Influences of Feeding of High-Iodine Eggs on Hypo- and Hyperthyroid Rats*

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Summary The effects of the feeding of high-iodine eggs to rats with an abnormal thyroid status were investigated. Rats were fed for one week on a commercial diet supplemented with propylthiouracil (PTU) (10 mg/100 g diet) or thyroxine-Na (240 µg/100 g diet) respectively, to induce hypo- or hyperthyroidism, and then further fed for 4 weeks on the respective drug-supplemented diets, containing 1% (w/w) of either ordinary or high-iodine egg powder. Control (euthyroid) rats were maintained on the commercial diet. The induction of a hypothyroid state resulted in thyroid hyperplasia, with decreased thyroid iodine content, altered serum thyroid relating hormone levels (increased TSH and decreased T₃ and T₄), elevated serum total cholesterol and reduced serum triacylglycerol (TG) levels, and also increased muscle and adipose tissue lipoprotein lipase (LPL) activities. In contrast, in the hyperthyroid animals, thyroid atrophy, as well as decreased serum TSH and increased T₃ and T₄ levels, was associated with reduced serum total cholesterol level and muscle LPL activity. There were no essential differences between animals given high-iodine and ordinary eggs in either hypo- or hyperthyroid state, although the effects of PTU treatment on the thyroid and serum TG level appeared to be slightly lesser in rats given high-iodine eggs than in those given ordinary eggs. It is concluded that high-iodine eggs did not have any side-effect on either hypo- or hyperthyroid rat in this study.

Key Words iodine, high-iodine egg, thyroid status, hypo- and hyperthyroidism, serum lipid, lipoprotein lipase, histopathological change, rat

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* Studies on Nutritional Consequences of High-Iodine Eggs (Part 5).
We have previously demonstrated that an administration of high-iodine eggs to rats lowered their serum triacylglycerol(TG) level, and elevated tissue lipoprotein lipase [EC 3.1.1.34](LPL) activity, without alterations in the serum levels of thyroid stimulating hormone (TSH), triiodothyronine (T₃) and thyroxine (T₄) (1-3). Another long-term study showed that the effects of high-iodine eggs appeared to suppress an age-dependent alteration of thyroid function and the brain lipid peroxide accumulation in rats (3). Histological features of the organs and tissues in rats given high-iodine eggs for a long time were not different from those of normal rats given ordinary eggs (4). Some other investigators recently suggested the possible hypocholesterolemic action of high-iodine eggs in rats fed on a cholesterol-rich diet (5). We also studied the utility of high-iodine eggs in humans (6, 7).

In the present study, we investigated the effects of feeding of high-iodine eggs on hypo- and hyperthyroidism in rats.

EXPERIMENTAL

Male Sprague-Dawley rats, 5 weeks old, weighing approximately 130 g, purchased from CLEA Japan Inc., Tokyo, were used.

Protocol and diets. After the rats were given a basal diet (Labo MR Stock; Nihon Nosan Kogyo K.K., Yokohama) for 1 week, they were divided into the control, hypothyroid (HYPO) and hyperthyroid (HYPER) groups with approximately the same mean body weight, and were meal-fed in individual cages between 17:00 and 09:00 h. The control group of animals continued to be on the same basal diet. The HYPO and HYPER groups of animals were given the basal diets supplemented either with an antithyroid drug, propylthiouracil (PTU) (10 mg/100 g diet) or with T₄-Na (240 μg/100 g diet). These two drugs were obtained from Sigma Chemical Co., Ltd., St. Louis, and premixed with a small amount of corn dextrin powder, corresponding to 1% weight of the diet, then added to the basal diet. One week later 4 to 5 rats of the control, HYPO and HYPER groups were sacrificed, and the remaining rats of the latter two groups were further divided into the two groups and maintained for an additional 4 weeks on the respective drug-supplemented diets to which ordinary (HYPO-OE and HYPER-OE groups) or high-iodine (HYPO-IE and HYPER-IE groups) egg powder previously reported (2) was added at the level of 1%(w/w). The general chemical compositions of the diets were almost the same among the groups: on the basis of a 100 g diet, 9.5–9.7 g of moisture, 19.2–19.4 g of crude protein, 4.8–4.9 g of crude fat, 6.6–6.7 g of crude fiber and 6.3–6.5 g of crude ash. Iodine contents of the diets used for the control, HYPO-OE, HYPO-IE, HYPER-OE and HYPER-IE groups were 33, 35, 392, 172 and 529 μg per 100 g of diet, respectively. The latter two diets contained 137 μg of iodine derived from T₄-Na supplemented. Animals were cared for in a room maintained at 24 ± 1°C with lighting from 07:00 to 19:00 h and allowed ad libitum access to tap water. Body weight was measured twice a week and food intake was checked on two successive days every week.

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Three weeks after the start of the experiment, all the rats were bled via the tail vein, which was followed by the sacrifice at the end of the 5-week experimental period. The blood sampling and sacrifice were carried out at 11:00h after the rats were given the experimental diets for 2h between 17:00 and 19:00h on the previous day, and then starved for 16h. From the data of food consumption, the rats of HYPO-OE and HYPO-IE groups were estimated to receive 650–910μg of PTU/100g body wt. per day and those of HYPER-OE and HYPER-IE groups 24–33μg of T4/100g body wt. per day during the latter 4-week experimental period.

Preparation of samples and analyses. Immediately after the sacrifice by decapitation, blood samples were collected, and the soleus muscle, epididymal fat pads and thyroid gland were removed and weighed. The liver and hypophysis were also excised. The right lobes of thyroid gland were pooled and determined for total and organic iodine content after fractionation by the trichloroacetic acid-precipitation method (3, 8). The left lobes, as well as the liver and hypophysis, were used for the histopathological examination with the hematoxylin-eosin staining (4). In order to estimate the amount of colloid in the thyroid gland, the Periodic acid-Schiff reaction was employed. Serum TSH, T3, T4 and total iodine were determined as described previously (2). The assays for serum TG and total cholesterol were performed by using the Triglyceride CII-Test Wako and Cholesterol C-Test Wako kits (Wako Pure Chemical Industries, Ltd., Tokyo), respectively. The LPL activity in the soleus muscle and epididymal adipose tissue was measured with the procedure described by Suzuki et al. (9), but the released free fatty acids during the enzymatic reaction were determined by the method of Laurell and Tibbling (10).

Statistical significance was evaluated by the analysis of variance (11).

RESULTS

Food intake, weight gain, and muscle and adipose tissue weights

As shown in Fig. 1, the animals treated with PTU became hypophagic about one week after the start of experiment, and exhibited no weight gain following the 2nd week of the experiment. On the other hand, the T4-treated animals showed a relatively small increase in body weight, when compared to the controls, despite hyperphagia. The soleus muscle weight was not affected by the treatment with either PTU or T4; however, the epididymal adipose tissue weight significantly decreased in rats treated with T4; i.e., at the end of the experiment, 3.51±0.27, 1.96±0.14 and 2.16±0.21 g/rat (mean±SEM) for the control, HYPER-OE and HYPER-IE groups, respectively. No differences between animals given high-iodine and ordinary eggs, however, were found in all the parameters studied.

Serum thyroid relating hormone and total iodine concentrations

Serum concentrations of TSH, T3 and T4 were markedly changed after 1 week of treatment with PTU or T4 (Table 1). In the PTU-treated rats, serum TSH was increased and serum T3 and T4 decreased. In the T4-treated rats, there were
Fig. 1. Food intake and weight gain in hypo- and hyperthyroid rats, given ordinary or high-iodine eggs. Rats were made hypo- and hyperthyroid by feeding them the PTU- and T4-supplemented diets, respectively. One week later, several rats of the control (euthyroid), hypothyroid (HYPO) and hyperthyroid (HYPER) groups were sacrificed. The remaining rats of the HYPO and HYPER groups were then fed for an additional 4 weeks on the respective drug-supplemented diets containing either ordinary (HYPO-OE and HYPER-OE groups) or high-iodine (HYPO-IE and HYPER-IE groups) egg powder. Vertical line represents standard error of the mean. Control (n=4), ---; HYPO-OE (n=7), -; HYPO-IE (n=7), ---; HYPER-OE (n=7), -; HYPER-IE (n=7), ---. a,b,c Significantly different from the control group (a: p<0.05, b: p<0.01 and c: p<0.001).

decreased serum TSH, and elevated serum T3 and T4 concentrations. No changes were found in the thyroid relating hormone concentrations after the administration of either high-iodine or ordinary eggs. Serum total iodine concentration was changed in parallel with serum thyroid hormone concentrations in the rats given ordinary eggs (HYPO-OE and HYPER-OE groups). However, it was significantly elevated in the rats given high-iodine eggs than in those given ordinary eggs in both hypo- and hyperthyroid states.

Thyroid weight and thyroid iodine content

As shown in Table 2, thyroid weight was increased in the PTU-treated rats, while it was decreased in the animals treated with T4. Total and organic iodine contents were markedly decreased with the PTU-treatment, whereas they were unchanged with the T4-treatment. In the hypothyroid state, the extent of thyroid
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Table 1. Effects of feeding of high-iodine eggs on serum thyroid relating hormone (TSH, T₃, and T₄) and total iodine concentrations in hypo- and hyperthyroid rats.

| Experimental period (wk) | Dietary group | TSH (μU/ml) | T₃ (ng/100 ml) | T₄ (μg/100 ml) | Total iodine (μg/100 ml) |
|-------------------------|---------------|------------|----------------|----------------|-------------------------|
| 1 (Before feeding of eggs) | Control (4)  | 3.1 ± 0.3 | 122 ± 12 | 5.8 ± 0.8 | 6.0 ± 0.6 |
|                         | HYPO¹ (5)     | 6.7 ± 0.4c | 60 ± 4b | 1.9 ± 0.2b | 4.1 ± 0.4 |
|                         | HYPER² (5)    | 1.2 ± 0.3b | 216 ± 15b | 15.2 ± 1.1c | 10.6 ± 0.9b |
| 5                       | Control (4)  | 3.0 ± 0.2 | 131 ± 14 | 5.3 ± 0.6 | 5.4 ± 0.5 |
|                         | HYPO-OE¹ (7)  | 11.3 ± 0.6c | 55 ± 2c | 1.0 ± 0.1c | 2.7 ± 0.3c |
|                         | HYPO-IE¹ (7)  | 11.6 ± 0.6c | 55 ± 1c | 1.0 ± 0.1c | 19.1 ± 1.1c,d |
|                         | HYPER-OE¹ (7) | 1.5 ± 0.1c | 225 ± 26a | 11.5 ± 0.7c | 10.8 ± 0.9b |
|                         | HYPER-IE¹ (7) | 1.6 ± 0.2b | 232 ± 20b | 11.2 ± 1.4c | 27.7 ± 1.6c,d |

Values are means ± SEM. Number of rats is shown in parentheses. ¹See legends to Fig. 1. a,b,c Significantly different from the control group (a: p<0.05, b: p<0.01 and c: p<0.001). d Significantly different from the corresponding ordinary egg-containing diet groups (HYPO-OE and HYPER-OE, respectively) (p<0.001).

Table 2. Effects of feeding of high-iodine eggs on thyroid weight and thyroid iodine content in hypo- and hyperthyroid rats.

Determinations for total and organic iodine were performed on the pooled thyroid sample from rats for each dietary group. Value for the thyroid iodine content represents the mean of triplicate analyses.

| Experimental period (wk) | Dietary group | Thyroid weight (mg/rat) | Thyroid iodine | % Organic |
|-------------------------|---------------|-------------------------|----------------|-----------|
|                         |               |                         | Total (μg/g of wet tissue) | Organic (μg/g of wet tissue) | % Organic |
| 1 (Before feeding of eggs) | Control (4)  | 16 ± 1¹ | 986 | 947 | 96 |
|                         | HYPO² (5)     | 37 ± 4a | 65 | 44 | 67 |
|                         | HYPER² (5)    | 12 ± 1a | 1,213 | 1,141 | 94 |
| 5                       | Control (4)  | 23 ± 1 | 1,092 | 1,037 | 95 |
|                         | HYPO-OE² (7)  | 54 ± 7a | 10 | 3 | 30 |
|                         | HYPO-IE² (7)  | 47 ± 3a | 48 | 19 | 40 |
|                         | HYPER-OE² (7) | 10 ± 1a | 1,050 | 981 | 93 |
|                         | HYPER-IE² (7) | 11 ± 1a | 1,216 | 1,143 | 94 |

Number of rats is shown in parentheses. ¹Mean ± SEM. ²See legends to Fig. 1. a Significantly different from the control group (p<0.001).

enlargement was slightly lesser, and the thyroid iodine contents were slightly higher in animals given high-iodine eggs than in those given ordinary eggs.

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Fig. 2. Effects of feeding of high-iodine eggs on serum lipid levels in hypo- and hyperthyroid rats. Number of rats is shown in parentheses. Bar represents the mean with standard error. For other explanations, see legends to Fig. 1. a,b,cSignificantly different from the control group (a: p<0.05, b: p<0.01 and c: p<0.001).

Serum lipid concentrations
Figure 2 shows the results of serum TG and total cholesterol concentrations. Serum total cholesterol was significantly increased in the rats treated with PTU, both on and after the 3rd week of the experimental period, while it significantly decreased in the rats treated with T4 on and after the 1st week, although there were no differences between animals given high-iodine and ordinary eggs in either thyroid state. The T4-treated rats exhibited a temporary fall in serum TG concentration in the 1st week of the experiment, but thereafter serum TG concentrations returned to the control levels. In the animals treated with PTU, the serum TG concentration decreased as the treatment period became longer. However, the serum TG concentration appeared to be higher in rats given high-iodine eggs than in those given ordinary eggs. The serum TG concentrations were 95.1±8.1, 72.8±9.4 and 63.1±4.2 mg/100 ml for the control, HYPO-IE and HYPO-OE groups in the 3rd week of experiment, respectively.

Tissue LPL activity
The soleus muscle and epididymal adipose tissue LPL activities were measured
Table 3. Effect of feeding of high-iodine eggs on tissue lipoprotein lipase activity in hypo- and hyperthyroid rats.

| Dietary group | Lipoprotein lipase activity (μmol FFA/h/g of wet tissue) |
|---------------|----------------------------------------------------------|
|               | Soleus muscle                                            | Epididymal adipose tissue |
| Control (4)   | 30.1 ± 2.0                                               | 12.9 ± 2.0                |
| HYPO-OE<sup>1</sup> (7) | 36.4 ± 1.5<sup>a</sup> | 28.6 ± 1.4<sup>c</sup> |
| HYPO-IE<sup>1</sup> (7)  | 32.3 ± 2.1                                               | 31.9 ± 3.9<sup>c</sup> |
| HYPER-OE<sup>1</sup> (7)  | 20.5 ± 1.9<sup>b</sup>                                   | 11.7 ± 1.3                |
| HYPER-IE<sup>1</sup> (7)  | 20.3 ± 1.6<sup>b</sup>                                   | 10.8 ± 0.8                |

Values are means ± SEM. Number of rats is shown in parentheses. See legends to Fig. 1. Significantly different from the control group (a: p < 0.05, b: p < 0.01 and c: p < 0.001).

at the end of the 5-week experiment. As seen in Table 3, the animals treated with PTU showed a higher LPL activity than the controls in both tissues, while those treated with T<sub>4</sub> showed a lower LPL activity in the soleus muscle, compared to the controls. In the hypothyroid state, animals given high-iodine eggs appeared to have a less increased activity in the soleus muscle than those given ordinary eggs.

Histopathological findings

Table 4 summarizes the histopathological findings in the liver, hypophysis and thyroid gland. The T<sub>4</sub>-treated rats hardly revealed histopathological changes except in the thyroid gland, but the PTU-treated rats showed marked histopathological abnormalities in the three organs examined. In the latter animals, the absence of colloid in the thyroid gland was noted in 2 out of 7 animals given ordinary eggs, while the decrease of colloid was present in 5 out of these 7 animals, and in all the animals given high-iodine eggs. The extent of decrease of colloid was lesser in the rats given high-iodine eggs as compared with those given ordinary eggs.

DISCUSSION

The present study was undertaken to see whether feeding of high-iodine eggs may affect experimentally induced hypo- and hyperthyroidism in rats. The dosages of PTU and T<sub>4</sub> used were sufficient to produce hypo- and hyperthyroid states in one week. The food consumption and weight gain in the PTU- and T<sub>4</sub>-treated animals were similar to those reported by several investigators who administered PTU or thyroid hormone to rats for 1–6 weeks (12–14). The administrations of PTU and T<sub>4</sub> both resulted in typical alterations in the serum levels of TSH, T<sub>3</sub> and T<sub>4</sub>, as demonstrated by Engelken and Eaton (13) in rats. The feeding of high-iodine eggs did not show any influence on the serum levels of these thyroid relating hormones.

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Table 4. Histopathological findings in hypo- and hyperthyroid rats given ordinary or high-iodine eggs.

| Dietary group              | Control | HYPO-OE\(^1\) | HYPO-IE\(^1\) | HYPER-OE\(^1\) | HYPER-IE\(^1\) |
|---------------------------|---------|----------------|----------------|----------------|----------------|
|                           | (4)     | (7)            | (7)            | (7)            | (7)            |
| Liver                     |         |                |                |                |                |
| Cellular infiltration     | 3       | 6              | 4              | 3              | 2              |
| Fatty liver or fatty infiltration | 3       | 2              |                |                |                |
| Hypophysis                |         |                |                |                |                |
| Vacuolar degeneration     | 7       | 6              |                |                |                |
| Proliferated basophilic cells | 6   | 4              |                |                |                |
| Proliferated eosinophilic cells | 3  | 7              |                |                |                |
| Swollen eosinophilic cells | 3       | 7              |                |                |                |
| Thyroid gland             |         |                |                |                |                |
| Decrease of colloid       | 5       | 7              |                |                |                |
| Absence of colloid        | 2       | 0              |                |                |                |
| Papillary adenoma         | 7       | 5              |                |                |                |
| Dilated vessels           | 6       | 6              |                |                |                |
| Proliferated interstitial tissue |       |                | 4              | 4              |                |
| Cuboidal epithelium of follicular cells | 4 | 7             | 7              | 1              | 3              |
| Columnar epithelium of follicular cells | 5 | 4              |                |                |                |
| Squamous epithelium of follicular cells |       |                | 5              | 7              |                |

Number of rats is shown in parentheses. Value is the number of rats showing respective histopathological changes. \(^1\) See legends to Fig. 1.

food consumption and weight gain in either hypo- or hyperthyroid state. However, the serum total iodine level in the rats given high-iodine eggs was markedly increased, as already shown in our previous studies with normal rats\(^2,3\).

Hyperplasia of the thyroid gland and reduction of thyroid iodine content noted in the present PTU-treated animals have been reported by Berthier and Lemarchand-Béraud\(^15\). The present study also revealed that an administration of \(T_4\) did not alter the thyroid total and organic iodine contents per unit tissue weight, but it caused the atrophy of the gland. In the hypothyroid state, high-iodine eggs seemed to be more or less effective in preventing the thyroid enlargement, the drop in the thyroid iodine content, and the disappearance of thyroid colloid. We have previously presented the similar results of observation that was done in rats

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administered with a smaller amount of PTU (40 \mu g/100 g body wt. per day) for 7 days following a long-term feeding of high-iodine eggs (3).

Serum total cholesterol was increased in the hypothyroid rats and contrarily decreased in the hyperthyroid rats. These findings have been shown by several workers (13, 16, 17), and the hyper- and hypcholesterolemia are well known as important clinical findings of the hypo- (18–20) and hyperthyroidism (20, 21), respectively. As to serum TG concentrations in rats with thyroid dysfunction, Keyes and Heimberg have shown that serum TG concentration remained normal in the rats given PTU, while it decreased in the rats given \( T_3 \) after a period of 1 week (12). On the other hand, the decreased or normal serum TG concentration has been observed by Engelken and Eaton in the rats administered, respectively, either with PTU or \( T_4 \) for 6 weeks (13). These data, however, can be explained by the present results demonstrating that the effect of both drugs on serum TG level has been time-dependent for the length of the treatment period. Although influences of high-iodine eggs on serum total cholesterol level were not found in rats with either hypo- or hyperthyroidism, the serum TG level in rats given high-iodine eggs tended to return toward that of the euthyroid controls in the hypothyroid state. This may suggest that the serum TG level is modulated more or less by feeding with high-iodine eggs in rats with hypothyroidism.

It has been shown that tissue LPL activity in the PTU-treated or thyroidectomized rats was increased in the skeletal muscle (22) and adipose tissue (23), but normal in the heart (22), whereas the exposure to the thyroid hormone resulted in a decreased and an increased LPL activity in the skeletal muscle (22) and the heart (22, 24), respectively. Similar results were obtained in the present study. The differences in these tissue LPL activities between the hypo- and hyperthyroid animals seemed partly to be related with the differences in the serum TG level.

In brief, the present study has demonstrated that the feeding of high-iodine eggs did not bring about any side effects on either hypo- or hyperthyroidism induced experimentally in rats.

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