Effects of Chronic Excess Salt Feeding: Demonstration of Normal Thyroid Function in Salt-Fed Dogs with Hypercholesterolemia[1] (35056)

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Some rats and dogs chronically fed excess amounts of salt develop a significant increase in plasma cholesterol concentration (1–4). Salt-induced hypercholesterolemia could be important in contributing to long-term perturbations in lipid metabolism and to the development and progression of atherosclerosis (4–8). Observations in mice (9–11) and dogs (12) indicate that under certain circumstances a high NaCl intake can interfere with thyroid function. Therefore, it is possible that salt-induced hypercholesterolemia may be secondary to hypothyroidism. We report here studies of thyroid function in a colony of dogs fed excess salt for more than 5 years. Our data suggest that it is unlikely that the salt-induced hypercholesterolemia is mediated through a depression of thyroid function.

Materials and Methods. 17 pure-bred beagles, 16 females and 1 male castrated at age 9 months, were studied. Each dog consumed a daily average of 200 g of kibble,[4] 100 g of canned horse meat, and 1 teaspoonful of a sodium-free mineral–vitamin supplement. The 12 "salt-fed" test animals had received excess salt diets (water containing 1% NaCl or kibble containing 6–10% NaCl) for more than 5 years, starting at 10–18 months of age (4), consuming 12–21 g of NaCl/day. The 5 *control* dogs had an estimated daily NaCl intake of 2.5 g.

^{131}I-thyroidal uptake studies. Each animal received 0.25 μCi of carrier-free ^{131}I intravenously as the sodium salt in 4–6 ml of 0.9% NaCl solution. Thyroid uptake was measured at both 24 and 48 hr after injection of isotope, using a thallium-activated 2 × 2-in. NaI crystal enclosed in a cylindrical lead shield 2 in. thick, placed below a shielded opening in the platform upon which the dogs were positioned with the thyroid directly over the crystal for optimal counting. Each dog was anesthetized with a slow intravenous infusion of sodium pentobarbital (13) before counting.

Other procedures. Blood was obtained by venipuncture in heparinized tubes for determination of plasma cholesterol (14), of urea nitrogen (15), and of protein-bound iodine (PBI) (16). For histological examination, biopsies of thyroids of 6 representative dogs were obtained under pentobarbital anesthesia, fixed immediately in neutral 10% formalin, and stained with hematoxylin and eosin. Statistical analyses were calculated using Student's *t* test (17).

Results. On the basis of diet and plasma cholesterol levels, there were four groups to be compared: (i) control group of 5 dogs on low-salt diet with normal cholesterol; (ii) "salt-fed" group of 12 dogs, some with normal and some with elevated cholesterol; (iii) "salt-fed" subgroup of 6 dogs with normal

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[4] Kibble = dry dog food. Made by Warner's Dog Food Company, Brooklyn, N.Y. Ingredients: Wheat flour, meat meal, bone meal, wheat middlings, wheat germ meal, dried tomato pomace, irradiated dried yeast, dried corn fermentation solubles, calcium phosphate, manganese sulfate, potassium iodide, iron sulfate, copper carbonate, salt, Vitamin A feeding oil, dried skim milk, Brewers dried yeast, Turmeric, charcoal. Analysis: Min. crude protein, 20%; min. crude fat, 3.5%; max. crude fibre, 3%; calcium, calcium, 1%; phosphorus, 0.7%; sodium, 0.35%; potassium, 0.4%; vitamin A (USP units/lb), 1000; vitamin D_2 (USP units/lb), 2000; vitamin E (IU/lb), 2.25; riboflavin, 1000μ/lb; thiamine, 300 μ/lb; niacin, 500 μ/lb; and pantothenic acid, 1800 μ/lb.

cholesterol values (within 3 standard deviations of the mean of the control group); and (iv) salt-fed subgroup of 6 dogs with elevated cholesterol values (at least 5 standard deviations greater than the control group mean value). All dogs had normal renal function (BUN 10 ± 1 mg/100 ml) and comparable body weights (9.7 ± 0.4 kg). There was no significant difference in ($p > 0.05$) systolic blood pressure (4) for the 5 control dogs (± 160.2, SE ± 2.65, range 152–168 mm Hg) and for the 12 salt-fed dogs (± 153.3, SE ± 7.56, range 98–200 mm Hg). The mean plasma cholesterol concentration in the group of 12 salt-fed animals (242 mg/100 ml) was significantly increased over that of the control group (179 mg/100 ml) ($p < 0.01$). This difference was due to an elevation in the 6 salt-fed animals classified in the subgroup "high-salt, elevated cholesterol" (293 mg/100 ml), since the mean concentration in the remaining 6 "high-salt, normal cholesterol" animals (190 mg/100 ml) was not significantly different from controls ($p > 0.05$).

The mean thyroidal ^{131}I uptake of the 5 control dogs was higher at 48 hr ($p < 0.05$) than that of the 12 dogs in the high-salt group, but there was no difference at either 24 or 48 hr between the mean ^{131}I uptakes of the 6 salt-fed dogs classified as normocholesterolemic and the 6 salt-fed dogs classified as hypercholesterolemic ($p > 0.05$). Because of the relatively high ^{131}I uptakes of dog No. 84, the data were reanalyzed with No. 84 excluded, but with similar conclusions.

All of the PBI values were within the normal range for dogs for this laboratory (Table I). There was no significant difference between the mean PBI values of the different groups and subgroups ($p > 0.05$), and there was no reciprocal relationship between PBI and cholesterol concentration.

Thyroid biopsies were obtained from dogs Nos. 11 and 93 (control group), 48 and 92 (high-salt, normal cholesterol), and 52 and 56 (high-salt, elevated cholesterol). All sections were reviewed by pathologists experienced in thyroid abnormalities, including those of the dog (18); no significant histological differences and no abnormalities could be recognized in a double-blind study.

Discussion. Among salt-fed dogs neither thyroidal ^{131}I uptake nor plasma PBI concentrations were significantly different between the group with and the group without hypercholesterolemia. Furthermore, histological review of thyroid biopsies indicated no differences. Thus, there was no evidence to support the suggestion that the hypercholesterolemia observed in salt-fed dogs and rats (1–4) is due to hypofunction of the thyroid. It is conceivable that the hypercholesterolemia was produced during a prior period of thyroidal hypofunction, with restoration of thyroid function to normal before correction of the hypercholesterolemia. However, the mean plasma cholesterol values of our hypercholesterolemic beagles were not significantly different at age 4.5 and 6 years. Similarly, in our salt-fed rats, repeated observations up to 22 months indicate that a spontaneous decline in plasma cholesteral seldom occurs, unless salt-feeding is stopped (unpublished data).

The somewhat lower ^{131}I uptakes observed in the salt-fed animals (group 2) as compared with the controls may be due to enhanced urinary excretion of iodide and a corresponding decrease in thyroid uptake, as observed in salt-fed mice (10). In the reports that iodine deficiency goiters could be produced by salt-feeding (9–11), special low-iodine diets were necessary. By neutron activation analysis, the iodine content of the dog kibble as found to be 3.9 mg/kg (±10%), so from this source alone each dog would received 750–800 μg of iodide/day, an amount in excess of normal requirements.

Certain other observations suggest that a high NaCl intake alone does not produce hypothyroidism. Daily ingestion of large amounts of NaCl increased the metabolic rate of young male Japanese (19), and salt-fed rats and rabbits had higher QO_2 values in liver and muscle slices *in vitro* (20). Chronic excess salt consumption had produced hypertension in several species [see (21)], while development of experimental hypertension can be blocked by inhibition of thyroid

TABLE I. ^{131}I-Thyroidal Uptake and PBI in Dogs According to Salt Intake and Plasma Cholesterol.

	12 dogs on high-salt diets				5 dogs on low-salt control diet				
Dog no.	Total cholesterol (mg/100 ml)	^{131}I uptake (%) 24 hr	^{131}I uptake (%) 48 hr	PBI (μg/100 ml)	Dog no.	Total cholesterol (mg/100 ml)	^{131}I uptake (%) 24 hr	^{131}I uptake (%) 48 hr	PBI (μg/100 ml)
	Subgroup with high cholesterol								
51	250	6.8	6.1	4.4	11	160	7.4	7.2	5.0
52	360	6.9	6.8	4.5	83	190	13.3	12.3	5.2
53	280	7.6	9.3	5.2	85	175	12.1	11.6	6.8
54	290	9.4	8.5	6.1	93	190	4.9	8.2	4.7
56	340	6.4	6.6	7.3	324	180	8.4	11.9	4.7
81	238	8.1	6.8	6.9		179 (±5.4)	9.0 (±1.4)	10.2 (±1.1)	5.3 (±0.4)
Mean (±SE)	293 (±20)	7.5 (±0.4)	7.4 (±0.5)	5.7 (±0.6)					
	Subgroup with normal cholesterol								
46	198	6.9	8.5	4.4					
48	160	5.6	7.1	3.8					
55	218	6.9	6.5	4.1					
84	200	19.3	10.1	6.1					
92	183	7.6	8.3	4.6					
95	183	7.3	3.9	4.3					
Mean (±SE)	193 (±8.1)	8.9 (±2.1)	7.4 (±0.9)	4.6 (±0.3)					
Group mean	242 (±18)	8.2 (±1.0)	7.4 (±0.5)	5.1 (±0.3)					

function surgically or chemically (22–24).

We have, therefore, concluded that the hypercholesterolemia which may be associated with chronic excess salt feeding is not the result of significant depression of thyroid function. The mechanism by which hypercholesterolemia may occur, as well as its significance to the development of atherosclerosis, remains unexplained. We attribute the observation that only half of the dogs on high-salt diets became hypercholesterolemic to a genetically-determined susceptibility to the effects of chronic excess salt ingestion, as has been demonstrated by inbreeding of rats to obtain strains resistant and susceptible respectively to salt-induced hypertension (25, 26). Since dogs are resistant to salt-induced hypertension, we are presently exploring in rats the question of whether these animals susceptible to salt-induced hypertension also are susceptible to salt-induced hypercholesterolemia and *vice versa*. Preliminary data suggest that this is indeed the case.

Summary. The hypercholesterolemia associated with chronic excess salt feeding is not due to hypothyroidism. As shown by thyroidal ^{131}I uptake, by plasma protein-bound iodine concentrations, and by histological studies of thyroid biopsy specimens, there were no significant differences in thyroid function between dogs with and dogs without hypercholesterolemia chronically consuming a high-salt diet.

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