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Nutritional Factors in Experimental Atherosclerosis of the Rabbit

By G. Sala and A. di Marco

Studies carried out on experimental cholesterol atherosclerosis in the rabbit showed the presence of different degrees of aortic atherosclerotic lesions in groups of animals fed with different diets.

This paper deals with the investigation of the role of nutritional factors and with the importance of endogenous factors in the development of experimental atherosclerosis. The complete work has been published elsewhere (1).

Material and methods

The detailed methods used in the present study are described elsewhere (1, 2).

Atherosclerosis was obtained in the male rabbit after the daily oral administration of 1 g of cholesterol and 3 ml of olive oil, mixed into the diet, for 60 days.

The atherosclerotic process was evaluated both by a subjective examination of the aorta and by chemical determination of cholesterol in the ether extract of the adventitia free aorta.

Serum cholesterol, total fats, phospholipids, turbidity and lipoproteins have been determined according to the methods already described. Thyroid function has been determined by histological methods and by the thyroid uptake of a tracer dose of I^{131} .

Results

1. A significant difference of atherosclerosis is evident in groups of rabbits fed different diets (Fig. 1). The lower degree of atherosclerosis meets with a decreased body weight and a lower concentration of fats in the serum. Thyroid function is higher in animals with inhibited atherosclerosis than in controls presenting a high degree of atheromasic process (Fig. 2).

2. The crude composition of the two diets is different (Table 1), but the

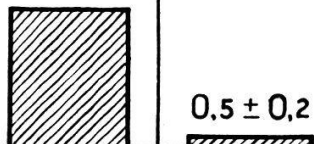
		I	II	
		Diet A	Diet B	
Serum	Fatty acids	mg %	2830 ± 433	709 ± 243
	C/P		71	45
	Phospholipids	mg %	35 ± 4.2	16 ± 2.5
	Cholesterol	mg %	2500 ± 179	721 ± 157
Body weight	Δ g	+110	-380	
Atherosclerosis Lesions +	8	8.6 ± 1.3		
	4		0.5 ± 0.2	

Fig. 1. Atherosclerotic pattern in two groups of rabbits fed different diets.

		I	II
		Diet A	Diet B
Fixation of I ¹³¹ thyroid %	5 hours	9 ± 0.8	35 ± 8
	24 hours	20 ± 2.2	32 ± 5.6
Weight of the thyroid gland	mg/kg	60 ± 23	184 ± 31
Follicle diameter	μ	50 ± 3	43 ± 4
Histological aspect		calm	activated

Fig. 2. Relationship between atherosclerosis and thyroid function.

chemical analysis does not show marked differences in carbohydrates, cellulose, proteins, fats, unsaponifiable matter and moisture content; the caloric value of the non-atherogenic diet (diet B) is eventually higher (Table 2). Diet A, however, is enriched with a salt mixture, vitamins and antibiotics.

3. When diet A is lacking in antibiotics or vitamins, the atheromasic process is not prevented; likewise the addition of single vitamins to the same diet appears to have no effect on atherosclerosis (Fig. 3).

4. In preliminary observations we could establish that rabbits fed diet B with low incidence of atherosclerosis, ingested a reduced quantity of food. Since this finding could be connected with atherosclerosis inhibition, it seemed necessary to consider separately cholesterol + olive oil and total food ingestion.

Table 1
Crude composition of diet A and B

Diet A	%	Diet B	%
Corn	26	Bran	50
Wheat meal	20	Corn	20
Alfa-alfa	22	Oats	20
Bran	30	Dry bread	10
Salt mixture*	2	Carrots	g 100
Vitamins**		Salad	g 50
Antibiotics***			

* Salt mixture = CaO = 46.8; CO₂ = 27.9; Na = 5.6; Cl = 8.7; P₂O₅ = 9.4; moisture = 1.2%.

** Vitamins = B₁ = 2810 U/kg; B₂ = 3435 γ/kg, A = 6600 U/kg; D₃ = 235 U/kg.

*** Antibiotics = Bacitracin = 1.73 γ/kg; Procaine Penicillin = 40 mg/kg.

Table 2
Chemical analysis and caloric equivalent of diet A and B

	Diet A %	Diet B %
Total carbohydrates	46	54.60
Cellulose	5.75	4.20
Proteins	15	13
Fats	3.03	4.50
Unsaponifiable matter	0.57	0.43
Total phosphorus	0.60	0.80
Ash	5.78	4
Moisture	12.60	11.80
Caloric equivalent	3800 Cal/kg	4130 Cal/kg

a) If groups of rabbits are obliged to eat amounts of cholesterol + olive oil equivalent to those of controls but still reduced quantity of food, an inhibition of atherosclerosis occurs, in spite of serum cholesterol and phospholipids being increased (Fig. 4).

b) An equivalent intake of food and of cholesterol + olive oil was obtained in both groups after the addition of a salt mixture to the non-atherogenic diet (diet B): finally the gravity and the incidence of atherosclerosis was the same in the groups fed different diets (Fig. 5).

Comment

From these results, it appears that atherosclerosis is inhibited in rabbits which were fed a lower amount of food and which consequently received a reduced caloric intake. These animals also showed an increased thyroid function, whose origin is still difficult to establish.

Diet A		I Com- plete	II -Vit. -Salts -Antib.	III +B ₁ B ₂ +A.D	IV +D	V +A
Serum						
Turbidity	T%	18,7	23,4	15,6	15,6	27,5
C/P		79	68	71	72	79
Phospholipids	mg%	33,3	35,7	31,7	37,9	25,2
Cholesterol	mg%	2613	2417	2347	2660	2018
Body weight	Δg	+270	+402	+167	+328	+520
Atherosclerosis						
Lesions +		7,3	9,1	8	5,8	8,9

Fig. 3. Vitamins and antibiotics do not modify the atherogenic effect of diet A.

		I Diet A	II Diet B
Food	g/24 h	80	40
Cholesterol	g/24 h	0,8	1
Olive oil	cc/24 h	2,4	3
Serum			
Turbidity	T%	18,7	29,7
C/P		79	59
Phospholipids	mg%	33,3	39,6
Cholesterol	mg%	2613	2407
Body weight	Δg	+ 270	- 50
Atherosclerosis			
Cholesterol		4 ± 0,6	1,8 ± 0,4
Aorta g %			

Fig. 4. Increased ingestion of cholesterol + olive oil does not favour atherosclerosis, when the food intake is low.

		I Diet A	II Diet B + Salts
Food	g/24 h	80	80
Cholesterol	g/24 h	0,8	0,8
Olive oil	cc/24 h	2,4	2,4
Serum			
Turbidity	T%	18,7	46,7
C/P		79	55
Phospholipids	mg%	33,3	24,7
Cholesterol	mg%	2613	1320
Body weight	Δg	+ 270	+ 320
Atherosclerosis			
Cholesterol		4 ± 0,6	4,6 ± 0,8
Aorta g %			

Fig. 5. High food intake is necessary for the development of atherosclerosis.

The increase of caloric intake seems to be responsible for the development of atherosclerosis, while the increased cholesterol + olive oil ingestion was not able per se, in absence of enough food intake, to favour the atherosclerotic process, in spite of the increased levels of serum fats.

In conclusion, these findings suggest that cholesterol experimental atherosclerosis in the rabbit depends both upon the exogenous cholesterol ingestion and upon metabolic and endogenous factors: prominent among them are the total caloric intake and thyroid function.

Summary

From the study of experimental atherosclerosis in groups of rabbits fed on different diets, the authors think that cholesterol atherosclerosis is determined both by exogenous cholesterol intake and by nutritional factors: low total caloric intake and increased thyroid function may inhibit the atheromasic process in the rabbit, even in presence of a high cholesterol intake.

Zusammenfassung

Auf Grund von Versuchen mit Kaninchen, bei denen durch verschiedenartige Fütterung eine experimentelle Atherosklerose erzeugt worden war, glauben die Verfasser, daß die Cholesterinatherosklerose sowohl durch exogene Cholesterinaufnahme als auch durch nahrungsbedingte Faktoren verursacht wird. Niedrige Kalorienzufuhr und steigende Schilddrüsenfunktion können den atheromatösen Prozeß beim Kaninchen hemmen, selbst bei gleichzeitiger reichlicher Cholesterinzufuhr.

Résumé

Se fondant sur une étude sur l'athérosclérose expérimentale chez des lapins soumis à des régimes différents, les auteurs pensent que l'athérosclérose cholestérolique dépend, d'une part, de l'apport exogène du cholestérol, d'autre part, de facteurs nutritifs; une ration alimentaire hypocalorique associée à une hyperfonction thyroïdienne peut inhiber l'athérosclérose, même si l'alimentation est riche en cholestérol.

1. Sala, G., and di Marco, A.: Atherosclerosi umana e sperimentale. CEA, Milano 1956, p. 189. – 2. Sala, G.: Minerva med. (Torino) 2, 251 (1956).