

Dietary Restriction and Regression of Atherosclerosis

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Summary. Rabbits were fed a cholesterol-enriched diet to render them atheromatous. After 3 months on this diet they were switched to a low-lipid stock diet. Some animals were killed at this point, while the rest were divided into (a) a group allowed to eat ad-libitum and (b) a restricted group allowed to eat half by weight of what the ad-libitum group consumed. Most animals were killed at 9 months (i.e. after 6 months' regression). The group allowed the restricted diet showed a 27% weight loss, but their serum cholesterol fell slightly more slowly than that of the ad-libitum animals. Likewise, atherosclerosis was slightly worse in the restricted than in the ad-libitum group. The results do not support the view that severe dietary restriction causes atherosclerosis to regress.

Introduction

Sporadic comments in the literature, particularly after the experiences of World War II, suggest that severe dietary restriction might have some beneficial effect on atherosclerosis (Katz and Pick, 1963; De Navasquez, 1964; Helwig-Larsen et al., 1952). Induced loss of weight before feeding cholesterol to the rabbit is reported to result in a lesser degree of atheroma (Firstbrook, 1950). This circumstantial and experimental evidence led us to see what effect calorie restriction would have on established atheroma in the rabbit.

Methods

Thirty-four New Zealand White male rabbits (~2.5 kg) were fed a stock cube diet (SG1) enriched with 1% cholesterol for 12 weeks (see Adams et al., 1973). Ten animals were baseline controls killed at the end of cholesterol feeding, while the other 24 were pair-matched for serum cholesterol levels and assigned to ad-libitum and restricted dietary groups. The restricted group, after a trial period, were fed approximately half by weight of that eaten by the ad-libitum group. Both groups received vitamin supplements of brewer's yeast (BPC) and Vionate (Squibb). The protein and mineral intake in the restricted group was adequate according to published tables (Short and Gammage, 1959; Altman and Dittmer, 1968).

Animals were weighed weekly and their serum cholesterol levels were estimated monthly. Some animals were killed at 3 months and others at 6 months of "regression" (i.e. after the cholesterol-enriched diet had been stopped). Aortas were removed in their entirety and their degree of atherosclerosis was estimated with a graticule, prepared by photographing suitable graph paper on glass plate (Morgan and Adams, 1974). The tissue was then sampled from the descending thoracic aorta and from the aortic arch as described previously (Adams et al., 1973). The samples were homogenized and their cholesterol contents determined as set out previously (Leffler and McDougald, 1963).

Results

Figure 1 shows that the ad-libitum group gained weight by a mean 26% at 6 months after stopping the cholesterol-enriched diet, whereas the restricted

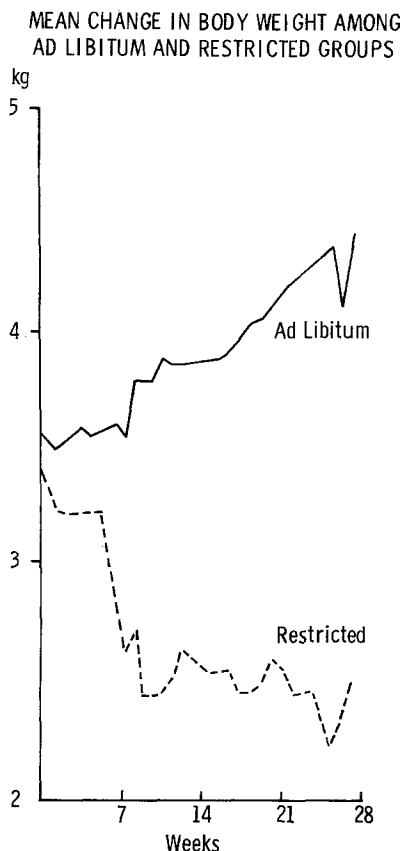


Fig. 1. Weight changes in rabbits fed a cholesterol-enriched diet and then transferred to ad-libitum and restricted low-lipid diets

group lost 27% over this period. Five animals died within a few weeks after stopping the cholesterol-enriched diet: two with otitis media and meningitis, two with biliary cirrhosis and one with undiagnosed jaundice.

Figure 2 shows the fall in blood cholesterol levels after stopping the cholesterol-enriched diet. From 8 weeks onwards the cholesterol level fell more slowly in the restricted group.

At 3 months three restricted animals and six ad-libitum animals were killed. The descending thoracic aorta in the restricted animals showed less atheroma as adjudged by cholesterol content (Table 1), histology and by graticule count of the gross specimen (94.7% area involvement versus 65.3%; $p \leq 0.03$; Table 2). The aortic arch, in the restricted animals, showed no improvement at this time and was in fact significantly worse (Table 1). This improvement in the descending thoracic aorta only and in such a small group seems unreliable evidence of regression. Moreover, if the cholesterol contents of arch and descending thoracic aorta were summated, then there was no improvement at 3 months.

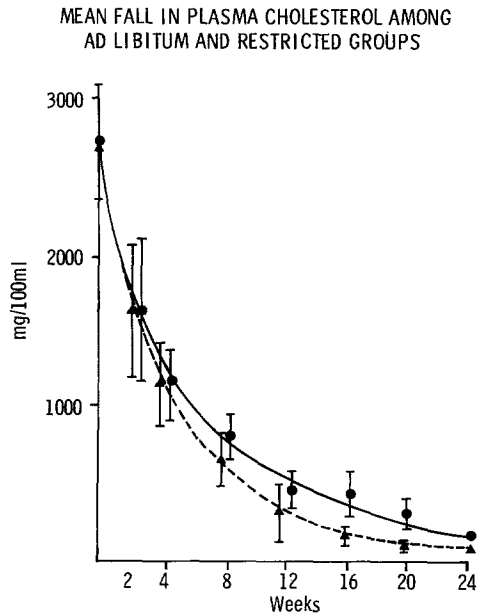


Fig. 2. Plasma cholesterol levels in rabbits on ad-libitum (▲) and restricted (●) diets

Table 1. Total aortic cholesterol among Dietary groups

	End of 1% cholesterol diet	Ad-libitum		Restricted	
		3 mo	6 mo	3 mo	6 mo
Number of animals	10	6	5	3	5
Thoracic (mg/g)	52.37	54.59	24.16 ^{a,d}	27.88 ^e	32.88 ^j
Arch (mg/g)	43.27	44.81	25.14 ^{b,e}	62.13 ^h	41.21 ^k
Thoracic (mg/5 cm*)	15.84	25.92	15.71 ^{c,f}	7.19 ⁱ	19.04 ^l
Thoracic and arch (% as ester)	64%	54%	38%	53%	40%

* The proximal 5 cm of the descending aorta (reference to length excludes possible error due to change in arterial weight during progression or regression of atherosclerosis)

Statistical analyses by courtesy of Dr. R. W. R. Baker:

6 mo ad-libitum vs. end of diet; $P \leq 0.002$ (a), 0.05 (b), NS (c) \bar{P} .

6 mo ad-libitum vs. 3 mo ad-libitum; $P \leq 0.001$ (d), 0.001 (e), 0.090 (f)

3 mo restricted vs. 3 mo ad-libitum; $P \leq 0.016$ (g), 0.0007 (h), 0.25 (i)

6 mo restricted vs. 6 mo ad-libitum; $P \leq 0.10$ (j), 0.006 (k), 0.20 (l)

The animals killed at 6 months of restricted diet showed no resorption in either arch or descending thoracic aorta, as adjudged by cholesterol content (Table 1,) by histology and by graticule count (94.2% area involvement versus 88.2% ; NS; Table 2. Aortic cholesterol retention was, in fact, more severe in the restricted than in the ad-libitum group, and in the arch this reached statistical significance (Table 1).

Table 2. Severity of aortic atheroma among dietary groups

	End of 1% cholesterol diet	Ad-libitum		Restricted	
		3 mo	6 mo	3 mo	6 mo
Graticule	91.9	94.7	94.2	65.3	88.2
Count*	<i>n</i> = 10	<i>n</i> = 6	<i>n</i> = 5	<i>n</i> = 3	<i>n</i> = 5

* % of squares involved in ascending aorta + arch + proximal 5 cm of descending thoracic aorta

Statistical Analyses:

3 mo restricted vs. 3 mo ad-libitum; $P \leq 0.03$

6 mo restricted vs. 6 mo ad-libitum; NS

3 and 6 mo ad-libitum vs. end of diet; NS

Comment

The somewhat increased atherosclerosis in the rabbit after 6 months' dietary restriction does not accord with reports on man from Leningrad (Katz and Pick, 1963), and from Belsen (De Navasquez, 1964) and Norwegian (Helwig-Larsen et al., 1952) concentration camps during or after World War II. However, post-mortem material obtained from Belsen at the end of the war may have been largely from those younger subjects who managed to survive that length of time. Of five hearts from Belsen prisoners in the museum at the Royal College of Surgeons, London (examined by courtesy of the Curator, Dr. Martin, Israel), only one is from a subject older than the third decade. This subject, a woman of 35 has two small yellow fibro-fatty plaques in her ascending aorta. Perhaps more to the point is the report based on 10,000 autopsies at Dachau of severe atherosclerosis in emaciated prisoners (Blaga, 1963). It could be argued that, if dietary restriction was sufficiently severe, muscle and other tissue would be resorbed and become wasted. Cholesterol would then be released from cell-membrane, would accumulate in the blood and might be expected to enhance atherosclerosis.

Chronic rabbit atherosclerosis, such as that encountered here at 9 months, resembles the uncomplicated fibro-fatty human disease (see Constantinides, 1965). These results in the rabbit may, therefore, have some relevance at the tissue level to the human disease. The findings reported here suggest, at least in the rabbit, that moderately severe dietary (calorie) restriction may actually exacerbate atherosclerosis, but this does not reflect—one way or the other—on the possible beneficial effect of lesser degrees of dietary restriction and controlled weight-loss.

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