PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 169, 110–112 (1982)

Hypocholesterolemic Effect of Alfalfa Meal in Monkeys Is Not Due to Thyroid Stimulation[1] (41317)

M. R. MALINOW

Oregon Regional Primate Research Center, Beaverton, Oregon 97006; and the University of Oregon Health Sciences Center, Portland, Oregon 97201

Abstract. Monkeys ingesting Purina Monkey Chow or semipurified foods containing alfalfa have plasma thyroxine levels similar to those in animals ingesting semipurified foods. The low plasma cholesterol levels in monkeys fed alfalfa are not due to induced hyperthyroidism.

Alfalfa meal incorporated into the diets of rabbits at levels between 25 and 75% (1) and in monkeys at the 51% level (2) lowers cholesterolemia. Several mechanisms have been hypothesized to be responsible for this effect, including intestinal binding of bile acids by fiber (3) and of cholesterol by saponins (4). Recently, Jackson (5) has demonstrated that alfalfa meal and laboratory chow both contain high levels of an immunoreactive thyrotropin-releasing-hormone-like material (Ir TRH), a finding that suggests another possible mechanism. Since the administration of D-thyroxine lowers plasma cholesterol in monkeys (6), the possibility that alfalfa Ir TRH may perturb thyroid function and thus modify cholesterolemia should be considered. In the study reported here, we ascertained thyroid function in monkeys fed chow or high amounts of alfalfa meal by measuring plasma thyroxine levels.

Materials and Methods. Adult female cynomolgus macaques (*Macaca fascicularis*), obtained in Indonesia and quarantined at the Oregon Regional Primate Research Center, were used in a series of experiments on atherosclerosis regression (2, 6). Only the pertinent features will be reported here. Over a 6-month period, 72 animals ingested semipurified diet I (Table I). The animals were then randomized into four groups of 18 monkeys each. The plasma cholesterol level was the blocking factor. Subsequently, the cholesterol content of the diet was reduced to 0.34 mg/kcal (diet II) and the following changes were made: group A, no change (controls); group B, 51% alfalfa meal substituted isocalorically (diet III); group C, added D-thyroxine, 3 mg/kg food; and group D, Purina Monkey Chow substituted for the semipurified food. Since monkeys weighed around 3.0 kg and ingested about 100 g of food/day, D-thyroxine intake approximated 0.3 mg/day or around 0.1 mg/kg body wt/day; the intake of alfalfa meal was approximately 50 g/day (2, 6). Plasma thyroxine levels (7) were determined 4, 10, 16, and 18 months later in all animals of group C and in three randomly selected monkeys from each of the remaining groups. At the end of 18 months, plasma thyroxine levels were determined in available samples from all animals. The commercially available antibody adequately detected D-thyroxine added to the diet, but the degree of cross-reactivity of D-T4 in the L-T4 assay was not determined. It seems likely, thus, that results in plasma assayed D- and L-thyroxine.

Results and Discussion. Table II indicates that throughout the observation period similar thyroxine levels were present in all groups of monkeys, with the exception of the D-thyroxine-treated animals, which had elevated levels. Body weights were similar in all groups of monkeys (2, 6), suggesting that thyrotoxicosis did not occur. Plasma cholesterol levels were decreased by alfalfa meal and by D-thyroxine in the presence of cholesterol added to the diet at a level of 0.34 mg/cal. However, mon-

[1] Publication 1179 of the Oregon Regional Primate Research Center, supported with Grants RR-00163 and HL-16587 of the National Institutes of Health.

0037-9727/82/010110-03$01.00/0

TABLE I. DIETS USED FOR *Macaca fascicularis*

Diet component	Diet I	Diet II	Diet III
	g/100 g of diet		
Casein	25	26.04	13.85
Sugar	43	41.04	15.58
Butter	25.5	24	15.80
Corn oil	—	2.5	—
Alfalfa meal	—	—	50.72
Vitamins (OWP)[a]	2	2.08	1.34
Salts (Hegsted IV)[a]	4	4.17	2.63
Vitamin D-3 (2000 IU/ml) (ml)	0.2	0.2	0.2
Cholesterol	0.5	0.096	0.06
	mg/100 cal		
Cholesterol	120	34	34
	% calories		
Protein	22	22	22
Fat	40	43	43
Carbohydrate	38	35	34

[a] ICN Nutritional Biochemicals, Cleveland, Ohio.

keys of group D fed chow without added cholesterol had even lower cholesterolemia.

If the alfalfa meal contained amounts of Ir TRH similar to those reported by Jackson (5), i.e., 157 ± 6 ng/g, our animals would have ingested around 7.5 μg/day. These amounts did not produce elevated levels of thyroxine in the blood. Purina Rat Chow contains an unspecified amount of alfalfa meal; its content of Ir TRH is 16 ± 1 ng/g (5); Purina Monkey Chow has not been analyzed for Ir TRH, but it may also contain this hormone since it probably comprises similar ingredients. However, the blood levels of thyroxine in chow-fed monkeys were not higher than those in monkeys ingesting semipurified foods. The Ir TRH in alfalfa described by Jackson (5) appears to have reduced biologic activity compared to synthetic TRH. Interpretation of our primate data suggests that Ir TRH is not absorbed intact, or if absorbed either does not

TABLE II. PLASMA LEVELS OF THYROXINE IN *Macaca fascicularis* AT VARIOUS INTERVALS

Group	Number of animals	Average thyroxine (μg/dl) after initiation of diets				Plasma cholesterol levels[b,c] (mg/dl)
		4 Months	10 Months	16 Months	18 Months[a]	
A, semipurified diet (SPD)	18	4.3 (3)	5.1 (3)	5.8 (3)	5.9 ± 0.3 (14)	341 ± 20[d]
B, SPD + 51% alfalfa meal	18	4.5 (3)	3.9 (3)	5.0 (3)	6.2 ± 0.6 (13)	202 ± 8
C,[d] SPD + 0.003% D-thyroxine	18	21.0 ± 1.5	17.7 ± 0.7	21.0 ± 1.7	14.3 ± 1.2	277 ± 11[d]
D, chow	18	5.4 (3)	5.1 (3)	5.6 (3)	6.7 ± 0.3 (17)	158 ± 4[d]

Note. Figures represent mean ± SE; number of animals are given in the parentheses.

[a] *P* (Student's *t* test): A versus C, <0.001; A versus B or D, not significant.

[b] Average of six determinations per monkey performed every other month during the last 12 months of the observation.

[c] *P:* A versus B, C, or D, <0.01.

[d] Values reported in Malinow *et al.* (6).

stimulate the thyroid gland or at least does not lead to chronic perturbation of thyroid function. Thus, the antihypercholesterolemic effects of alfalfa meal in monkeys are not associated with induced hyperthyroidism.

1. Cookson FB, Altschul R. Brit J Exp Pathol **49**:348, 1968.
2. Malinow MR, McLaughlin P, Naito HK, Lewis LA, McNulty WP. Atherosclerosis **30**:27, 1978.
3. Kritchevsky D. Lipids **13**:982, 1979.
4. Malinow MR, McLaughlin P, Stafford C. Amer J Clin Nutr **31**:814, 1978.
5. Jackson IMD. Endocrinology **108**:344, 1981.
6. Malinow MR, McLaughlin P, McNulty WP, Naito HK, Lewis LA. Atherosclerosis **31**:185, 1978.
7. Tetrasorb-125, Instruction Manual, North Chicago, Ill. Radio-Pharmaceutical Products Division, Abbott Laboratories.

Received February 27, 1981. P.S.E.B.M. 1982, Vol. 169.