 ± 24 (5)	22·5 ± 3·2 (4)	339 ± 60 (4)
14	47·1 ± 4·9 (9)	261 ± 61 (7)	11·0 ± 0·9 (8)	621 ± 94 (8)
20	69·9 ± 3·7 (5)	364 ± 18 (5)	7·5 ± 0·5 (5)	2406 ± 360 (5)

Histology of the Thyroid Gland

The histological appearance of the thyroid glands indicated an intense degree of hyperplasia and hypertrophy in the iodine-deficient thyroids compared to controls. There were barely detectable amounts of colloid in the follicles, and the epithelial cells lining the narrowed follicular spaces consisted of columnar cells instead of the normal cuboidal cells.

Discussion

There is considerable information already available on the influence of the low-iodine diet developed by Remington (1937) on laboratory rats (Studer and Greer 1965, 1968; Riesco, Taurog and Larson 1976; Naeije *et al.* 1978; Rosman *et al.* 1978). This commercially prepared, iodine-deficient diet is formulated primarily from cornstarch (78%) and wheat gluten (18%). However, the cost and the unpredictable variability of the iodine content of the diet have created problems in studies related to iodine deficiency. Also, the diet has not been found suitable for optimal growth in the rat (Riesco *et al.* 1976; Rosman *et al.* 1978). These issues are of significance when studying the influence of iodine deficiency on growth and development in the rat.

The alternative diet presented here, based essentially on maize, peas and torula yeast, has been shown to effectively produce severe iodine deficiency in rats, while

maintaining normal growth and development, fertility, pregnancy and lactation. The extent of iodine deficiency is illustrated by the low urinary iodine excretion, goitre, and the increased avidity of the thyroid for iodine. The concentration of plasma T_4 fell to a plateau level of about 10% of the control value, and this was associated with a high TSH concentration relative to the controls. Concentrations of plasma T_3 were only assessed at 20 weeks because of shortage of samples. At this stage, there was no difference between iodine-deficient and control values, an observation consistent with those of Riesco *et al.* 1977. Because of their greater sensitivity to iodine status, plasma T_4 and TSH were considered to be the best predictors of iodine deficiency. It is apparent from these results that the hypothyroidism observed was the result of iodine deficiency alone, and not caused by any other nutritional difference.

The selection of suitable ingredients for the diet has provided some interesting, alternative, high-protein sources to gluten which, in our experience, is a poor-quality protein for somatic growth and has a relatively high iodine content. Although different geographic regions undoubtedly influenced the iodine status, peas from our property in South Australia and mung beans from Queensland appeared to be consistently low in iodine, and as such are reliable protein sources. Torula yeast from a specific U.S. source also was low in iodine, whereas that from a number of other sources was not as reliably low. Of the main suppliers of carbohydrate, oats and maize were the two consistently low in iodine and, of these, maize from southern central Queensland was undoubtedly the most deficient source discovered.

It is evident therefore that the prepared diet is very suitable for producing severe iodine deficiency and can be compiled from readily available ingredients. It should enable a complete study of iodine deficiency on somatic and brain development in the rat to be made in the future.

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