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Effect of Chondroitin Sulfate A and Flavonoids on Hypervitaminosis D in Rats* (33960)

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Excess intake of vitamin D has been reported to have a number of adverse effects in several mammalian species. Among these effects are the toxicity response of some subjects to a relatively small excess intake of vitamin D 1), placental transfer and sensitization of the fetus to vitamin D (2), the phenomenon of calciphylaxis (3), involvement of vitamin D in cardiovascular injury (4), and interrelations of a high vitamin D intake with high lipid intake in the induction of atherosclerosis (5). These diverse effects of high vitamin D intake on the mammalian body point to a need for further studies to determine blood and tissue changes in hypervitaminosis D. The elucidation of blood parameters sensitive to vitamin D might enable detection of toxic conditions and institution of therapy before irreversible tissue changes occur. Also further work on treatments to prevent or alleviate vitamin D toxicity is needed. Two classes of substances warrant investigation. Chondroitin sulfate A (CSA) was reported to inhibit calcification (6), and flavonoids to protect animals against certain nutritional stresses (7).

In view of the above evidence the purpose of the present investigation was (i) to determine whether the erythrocyte sedimentation rate (ESR) and hematocrit are sensitive to excess vitamin D intake, (ii) to determine relations between hypervitaminosis D and blood lipid levels at normal lipid intakes, and (iii) to determine whether CSA or flavonoids (rutin and hesperidin) exert any protective effect against hypervitaminosis D.

Materials and Methods. One hundred twelve female Wistar rats, 5–6 weeks old averaging 164 g in weight, were used as experimental animals. The basal diet consisted of

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the following (g/kg of diet): sucrose, 610; vitamin-free casein, 240; cottonseed oil, 100; HMW salt mix, 50. Vitamins (mg/kg of diet): thiamine HCl, 20; riboflavin, 20; pyridoxine, 20; calcium pantothenate, 60; nicotinic acid, 100; ascorbic acid, 200; biotin, 4; folic acid, 10; para-aminobenzoic acid, 400; inositol, 800; 2-methyl-1,4-napthoquinone, 5; alpha-tocopherol, 100; choline chloride, 2000; vitamin B_{12}, 0.150; vitamin A, 5000 USP units; and vitamin D_2, 500 USP units. The choline chloride was incorporated into cornstarch (1 part choline chloride to 4 parts cornstarch) and added to the diet in this form. The fat soluble vitamins (except K) were dissolved in the cottonseed oil. The high vitamin D diets were formulated by adding 1,500,000 USP units of vitamin D_2 (Viosterol) per kg of diet. The hesperidin[1], rutin[2] and CSA[3] were added to the diet at a 1% level, replacing an equal amount of sucrose.

The treatments were as follows: normal control (vitamin D_2, 500 USP units), 16 rats; high vitamin D_2 (1,500,000 USP units), 24 rats; high vitamin D_2 + CSA, 24 rats; high vitamin D_2 + rutin, 24 rats; and high vitamin D_2 + hesperidin, 24 rats. The 112 rats were divided into 28 groups of 4 rats, with equal body weights among and within groups. Four rats were placed in a double open wire mesh cage. The groups of 4 were assigned randomly to the 5 treatments. Average animal weight was 164 g at the start of feeding the experimental diets. The rats' ears were notched for identification of the individual animals.

At the end of the 102 day trial, the animals were anesthetized with 0.5–1 ml of sodium pentabarbital by intraperitoneal injection. The chest cavity was opened and blood was drawn by heart puncture, using heparinized syringes. Erythrocyte sedimentation rates (ESR) and hematocrits were determined with the Wintrobe method (8).

A blood sample was centrifuged and a plasma aliquot was obtained for cholesterol analysis, using a 1:10 isopropanol extract of the plasma. The cholesterol determinations were carried out with a Technicon AutoAnalyzer using the method of Zlatkis *et al.* (9). At necropsy, the heart, abdominal aorta, and kidneys were removed and fixed in 10% neutral formalin. Five-micron sections were cut from the left ventricle, aorta, and kidneys and stained with hematoxylin and eosin.

The data were analyzed using analysis of variance for all treatments except ESR which was analyzed using a nonparametric test, (Mann-Whitney U test) (10).

Results. During the 102-day experimental period 3 rats died, one each on high D_2 + CSA, high D_2 + rutin, and high D_2 + hesperidin treatment. Average animal weight gains (g), (Table I) were as follows: normal control, 133; high vitamin D_2, 16; high D_2 + CSA, 18; high D_2 + rutin, 18 and high D_2 + hesperidin, 20. There was a significant difference ($p<0.01$) in weight gain between normal control rats and those fed diets high in vitamin D_2, but no statistically significant difference in weight gain of the rats among the 4 treatments receiving high vitamin D.

Serum cholesterol levels (Table I) were as follows (mg/100 ml): normal controls, 120; high vitamin D_2, 131; high D_2 + CSA, 137; high D_2 + rutin, 136; and high D_2 + hesperidin, 130. There was a significant rise ($p<0.01$) in serum cholesterol levels in rats fed diets high in vitamin D_2, but no significant effect of CSA, rutin, or hespiridin on serum cholesterol levels.

Hematocrits (Table I) did not differ significantly, as a consequence of dietary treatments. Controls had a packed cell volume of 48%. The high D_2- and high D_2 + hesperidin-treated animals had cell volumes of 44% and the high D_2 + CSA and high D_2 + rutin each had 47% cell volumes.

The ESR (Table I) varied from an average of 0.1 mm/hr for the normal controls to 1.7 mm/hr for the high D_2-treated rats. The ESR of the high D_2 + CSA-treated rats

[1] Hesperidin 99+% pure, supplied by J. Kesterson, Chemist, Citrus Experiment Station, Lake Alfred, Florida.

[2] Rutin obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[3] Chondroitin sulfate A supplied by The Institute for Arteriosclerosis Research, Los Angeles, California.

TABLE I. Effect of Chondroitin Sulfate A, Rutin, or Hesperidin on Weight Gain, Serum Cholesterol, Hematocrit, and Erythrocyte Sedimentation Rates of Female Rats Fed Vitamin D_2 (1,500,000 USP Units /kg of diet).

	Body wt		Cholesterol		Hematocrit		
	Start	Gain	(mg/100 ml)	SD	Cell volume (%)	SD	ESR (mm/hr)
Vit. D_2 (500)[a]	165	133	120	14.1	48	3.1	0.1
Vit. D_2 (1,500,000)[a]	168	16[b]	131[b]	5.5	44	5.0	1.7
+ CSA	164	18[b]	137[b]	21.6	47	3.6	0.2[b]
+ Rutin	165	18[b]	136[b]	20.1	47	5.1	0.3[b]
+ Hesperidin	159	20[b]	130[b]	27.9	44	3.5	0.3[b]

[a] USP units/kg of diet.

[b] Body weight and serum cholesterol significantly different ($p < 0.01$) from Vit. D_2 (500); and ESR significantly different ($p < 0.01$) from Vit. D_2 (1,500,000).

averaged 0.2 mm/hr while those of the high D_2 + rutin-, and high D_2 + hesperidin-treated rats averaged 0.3 mm/hr. Rutin, hesperidin, and CSA showed a highly significant effect ($p<0.01$) on reduction of the increased ESR due to the excessive intake of vitamin D, but showed no stastically significant effect on the other parameters measured.

The tunicae intima and media of the aorta and branches of the coronary arteries contained calcified and necrotic material (Figs. 1, 2), without evidence of intimal hyperplasia.

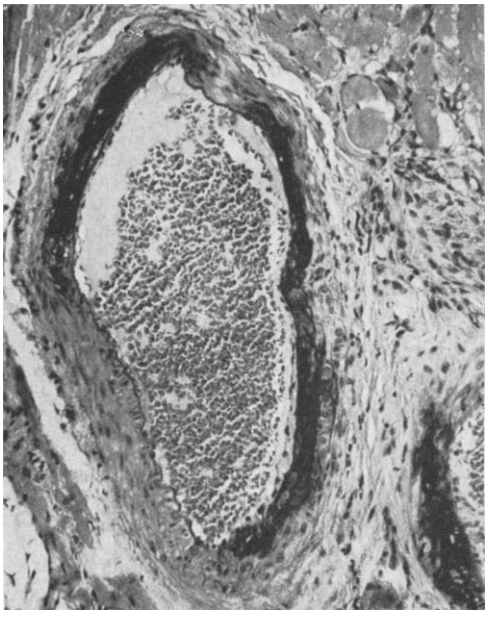

FIG. 1. Coronary artery of rat fed vitamin D and chondroitin sulfate: there is extensive calcification of the tunica media; H and E; ×65.

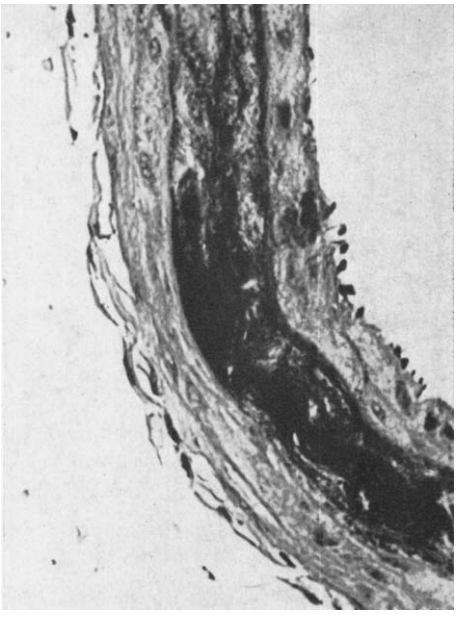

FIG. 2. Aorta of rat fed vitamin D and chondroitin sulfate: there is extensive calcification of the tunica media; H and E; ×130.

Numerous deposits of calcium were present in the kidneys; mineral was present in lumens of the distal convoluted tubules and collecting tubules (Fig. 3). Such deposits sometimes not only filled these lumens but also extended as deeply as the basement membrane of the tubules. The tunicae intima and media of branches of the renal artery often contained calcium, although frequently

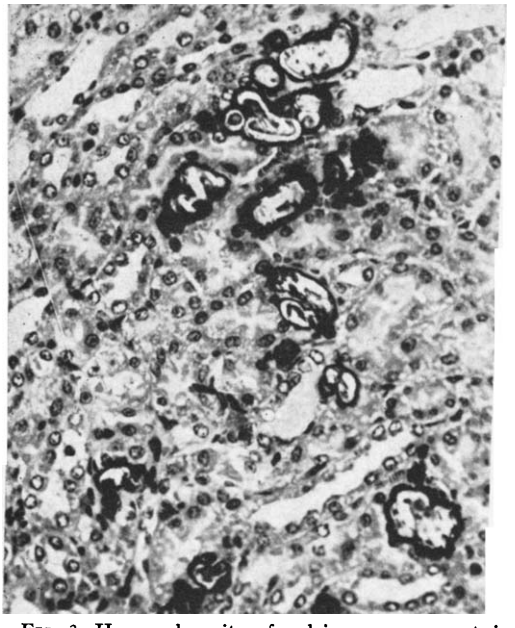

FIG. 3. Heavy deposits of calcium are present in the kidney tubules of a rat fed vitamin D and chondroitin sulfate; H and E; ×130.

only the internal elastic membrane of the arterial wall was mineralized. There were no differences attributable to CSA or flavonoids.

Discussion. The levels of vitamin D fed were highly toxic as evidenced by an average weight gain of only 18 g for the rats fed diets containing high levels of vitamin D compared to a weight gain of 133 g for the normal controls. The adverse effect of excess vitamin D on weight gain was not prevented by the CSA or flavonoids.

The high vitamin D intake was associated with an elevation of serum cholesterol levels (Table I). However, despite tissue and blood vessel degeneration (Figs. 1–3), no lipid deposition was found in the coronary arteries or the aorta of the rats fed high vitamin D. Although elevated above normal controls, blood lipid levels were apparently below the thresholds necessary for lipid deposition to occur (5). The CSA or flavonoids did not appear to alter the serum cholesterol levels or calcification of tissue.

The high vitamin D intake was associated with a significant increase in the ESR; that is, there was a decrease in the suspension stability of the blood. A decreased suspension stability is usually caused by alteration of the macromolecular composition of the blood, particularly the plasma proteins (11). The evidence that excess intake of vitamin D causes alteration of the macromolecular composition of the blood may be of some significance. Elucidation of these biochemical changes may enable development of sensitive tests to determine minimum, adequate, and excess intake levels of vitamin D for a particular subject. The use of tissue alterations as a criteria of intake is not satisfactory as irreversible changes may occur before the dietary or metabolic abnormality becomes apparent. Although both CSA and the flavonoids returned the ESR towards normal (Table I) the physiological significance is not apparent from these data.

Summary. A high vitamin D intake 1, 500,000 units/kg of diet) severely decreased weight gain, caused necrosis and calcification of blood vessels and kidneys, and decreased the suspension stability of the blood of rats. Chondroitin sulfate A and the flavonoids (rutin and hesperidin) showed no effect on weight loss, serum cholesterol, tissue calcifications, or hematocrits, but returned the suspension stability of the blood towards normal.

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