

Original Research

Cholesterol Vehicle in Experimental Atherosclerosis 24: Avocado Oil

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Key words: atherosclerosis, avocado oil, cholesterol, coconut oil, corn oil, olive oil, rabbits

Objective: To determine atherogenicity of avocado oil relative to saturated (coconut oil), monounsaturated (olive oil) and polyunsaturated (corn oil) fats.

Methods: New Zealand White rabbits were fed a semipurified diet containing 0.2% cholesterol and 14% fat for 90 days. They were then necropsied and severity of atherosclerosis was determined visually.

Results: Coconut oil was the most atherogenic fat. Corn oil was only slightly less atherogenic than either olive or avocado oils. Percentage of serum HDL cholesterol was highest in the rabbits fed the two monounsaturated fats.

Conclusion: Avocado oil is of the same order of atherogenicity as corn oil and olive oil.

INTRODUCTION

In our initial studies [1,2], we demonstrated that saturated fat (coconut oil) was more atherogenic for rabbits than unsaturated fat (corn oil). Subsequent studies showed that a monounsaturated fat (olive oil) was only slightly more atherogenic than corn oil in rabbits fed cholesterol-rich [3] or cholesterol-free [4] diets. Avocado oil is a monounsaturated oil similar to olive oil (both contain high levels of oleic acid), and it was deemed of interest to determine if avocado oil exerted an effect on experimental atherosclerosis similar to that of olive oil. We also compared the atherogenic effect of avocado oil with those of a saturated fat (coconut oil) and a polyunsaturated fat (corn oil).

MATERIALS AND METHODS

Male rabbits of the New Zealand White strain were randomized into four groups of seven rabbits each. The average starting weight of the four groups was similar (2610 ± 1 gm). The rabbits were fed an atherogenic diet containing 0.2% cholesterol (Table 1) for 90 days. The corn oil diet contained

14% of fat. The others contained 13% of the test fat plus 1% corn oil to insure against essential fatty acid deficiency. The fatty acid composition of the test fats is given in Table 2. The animals were maintained individually in stainless steel cages in an air-conditioned, humidified room on a 12 hour light/dark cycle. Food and water were provided *ad libitum*. The diets were stored under refrigeration. Portions taken to provide weekly feeding were kept in an air conditioned room. The diets were prepared to our specifications and pelleted by Dyets, Inc. (Bethlehem, PA). After 90 days the rabbits were bled under deep sedation and necropsied. Serum total and HDL-cholesterol and triglycerides were determined using appropriate kits (Sigma, St. Louis, MO). Livers were weighed, and one gram aliquots were extracted with chloroform-methanol 2:1 [5]. The extracts were used for analysis of total and free cholesterol. Aortas were removed, cleaned and severity of lesions graded visually on a 0–4 scale [6]. When visual grading was compared with newer morphometric methods under double blind conditions we observed excellent correlation ($r > 0.90$) (D. Kritchevsky and D. M. Klurfeld, unpublished observation). The aortas were also subjected to chloroform-methanol (2:1) extraction. The lipid extracts of liver and aorta were taken to dryness under nitrogen and the residual lipid solubilized by

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Table 1. Atherogenic diet

Ingredient	%	% Calories
Casein	25.00	25.8
DL-Methionine	0.20	
Sucrose	20.48	21.1
Starch	20.00	20.6
Test fat ^a	13.00	30.2
Corn oil	1.00	2.3
Cellulose	15.00	
Mineral mix	4.00	
Vitamin mix	1.00	
Choline bitartrate	0.12	
Cholesterol	0.20	

^a Corn, coconut, olive or avocado oil.

emulsification with an appropriate surface active agent [7]. In this case 1% Triton in chloroform was used. The Triton-lipid solution was taken to dryness under nitrogen and reconstituted in 200 μ L of distilled water. Aliquots (25 μ L) of the lipid solution were analyzed enzymatically in triplicate for total and free cholesterol (Wako Chemical, Richmond, VA). Ester cholesterol concentrations were taken as the difference between the total and free cholesterol levels obtained by analysis. This enzymatic assay has been used for analysis of tissue lipids in hamsters [8]. All experimental procedures were approved by the Wistar Institutional Animal Care Use Committee (IACUC).

RESULTS

The necropsy data are summarized in Table 3. Average weight gain among the four groups was 215 ± 44 g; rabbits fed coconut oil for 90 days showed the lowest weight gain, but none of the differences were significant. Liver weight ranged from 56.6 g (corn oil) to 75.7 g (olive oil), weights for the other two groups being intermediate. Relative liver weight (g/100 g body wt) was significantly lower in the rabbits fed corn oil than in the other three groups. However, the concentration of cholesterol was significantly higher in the corn oil group. Total

Table 2. Fatty Acid Composition (%) of Vegetable Oils

Fatty Acid	Corn	Coconut	Olive	Avocado
8:0	—	1.8	—	—
10:0	—	5.6	—	0.2
12:0	—	51.8	—	1.8
14:0	—	20.6	—	0.8
16:0	10.8	9.3	13.7	15.8
16:1	0.1	—	1.5	4.9
18:0	1.6	2.7	1.9	0.4
18:1c	25.1	6.2	68.2	53.7
18:1t	0.7	0.1	2.4	5.9
18:2	59.8	1.8	10.9	15.4
18:3	1.3	0.1	0.7	1.1
Other	1.3	—	3.1	—
Iodine value (calc.)	135	9	86	89

liver cholesterol levels (g/liver) were 13.9 in rabbits fed corn oil and 8.8, 12.5 and 11.3 in rabbits fed coconut, olive or avocado oils, respectively. The percentage of liver cholesterol ester (an indicator of cholesterol deposition) was in the same range for all groups ($64.6 \pm 1.7\%$) but was highest in animals fed corn oil.

Serum cholesterol levels were significantly higher in the rabbits fed coconut oil than in any of the other groups. High density lipoprotein cholesterol (HDL-C) was significantly lowest in the coconut oil fed group. The two groups fed the primarily monounsaturated fats exhibited higher levels of serum HDL-C than did the rabbits fed corn oil. Triglyceride levels in all four experimental groups were similar.

The atherogenic effects of the various oils are shown in Table 4. Consistent with previous findings rabbits fed coconut oil exhibited the most severe atherosclerosis in both the aortic arch and thoracic aorta. Severity of lesions in the olive oil-fed group was similar to that seen in the corn oil-fed group. The atheromata observed in the rabbits fed avocado oil were somewhat less severe than those seen in the corn oil-fed or olive oil-fed groups, but the differences were not significant.

The amount of ester cholesterol in the artery increased with age and with increasing severity of atherosclerosis [9–11]. Thus, a low free to esterified cholesterol ratio is indicative of increased atherosclerosis, and, conversely, the free/ester cholesterol ratio is inversely correlated with severity of atherosclerotic lesions. The aortic free/ester cholesterol ratio was lowest in the aortas of the rabbits fed coconut oil, indicating increased ester deposition, and highest in the aortas of the corn oil fed group. The difference among groups was significant ($p < 0.007$).

DISCUSSION

The literature related to atherosclerosis has emphasized the beneficial effects of polyunsaturated fat (PUFA) and decried the deleterious influence of saturated fat. Until relatively recently, monounsaturated fats (MUFA) were regarded as neutral; however, demonstration that these fats lower total serum cholesterol without also reducing the HDL-cholesterol levels [12] and their prominent role in the “Mediterranean” diet have focused interest upon their dietary effects. Studies in experimental atherosclerosis have followed the same pattern, with emphasis being placed on saturated or polyunsaturated fats. The study reported here confirms our earlier observations that olive oil is of the same order of atherogenicity as corn oil and demonstrates that another monounsaturated fat, namely, avocado oil behaves similarly. Both olive and avocado oils contain other substances with possible anti-atherogenic potential (phenolic antioxidants in olive oil and phytosterols and carotenoids in avocado oil), but not enough to have had a serious effect on the outcome of this study. Peanut oil is a monounsaturated fat that has demonstrated atherogenic properties [13–15], but the

Table 3. Necropsy Results in Rabbits Fed 14% Fat and 0.2% Cholesterol for 90 Days

	Group				ANOVA <i>p</i> <
	Corn Oil	Coconut Oil	Olive Oil	Avocado Oil	
Number	7	7	7	7	—
Weight gain (g)	229 ± 149	102 ± 94	316 ± 82	213 ± 57	NS
Liver wt. (g)	56.6 ± 4.37	64.0 ± 3.75	75.7 ± 4.75	66.2 ± 3.99	NS
Relative liver wt. (%)	1.98 ± 0.07	2.34 ± 0.08	2.58 ± 0.10	2.34 ± 0.09	NS
Serum lipids (mg/mL)					
Cholesterol (C)	247 ± 30	579 ± 23	232 ± 18	384 ± 69	0.0001
% HDL-C	16 ± 1.5	8 ± 0.9	28 ± 2.7	21 ± 4.2	0.0001
Triglycerides	54 ± 5	50 ± 10	44 ± 3	70 ± 13	NS
Liver cholesterol (mg/g)					
Total	246 ± 17	138 ± 9	165 ± 14	171 ± 11	NS
Free	77 ± 9	47 ± 2	65 ± 9	63 ± 5	NS
Ester	169 ± 8	91 ± 8	100 ± 7	108 ± 3	NS
% Ester	68.7 ± 1.76	65.9 ± 1.15	60.6 ± 2.78	63.2 ± 1.28	NS

NS = not statistically significant.

lesions produced are more fibrous than fatty and may be due to the high lectin content of the oil [16].

There is still no consensus regarding the relative lipidemic and atherogenic roles of monounsaturated and polyunsaturated fats. This may be due, in part, to the fact that results have been obtained using different subjects (humans, monkeys, rabbits, transgenic mice) and may not be justifiably comparable. In humans, Mattson and Grundy [12] showed that dietary PUFA reduced serum levels of all lipoproteins, whereas MUFA exerted their major effect only on the LDL fraction. Mensink and Katan [17] and Kris-Etherton *et al.* [18] made similar observations. It should be noted, however, that a meta analysis by Gardner and Kraemer [19] suggests that MUFA and PUFA exert similar effects on human plasma lipoproteins. Rudd *et al.* [20] and Merkel *et al.* [21] have reported that MUFA promote atherosclerosis in LDL-receptor-null, human ApoB-100-over-expressing and LDL receptor deficient mice, respectively. Rudel *et al.* [22] have also reported that, compared to MUFA, PUFA protects African Green monkeys from coronary artery atherosclerosis. On the other hand, Toborek *et al.* [23] have shown that PUFA induce inflammatory processes in human

endothelial cells *in vitro* and Nicolosi *et al.* [24] have demonstrated that, compared with MUFA, PUFA enhance LDL oxidation and increase aortic fatty streak formation in cholesterol-fed hamsters.

Is there really a dichotomy? It would appear that MUFA are protective in the early stages of atherogenesis and PUFA in the later stages. Aguilera *et al.* [25] studied progression of atherosclerosis in atherosclerotic rabbits fed sunflower, olive or fish oils. They were essentially studying effects of fats on formation of new atherosclerotic lesions. Sunflower oil feeding significantly increased the severity of lesions in the aortic arch, thoracic aorta and abdominal aorta by 28%, 28% and 22%, respectively. Fish oil increased severity of lesions at the three sites by 15%, 3% and 15%, respectively. In contrast, olive oil decreased severity of lesions in the aortic arch and in the thoracic aorta by 10%, while increasing severity of lesions in the abdominal aorta by 15%.

Rudel *et al.* [22] pointed out that arterial cholesteryl ester, particularly cholesteryl oleate, was higher in monkeys fed MUFA compared to those fed PUFA. Oleic acid is the preferred substrate for cholesteryl ester formation [11,26,27]. Increasing cholesteryl ester content of aortic tissue with age and

Table 4. Severity of Atherosclerosis (0–4 Visual Scale) in Rabbits Fed 14% Fat and 0.2% Cholesterol for 90 Days

	Group				ANOVA <i>p</i> <
	Corn Oil	Coconut Oil	Olive Oil	Avocado Oil	
Number	7	7	7	7	
Visual grade					
Aortic arch	0.79 ± 0.22	1.57 ± 0.20	0.75 ± 0.033	0.50 ± 0.10	0.02
Thoracic aorta	0.36 ± 0.17	0.86 ± 0.14	0.43 ± 0.19	0.29 ± 0.14	NS
Aortic Cholesterol (mg/g)					
Total	72.8 ± 7.7	77.1 ± 15.6	97.2 ± 9.1	86.5 ± 9.6	NS
Free	53.5 ± 2.9	45.8 ± 9.0	60.4 ± 7.5	56.5 ± 4.5	NS
Ester	19.3 ± 5.1	31.3 ± 7.1	36.7 ± 4.1	30.0 ± 7.3	NS
% Ester	23.9 ± 4.7	39.0 ± 5.1	38.5 ± 3.7	32.8 ± 4.6	NS
Free/Ester	5.70 ± 1.47	1.61 ± 0.32	1.74 ± 0.27	2.60 ± 0.68	0.007

NS = not statistically significant.

atherogenesis is well known [10]. The differences in effects of MUFA and PUFA on atherogenesis may be a function of which end of the atherogenic process is being studied. MUFA may be more protective in early stages which involve inflammatory processes and LDL oxidation and PUFA more effective in later stages when they do not provide aortic cholesterol with its preferred substrate for esterification.

ACKNOWLEDGMENT

This work was supported, in part, by a grant from the California Avocado Commission, Santa Ana CA, and by a Research Career Award (HL00734) from the National Institutes of Health.

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Received March 27, 2002; revision accepted August 16, 2002.