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Accelerated atherosclerosis in apolipoprotein E-deficient mice fed Western diets containing palm oil compared with extra virgin olive oils: A role for small, dense high-density lipoproteins

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Abstract

To test the hypothesis that extra virgin olive oils from different cultivars added to Western diets might behave differently than palm oil in the development of atherosclerosis, apoE-deficient mice were fed diets containing different cultivars of olive oil for 10 weeks. Female mice were assigned randomly to one of the following five groups: (1–4) fed chow diets supplemented with 0.15% (w/w) cholesterol and 20% (w/w) extra virgin olive oil from the Arbequina, Picual, Cornicabra, or Empeltre cultivars, and (5) fed a chow diet supplemented with 0.15% cholesterol and 20% palm oil. Compared to diets containing palm oil, a Western diet supplemented with one of several varieties of extra virgin olive oil decreased atherosclerosis lesions, reduced plaque size, and decreased macrophage recruitment. Unexpectedly, total plasma paraoxonase activity, apoA-I, plasma triglycerides, and cholesterol played minor roles in the regulation of differential aortic lesion development. Extra virgin olive oil induced a cholesterol-poor, apoA-IV-enriched lipoparticle that has enhanced arylesterase and antioxidant activities, which is closely associated with reductions in atherosclerotic lesions. Given the anti-atherogenic properties of extra virgin olive oil evident in animal models fed a Western diet, clinical trials are needed to establish whether these oils are a safe and effective means of treating atherosclerosis.

Keywords: Olive oil; Apolipoprotein; Mice; Atherosclerosis; Paraoxonase

1. Introduction

Atherosclerosis is a disease that has a multi-faceted aetiology, and diet is one of the most important environmental factors influencing the progression of the disease. Adherence to a Mediterranean diet is associated with reduced rates of coronary disease, diabetes, and cancer [1–3], and a reduction in all-cause of mortality [4–5]. The Mediterranean diet, which has been used by populations in the Mediterranean Basin for more than 2000 year, is rich in cereals, vegetables, fruits, and legumes, and low in cholesterol and saturated fatty acids, and the major source of fat is virgin olive oil. Unlike other oils that

Abbreviations: Apo, apolipoprotein; CAD, coronary artery disease; DCF, dichlorofluorescein; EVOO, extra virgin olive oil; FPLC, fast performance liquid chromatography; HDL, high density lipoproteins; HDL-C, high density lipoprotein cholesterol; LDL, low density lipoproteins; MCP-1, monocyte chemoattractant protein-1; MUFA, monounsaturated fatty acids; PON-1, paraoxonase 1; TC, total cholesterol; TG, triglycerides; VLDL, very low density lipoproteins

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have a fatty acid composition similar to olive oil, which have to be washed and refined before consumption, virgin olive oil, especially, the first-press "extra virgin" olive oil (EVOO) is a natural juice; therefore, it retains important minor compounds that have anti-atherosclerotic properties [6]. Considerable attention is being paid to the potential health benefits of olive oil. Human consumption of olive oil decreases the major risk factors of atherosclerosis by improving the lipoprotein profile, blood pressure, glucose metabolism, and oxidative stress (reviewed in ref. [7]). Yet, the effects of olive oil on the anti-atherogenic properties of high-density lipoproteins remain controversial because studies have shown no effects [8–9], augmentation [10], and a reduction [11] in HDL-C after supplementation with olive oil. The apparent conflict might exist because these particles are a heterogeneous class of lipoproteins [12]. Not all HDL particles have the same biological properties, and there are subtypes that more efficiently promote cholesterol efflux from vascular cells, as well as anti-inflammatory, pro-fibrinolytic and antioxidant activities [13]. Those anti-atherogenic particles mainly consist of the antioxidant enzyme paraoxonase [14] and the apolipoproteins A-I and A-IV [13–16].

To better understand the beneficial anti-atherogenic properties and mechanisms of EVOO, the interactions between its components and diet should be examined using experimental models. Previously, we showed that EVOO in a low cholesterol diet decreased atherosclerosis in female apoE-deficient mice [17], and similar results were observed in rabbits [18]. We demonstrated how dietary cholesterol suppressed the beneficial effects of EVOO on mice [19], and others have shown that common olive oil (which lacks minor compounds) loses its anti-atherosclerotic effects when compared with saturated fat when dietary cholesterol (0.2%) was present [20]. While it seems clear that high cholesterol intake impairs the atheroprotective capabilities of EVOO, the potential differing effects of EVOO and saturated fat in the presence of dietary cholesterol and their effects on antioxidant HDL proteins need to be addressed. To that end, we examined the effects of a Western diet (high fat, high cholesterol) enriched with an EVOO or palm oil on serum lipids, HDL-borne antioxidant proteins, and the development of atherosclerosis in apoE-deficient mice. EVOO from different cultivars have subtle differences in fatty acids and minor compounds; therefore, we tested the effects of four cultivars. We used apoE-deficient mice, a well-characterized model that spontaneously develops atherosclerosis that has features similar to those observed in humans and is widely used to study the effect of diets on lipid metabolism [21].

2. Methods

2.1. Animals, dietary intervention, and procedures

Seventy female apoE-deficient mice were used. The animals were bred in the *Unidad Mixta de Investigación*, Zaragoza (Spain), housed in sterile filter-top cages under a 12-h light/12-dark cycle, and given ad libitum access to food and water. All experimental groups of mice had similar baseline plasma cholesterol concentrations.

In the first experiment, 54 mice were randomly assigned to one of five groups and, for 10 week, fed a diet of standard mouse chow diet (B&K Universal Ltd., Humberside, UK) supplemented with 0.15% (w/w) of cholesterol and 20% (w/w) of fat. In four of the groups, the fat source was an extra virgin olive oil from one of the following four Spanish cultivars: Arbequina, Picual, Cornicabra, and Empeltre. In the fifth group, the fat source was palm oil. In the second experiment, 16 mice were assigned to one of two groups and, for four weeks, fed the standard chow-based diet with 0.15% (w/w) of cholesterol and enriched with 20% of either EVOO from the Picual cultivar or palm oil. Blood samples were collected from the tail weekly while the mice were under isofluorane anaesthesia. The Ethics Committee for Animal Research of the University of Zaragoza approved the experimental protocols.

The diets were prepared weekly, stored in N_2 at $-20\,^{\circ}C$, and their composition analyzed (Table 1, see also [17]). At the end of the experiments, the animals were sacrificed by

Table 1
Chemical composition of experimental diets fed to five groups of apoE-deficient mice

	Extra virgin olive oil varieties				Palm oil
	Arbequina	Picual	Cornicabra	Empeltre	
Energetic content (kJ/g)	18	18	18	18	18
Carbohydrate	46	46	46	46	46
Protein	13	13	13	13	13
Fat	22	22	22	22	22
Vitamin E (UI %)	21	24	23	22	21
Cholesterol (mg %)	175	175	175	175	175
Fatty acids					
C16:0	13.14	11.39	8.58	11.68	39.72
C16:1	1.16	0.91	0.67	0.90	0.17
C17:0	0.14	0.05	0.08	0.09	0.09
C17:1	0.22	0.09	0.12	0.2	0.03
C18:0	2.11	2.93	3.06	1.81	4.96
C18:1	69.45	77.26	78.42	73.69	38.88
C18:2	12.11	5.82	7.44	9.98	14.86
C18:3	0.71	0.72	0.64	0.76	0.56
C20:0	0.38	0.36	0.42	0.31	0.33
C20:1	0.35	0.28	0.36	0.4	0.17
C22:0	0.13	0.10	0.12	0.11	0.09
C24:0	0.06	0.62	0.08	0.06	0.13
Monounsaturated	71.18	78.54	79.57	75.19	39.25
Polyunsaturated	12.82	6.54	8.08	10.74	15.42
Saturated	15.96	15.45	12.34	14.06	45.32
P/S ratio	0.80	0.42	0.65	0.76	0.34
Total polyphenols (mg/kg)	25	90	15	45	2

Dietary components are expressed as g% (w/w). Other components of the chow diet include crude fibre 4.5% and minerals 6.8%. A total dry matter of 87.5%. P/S ratio: polyunsaturated/saturated fatty acid ratio.