

Morphologic Changes in the Thyroid Glands of Puppies Fed a High-Iodine Commercial Diet

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[Vol 2 Issue 4](#)

[Vol 2 Issue 3](#)

[Vol 2 Issue 2](#)

[Vol 2 Issue 1](#)

[Vol 1 Issue 4](#)

[Vol 1 Issue 3](#)

[Vol 1 Issue 2](#)

[Vol 1 Issue 1](#)

[Reprint Information](#)

[Back to The
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ABSTRACT

Iodine excess causes alterations in thyroid activity, blocking both its characteristic functions and cell proliferation. Depending on the dose of iodine and on the previous conditions of the gland, iodine excess can have a goitrogenic effect and induce the blockade of hormone biosynthesis and secretion-provoking hypothyroidism. Three groups of puppies younger than 3 months old were fed different diets: 1) a home-prepared diet (control group), 2) a commercial diet (containing 5.6 mg potassium iodide/kg dry food), and 3) a home-prepared diet supplemented with 5.6 mg potassium iodide/kg dry food. Thyroid volume was evaluated by sonography and by weight, histopathology, and morphometry, and thyroid hormones were measured (thyroid stimulating hormone [TSH] and thyroxine T₄). Volume, weight, and diameter of the thyroid follicles were increased (P<0.05) in the 2 iodine-supplemented groups as compared with the control group. TSH was increased (P<0.05) and T₄ was lower (P<0.05) in groups with a high amount of iodine in diets than in the control group. It was concluded that increased dietary iodine alters thyroid morphology and function in puppies younger than 3 months old.

Introduction

Thyroid function is controlled by multiple factors. Among these, the supply of iodine plays a key role because it is not only a limiting factor for thyroid hormone biosynthesis, but is also a regulator of thyroid function and growth.^{1,2} Both low- and high-iodine intakes can cause changes in thyroid hormone biosynthesis, as well as in

its morphology. Excessive iodine intake can impair thyroid hormone biosynthesis (the Wolff-Chaikoff effect) and growth and lead to hypothyroidism.^{3–5} Overdosage of dietary iodine affects the normal function of the thyroid gland, particularly during the first 3 months of life in puppies, as was previously described.^{6,7} Thyroid dysfunction was found to occur when iodine was administered at levels 10 times the physiologic requirement. Thus, levels of thyroxine (T_4), thyroid-stimulating hormone (TSH), and both iodine uptake and urinary excretion were altered.⁷ The daily dietary intake of iodine in dogs ranges from 100 to 150 $\mu\text{g}/\text{day}$;^{8,9} however, several commercial brands of puppy food have been found to contain high amounts of iodine (such as potassium iodide).⁷

Staff at the Small Animal Clinic of the University of Buenos Aires School of Veterinary Medicine noticed that a number of dogs showed an increasing thyroid volume, measured by ultrasonography.¹⁰ After questioning the owners, it was concluded that these animals were usually fed commercial diets, while dogs fed a home-prepared diet showed normal volume thyroid.

In human patients treated with excess iodine, a decrease in thyroid blood flow¹¹ occurs, due in part to an increase in colloid content that raises intrathyroidal pressure and consequently reduces the volume of the vascular space. The increase in colloid content is due to a decrease in thyroid hormone secretion, caused by the inhibition of the pinocytosis-endocytosis processing of thyroglobulin from the colloid.^{12–14} There is also evidence that excess iodine decreases the expression of vascular-endothelial growth factor (VEGF).⁵ These changes have been evaluated in human patients by sonography and calculation of the thyroid volume.^{15,16}

The goal of this study was to analyze the morphologic changes in the thyroid gland of puppies younger than 3 months of age receiving a diet with normal amounts of iodine and a commercial diet with a high iodine content.⁷

Materials and Methods

Animals and Diet

Eighteen mongrel puppies, aged from 45 days (after delivery) to 3 months were studied. The animals were divided into 3 groups (6 puppies per group). Each group comprised litter mates. The puppies were born from mothers that were known not to have eaten a high iodine diet.

Average body weights at 45 days of age were 2.20 ± 0.07 kg (mean \pm SEM) in group A, 2.13 ± 0.06 kg in group B, and 2.33 ± 0.06 kg in group C. At 3 months of age, the body weights were 4.64 ± 0.14 kg for group A, 4.78 ± 0.11 kg in group B, and 4.30 ± 0.20 kg in group C.

Group A puppies were fed a home-prepared diet consisting of 55% rice, 25% meat, 10% milk, 5% oil, and 5% fiber, with the addition of purified calcium carbonate (1400 mg/kg dry matter), potassium iodide (250 mg/kg dry matter) and vitamins. Group B puppies were fed a commercial diet containing 5.6 mg potassium iodide/kg dry matter,⁷ and group C puppies were fed on a home-prepared diet supplemented with 5.6 mg potassium iodide/kg dry matter. The respective diets were given from day 45 until day 90 after birth. The amount of food offered was 200 to 250 g dry food/d, divided into 4 daily meals. The animals in groups B and C received about 1.2 to 1.8 mg potassium iodide/d, and those in group A received 80 to 100 μg potassium iodide/d. The mothers of the puppies (body weight 12.30 ± 0.43 kg) were fed on a home-

prepared diet containing 300 µg potassium iodide/kg of dry matter. The amount of food offered was 400 g dry food/d, divided into 2 daily meals. Mothers received about 130 to 150 µg potassium iodide/day.

Thyroid Hormones In Serum

Total T₄ levels were determined using standard radioimmunoassay (RIA) techniques. Thyroid-stimulating hormone (TSH) was measured by a specific TSH-IRMA canine kit (cTSH, DPC©, USA). Thyrotropin releasing hormone (TRH)-TSH tests were performed by injecting 200 µg of TRH (TRHFerring®, Germany) intravenously: serum samples were collected at 0 and 15 minutes after TRH administration for TSH estimation.^{17,18} A normal response is characterized by a TSH concentration of <0.55 ng/mL after stimulation.¹⁸

Thyroid Sonography Study

Thyroid volume was evaluated using ultrasound with a transducer of 7.5 mHz,¹⁹ using the formula proposed by Rezzonico et al,¹⁶ in which $LV = TD \times LD \times 0.36$ is the total thyroid volume (TTV), the sum of the volumes of both lobes. (LV: lobe volume; TD: transversal diameter; LD: longitudinal diameter; 0.36: elliptic correction factor). Sonography studies were performed before feeding and at the end of the study.

Morphologic study

At the end of the study, the 3-month-old puppies were euthanized and their thyroid glands were weighed (TW) and related to body weight (BW): $TW/BW \times 1000$. Thyroid samples were fixed in 10% formalin, embedded in paraffin wax, and stained with hematoxylin and eosin (H&E). The follicular diameter was determined in micrometers (µm). Photomicrographs of 10 typical sections were obtained. In each one, 10 follicles were selected randomly and the follicle diameters measured. A total of 500 follicles/group were measured.

Statistical Analysis

The values were expressed as average ± SEM, and comparison among the 3 groups was performed by ANOVA, multiple comparison, and Bonferroni's test (control vs treatment groups) as well as by Student t-test. The level of significance was set at 0.05.

Ethical Approval

All animal studies followed the recommendations of the Committee on Animal Care and Use of the Faculty of Natural Sciences, Buenos Aires University (UBACYT approval number 3119/97, under the Argentine Government law 6344/96).

Results

The serum levels of thyroid hormone are shown in Table 1. Group C showed hypothyroidism, with elevated TSH levels and a significant decrease in circulating T₄ levels. In group B, both hormone levels were within the normal range or were slightly above (TSH) or below (T₄) normal. TSH (P<0.002) and T₄ (P<0.0001) concentrations were significantly different from those of group A. A hyper-response to TRH was observed in group B, because TSH levels after TRH were higher than 0.55 ng/mL (10 ca). This test was not performed in animals from group C because their basal TSH

values were already significantly elevated. The thyroid status of group A did not change after stimulation with TRH. Therefore, we may conclude that hypothyroidism was present in group C as in group B, being more severe in the first one.

The TTV (Fig. 1) was significantly increased ($P<0.05$) in groups B and C (diets with iodine excess) compared with group A. The differences between B and C were not significant. The TW/BW ratio was larger ($P<0.05$) in groups B and C compared with group A (Fig. 2).

Histological study of the thyroid showed a flat thyroid epithelium and an increased number of colloid vacuoles in the follicular lumen in groups B and C, suggesting a resting thyroid. The follicular diameter was significantly larger ($P<0.0001$) in groups B and C compared with group A (Figs. 3 and 4).

Discussion

Many dog owners feed their dogs commercial diets with the assumption that they provide a more balanced diet. The finding of functional thyroid abnormalities in dogs from Buenos Aires and surrounding areas implies that this is not necessarily the case and led to the present investigation. We found that most commercial canine diets had a very high content of iodine,^{6,7} and it was important to determine whether an excessive iodine intake was the cause of these abnormalities.

That iodine deficiency causes serious alterations in the thyroid function of embryos and neonatal animals is well known. These alterations are evident in endemic goiter regions. In these regions, the incidence of neonatal cretinism and hypothyroidism in humans are significantly increased compared with areas in which the iodine supply is adequate.²⁰ On the other hand, iodine excess inhibits the biosynthesis and secretion of thyroid hormones, causing hypothyroidism.^{21,22} In extreme situations, this excess iodine causes hypothyroidism and increases the levels of circulating TSH, with hypertrophy and hyperplasia of the gland.^{3,12}

A significant decrease in circulating total T_4 and a corresponding increase in serum TSH were observed in this study (Table 1). Moreover, the level of TSH after TRH administration was significantly increased, while the level of T_4 was decreased. These data fit the concept that excess iodide, whether in commercial or home-prepared diets, affects thyroid status in these dogs.^{6,7} The results showed that feeding a diet containing a similar amount of iodine as contained in the commercial diet could result in hypothyroidism.

The goitrogenic effect of iodine excess has been noted in humans.²³ It has been shown that thyroid volume increases in normal subjects receiving excess iodine.¹³ In rats, the administration of excessive amounts of iodine causes an increase in follicular diameter.^{24,25} In previous studies, excess iodine in food, in both commercial and home-prepared diets, caused changes in thyroid structure demonstrable by ultrasound in puppies, pregnant bitches, and neonatal puppies.^{10,26} To our knowledge, this was the first demonstration of an action of this kind in dogs, although a similar effect has been shown in horses.^{27,28} It is interesting to note that these alterations are evident at the histologic level by a flattening of the follicular epithelium and the presence of colloid vacuoles, indicating thyroid inhibition (Figs. 3 and 4). This effect is due to the impairment of thyroid function caused by iodine excess.^{2,7,22,29} The inhibition of phagocytosis/pinocytosis of the colloid that contains thyroglobulin causes it to accumulate in the follicular lumen, thereby increasing the follicular diameter and diminishing the height of the follicular epithelium.^{11,14,23,24}

In summary, this study has shown that raised dietary iodine modifies both thyroid function and morphology in young puppies and can cause hypothyroidism in dogs. The effects seen in animals fed commercial diets containing excess iodine were reproduced in dogs fed a home-prepared diet supplemented with an equivalent amount of iodine. These results suggest that supplementing commercial diets with iodine should be carefully controlled to avoid undesirable effects.

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Table 1. Concentrations of TSH and T₄ in Puppies Fed Diets With Different Iodine Contents

	Group A	Group B	Group C
TSH (ng/mL)	0.20 ± 0.02*	0.30 ± 0.03†	1.03 ± 0.12‡
TSH 15 min (ng/mL)	0.30 ± 0.02§	0.62 ± 0.03	
Total T ₄ (nmol/L)	38.78 ± 2.83¶	20.85 ± 0.77**	12.87 ± 1.03††

Three months old puppies were fed a home-prepared diet (group A); a commercial diet with 5.6 mg potassium iodide/kg dry food (group B); or a home-prepared diet supplemented with an amount of iodine equivalent to that in the commercial diet (Group C).

Each value is the average ± SEM of six animals.

TSH: *P<0.02 (A vs B); †P<0.001 (B vs C) and ‡P<0.0001 (C vs A). §P<0.0001 (A vs B).

T₄: ¶P<0.0001 (A vs B); **P<0.001 (B vs C) and ††P<0.0001 (C vs A) According to

Student t-test.

Figure 1. Total thyroid volume (TTV) calculated by ultrasonography in puppies fed different diets. (A) Home-prepared diet. (B) Commercial diet containing 5.6 mg potassium iodide/kg dry food. (C) Home-prepared diet containing a supplement of 5.6 mg potassium iodide/kg dry food. * $P < 0.05$ A vs B and C (Bonferroni's test); ** $P < 0.04$ B vs A; *** $P < 0.0001$ C vs A. The differences between B and C are not significant (Student t-test). Values are expressed as mean \pm SEM; $n = 6$ puppies per group.

Figure 2. Ratio of thyroid weight:body weight (TW/BW) in puppies fed different diets: Group A, home-prepared diet; Group B, commercial diet containing 5.6 mg potassium iodide/kg dry food; Group C, home-prepared diet containing a supplement of 5.6 mg potassium iodide/kg dry food. * $P < 0.05$ A vs B and C (Bonferroni's test); ** $P < 0.002$ B vs A; *** $P < 0.0001$ C vs B and C vs A (Student t-test). Values are expressed as mean \pm SEM; $n = 6$ puppies per group.

Figure 3. Thyroid follicle diameters in puppies fed different diets. Group A, home-prepared diet; Group B, commercial diet containing 5.6 mg potassium iodide/kg dry food; Group C, home-prepared diet containing a supplement of 5.6 mg potassium iodide/kg dry food. * $P < 0.05$ A vs B and C (Bonferroni's test). ** $P < 0.0001$ A vs B and A vs C; ** $P < 0.02$ B vs C (Student t-test). Values are expressed as mean \pm SEM; $n = 500$ follicles per group.

Figure 4A

Figure 4B

Figure 4C

Figure 4. Thyroid histology of 90-day-old puppies fed with different diets. Group A, home-prepared diet; Group B, commercial diet containing 5.6 mg potassium iodide/Kg dry food; Group C, home-prepared diet supplemented with 5.6 mg potassium iodide/kg dry food. Group A showed variable follicular size, with a predominance of smaller follicles (active). Group B showed mainly larger follicles with a flat epithelium and colloid vacuoles (V). Group C shows essentially the same features as group B, but smaller follicles.

