The effects of olive oil and Cholesterol enriched diet on aortic fatty streak development and lipid peroxidation in Rabbits

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Abstract

Aim: High plasma cholesterol levels, mainly LDL is a widely recognized major risk factor for Coronary Heart Disease(CHD). According to the epidemiologic studies findings, people from the Mediterranean countries, have lower CHD rates than other countries, in these countries the usual diet is high in olive oil. The present study compares the effects of cholesterol-enriched diet with or without adding olive oil on serum Lipoproteins, lipid peroxidation, and atherosclerosis development. Methods: Twenty Dutch male rabbits were categorized into four groups (one group as Control, and others as Experimental). They received one of Standard (I), Olive oil rich (II), Cholesterol – rich (III), and Cholesterol + Olive oil (IV) diet for Twelve weeks. Fasting blood samples from the heart were collected at the beginning, and the end of the experimental period. Results: Means of serum lipids were not significantly different at the beginning of the experimental period. After intervention, significant differences were showen in total cholesterol, HDL-C, triglyceride and MDA between groups. The comparison of III and the IV groups showed a higher mean of MDA in group III ($P< 0.001$). Any aortic lesion was not observed at I and II groups. Aortic lesions in IV group showed a significantly lower degree than group III ($p=0.02$). Conclusions: These findings showed the preventive effect of olive oil against atherosclerosis development which is independent of plasma lipoprotein effect, and suggested that probably olive oil acts on arteries directly.

What's already known about this topic?

* Some previous studies showed the beneficial effects of olive oil.

* Studies regarding the effect of olive oil on atherosclerosis development in rabbits are limited.

What does this article add?

- This study is the first investigation that was designed to evaluate the effect of olive oil on serum lipoproteins, lipid peroxidation, and atherosclerosis development in rabbits.
- The present study indicated the preventive effect of olive oil against atherosclerosis development which is independent of plasma lipoprotein effect, and suggested that probably olive oil acts on arteries directly.

1. Introduction

Atherosclerosis is the result of dyslipidemia and lipid oxidation, and the main causes of worldwide deaths are directly related to atherosclerosis [1, 2]. Dietary fat composition plays an important role in the development of cardiovascular disease (CVD)[3].

Epidemiological surveys showed low cholesterol levels and low prevalence of CHD in a population consuming a diet low in total fat, saturated fatty acids and cholesterol in the Mediterranean countries, despite of high average fat intake (40% of total calorie), CHD rates and plasma cholesterol levels are relatively low[4]. In

these countries the usual diet is high in olive oil, which is rich in monounsaturated fat content [5, 6]. For the same total cholesterol, the death rate for CHD is lower in the Mediterranean than other countries[7], suggesting that the influence of diet may not only be simply related to plasma cholesterol levels but also to other risk factors. Several studies support the hypothesis that lipid peroxidation and impaired antioxidant status is implicated in the initiation of atherosclerosis[8-10].

The beneficial effects of olive oil on atherosclerosis are related to the high levels of monounsaturated fatty acids, mainly oleic acid and to other constituents such as phytosterols and terpenes[11]. Kasdallah-Grissa et al. reported that olive oil ingestion by rats protects the liver from ethanol-induced oxidative damage by improving the antioxidant status[12]. In another study, Coni et al. indicated that extra virgin olive oil biophenols increased the ability of LDL to resist oxidation in rabbits[13].

To the best of our knowledge, studies regarding the effect of olive oil on atherosclerosis development in rabbits are limited. Therefore, the aim of this study was to evaluate the effects of olive oil on serum lipoproteins, lipid peroxidation, and atherosclerosis development in rabbits.

2. Materials and Methods

2.1. Animal

Twenty Dutch male rabbits with the average weight of 1951 g (from Pasteur Institute Iran) were housed individually 14 weeks (2 weeks: adaptation period, and 12 week experimental period). Animals were housed for 2 weeks before experiments under standard laboratory conditions with a 12 h light/12 h dark cycle and constant temperature (22-25°C).

2.2. Diet

The pellet rabbit diet was provided by " Pars Domestic Food Co" . Once weekly pellets were used to prepare an olive oil rich (8% $^{w}/_{w}$), cholesterol rich (1% $^{w}/_{w}$), and cholesterol and olive oil together. Olive oil enriched diet was prepared by using olive oil, and cholesterol rich diet by dissolving the cholesterol in diethyl ether[14], cholesterol+olive oil by dissolving the cholesterol in warm olive oil, and pouring them over the pellets and mixing thoroughly (for cholesterol rich diet, the hood was needed to evaporate diethyl ether). Mixed diet was kept at 4° C[4].

2.3. Experimental Design

After 2 weeks of adaptation, the rabbits were allocated to 4 groups of 4-6 animals: (I): standard diet (pellets); (II) olive oil; (III) cholesterol; (IV) cholesterol+olive oil. All rabbits received 100 g diet daily. The food intake for each animal was recorded daily. They had free access to tap water and were weighed every ten days.

2.4 Histological study

At the end of the study, rabbits were anesthetized and their aortas were removed and washed with normal saline, and placed in formalin 10% for further histologic preparations. The evaluation of atheromatous changes was made by macroscopic and microscopic (light) observation and expressed based on frequency and severity of lesions and graded 0-411* *Grade 0 :* ***Without any lesion*** *Grade I :* ***a little fatty streaks or fatty dots.*** *Grade II :* ***Moderate fatty streaks or fatty dots.*** *Grade III :* ***The most part of surface include fatty streaks or fatty dots.*** *Grade IV :* ***Thoughout of surface include fatty streaks or fatty dots.*** .

2.5. Biochemical assays

Blood samples from the heart were collected after 13-15 hrs fasting at the beginning and at the end of the experimental period. The blood is centrifuged and kept at -40 oC. The concentration of serum total cholesterol (TC), triglycerides (TGs), high-density lipoprotein-cholesterol (HDL- C), were determined by enzymatic procedures (enzymatic Kits, pars Azmun). The malondialdehyde (MDA) was measured using the thiobarbituric acid method[15] and total antioxidant capacity (TAC) using ferric reduction of antioxidant power[16].

2.6. Statistical analysis

Data were analyzed using the SPSS 24 statistical software package. The normality of the distribution of data was first checked by one-sample Kolmogorov-Smirnov test. One way ANOVA, Independent T-test, paired sample T test, Mann–Whitney U, and Kruskal- Wallis test were used to compare the treatment effect. $P<0.05$ is considered statistically significant.

3. Results

3.1. Body weight and food intake

Body weight showed no significant differences between groups at baseline and at the end of the study. However, there was a significant difference in food consumption between groups ($P<0.007$) (**Table 1**).

3.2. Serum lipid profile

The serum concentration of biochemical parameters in rabbits at the beginning and at the end of the experimental period are shown in**Table 2** . There was no significant difference between groups at the beginning of the study ($P>0.05$). Multiple comparisons of those biochemical factors showed a significant difference in total cholesterol ($P<0.001$), HDL-C ($P=0.04$), and triglyceride ($P<0.001$) between groups at the end of the study. In within group comparisons, olive oil intake significantly decreased triglyceride levels ($P=0.01$), however, in group III and IV total cholesterol and triglyceride significantly increased ($P<0.05$) (Table 2).

3.3. Serum oxidative stress markers

There was no significant difference between groups at the beginning of the study ($P>0.05$) (**Table 3**). After 12 weeks, the serum concentration of MDA was the highest in group III, and total antioxidant capacity (TAC) was the highest in group I. Multiple comparisons showed a significant difference in MDA ($P<0.001$) between groups at the end of the study. However, there was no significant difference in serum concentration of TAC between groups after interventions ($P<0.104$). Within group comparisons showed that MDA levels significantly increased in group III ($P<0.001$), and TAC significantly decreased in group IV ($P=0.04$) (**Table 3**).

3.4. Aortic lesion

The Aortic lesion degree showed no difference between group I and II, but group III showed the highest degree. Overall, there was a significant difference in aortic lesion degree between groups at the end of the study ($P<0.001$) (**Table 3, Figure 1**).

3.5. Comparision of cholesterol and cholesterol+olive oil rich diet.

The Comparison of biochemical factors, and aortic lesion degree in cholesterol and Cholesterol + Olive oil fed rabbits after 12 weeks are shown in **Table 4.** The results showed that MDA levels was significantly higher in group III than in the IV group ($P<0.001$). Also, aortic lesions in group IV showed a significant lower degree than group III ($P=0.02$). However, serum triglyceride concentration and total cholesterol were significantly higher in the IV group ($P<0.05$). There was no significant difference in other variables between groups (**Table 4**).

Two rabbits from cholesterol riched diet died because of jaundice in the last week of study.

4. Discussion

In this study, a cholesterol-rich diet increased the total cholesterol, HDL- C, T.G in rabbits. The III and IV groups showed that cholesterol+olive oil rich diet significantly increased T.G, and decreased MDA and aortic lesion degree in compared with the cholesterol rich diet. This finding is confirmed by Mahfouz et al. study that reported cholesterol feeding led to an increase in lipid peroxidation in rabbits [17]. Simon et al. indicated that the replacement of a high-fat diet with olive oil can recover normal blood cholesterol values in hypercholesterolemic rabbits[18].

In our study, less severe degree of the lesion in the cholesterol + Olive Oil group in spite of high blood cholesterol, showed the preventive effect of olive oil against atherosclerosis, which is independent of plasma lipoprotein effect, and suggested that probably olive oil acts on arteries directly[19]. In line with our study, previous human clinical studies emphasize the beneficial effects of virgin olive oil on cardiovascular health[20, 21]. It has been suggested that the consumption of olive oil rich in phenolic compounds can reduce the risk of atherosclerosis development by reducing inflammation in the vascular wall, and protecting against endothelial dysfunction[22, 23]. Konstantinidou et al. reported that olive oil plays an important role in the down-regulation of inflammatory genes implicated in atherosclerosis[24].

The antioxidant activity of olive oil has been widely documented in vivo and in vitro studies. Amamou et al. showed that olive oil intake could protect the rat liver against Cd-induced injury by increasing the activities of antioxidant enzymes and reducing MDA levels[25]. In another study, Oliveras-Lopez et al. reported that a daily dose of 50 ml olive oil consumption leads to a significant increase in TAC levels and catalase (CAT) activity after six weeks[26].

The mechanism proposed to explain the beneficial effects of olive oil may be related to its richness in phenolic compounds, and MUFA (mainly oleic acid). Phenolic compounds including, oleuropein, hydroxytyrosol, tyrosol, and caffeic acid have been shown to exert protective effects on lipid peroxidation, LDL oxidation (oxLDL), and DNA oxidative damage[27, 28]. OxLDL induces plaque formation within the arterial wall, and subsequently causes atherosclerosis and CVD [29]. Olive oil phenolic compounds bind to LDL and this may account for the LDL oxidation resistance[30].

To the best of our knowledge, this study is the first investigation that was designed to evaluate the effect of olive oil on atherosclerosis development in rabbits. However, a limitation of this study includes the small sample size in each group. Therefore, the inclusion of a greater number of subjects is suggested to improve the power of future studies.

Conclusion

The results of the present study indicated a protective effect of olive oil against atherosclerosis development which is independent of plasma lipoprotein effect, and suggested that probably olive oil acts on arteries directly.

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Table 1. Baseline weight, weight gain, and total food consumption of rabbits during the experimental period.[1]

Group	Baseline weight (g)	Weight gain (g)	Total food consumption (g)
Group I (Standard)	2065.0 ± 109.7	585.0 ± 76.212	7067.1 ± 135.65
Group II (Olive oil)	1992.5 ± 64.08	492.5 ± 1.2412	4944.9 ± 472.66
Group III (Cholesterol)	1873.3 ± 143	440.0 ± 312.17	5348.3 ± 583.35
Group IV (Cholesterol + Olive oil)	1875.0 ± 129	428.0 ± 1.39	4544.8 ± 348
P	0.68	0.73	0.007*

[1]All values are mean±SD.

*P < 0.05 indicates a significant difference between groups. P was obtained from ANOVA

Table 2. The effects of intervention on serum lipid profile in four exprimental groups.[1]

Variable Group	**Total Cholesterol *(mg/dl)***	**Total Cholesterol *(mg/dl)***	**Total Cholesterol *(mg/dl)***	**Total Cholesterol *(mg/dl)***	**Triglyceride *(mg/dl)***	**Triglyceride *(mg/dl)***	**Triglyceride *(mg/dl)***	**Triglyceride *(mg/dl)***	**HDL–C *(mg/dl)***	**HDL–C *(mg/dl)***	**HDL–C *(mg/dl)***	**H C**

	Baseline	12th week	[?]**	P[1]	Baseline	12th week	[?]**	P	Baseline	12th week	[?]**	P
Group I (Standard)(n=4)	56.00 ±14.27	27.75 ±4.83	-28.25 ±15.91	0.81	122.50 ±20.05	65.00 ±12.21	-57.50 ±13.97	0.01*	16.75 ±6.27	16.00 ± 1.47	-0.75± 6.03	0
Group II (Olive oil) (n=4)	53.75 ±20.71	19.75 ±2.62	-34.00 ±18.28	0.08	151.50 ±25.02	71.75 ±6.23	-79.75 ±19.71	0.01*	13.75 ±3.47	10.25 ±1.70	-3.50 ±2.62	0
Group III (Cholesterol)[2]	58.66 ±20.1	1757.20 ±149.62	1698.60 ±149.70	<0.001*	130.40 ±11.83	244.20 ±44.45	113.80 ± 47.37	0.04*	13.80 ±2.85	22.20 ± 3.83	8.40 ±16.20	0
Group IV (Cholesterol + Olive oil)[2]	69.4 ±11.07	2906.40 ±421.01	2837.00 ±423.86	0.001*	147.80 ±9.13	775.60 ±105.07	627.80 ± 111.03	0.002*	13.40 ±1.80	28.60 ±6.27	15.20 ±7.53	0
P Between	0.90	<0.001*	<0.001*	-	0.40	<0.001*	<0.001*	-	0.80	0.04 *	0.17	-

All values are mean ± SEM.

[2] n=6 at baseline and n=5 at 12thweek.

P<0.05 indicates a significant difference in related variables between groups. P Between was obtained from ANOVA.

P[1] was obtained from paired sample t test.

** differences between 12 week and baseline levels

Table 3. The effects of intervention on serum oxidative stress markers in four exprimental groups.[1]

Variable Group	**MDA** (mg/dl)	**MDA** (mg/dl)	**MDA** (mg/dl)	**MDA** (mg/dl)	**Antioxidant capacity** (%)	**Antioxidant capacity** (%)	**Antioxidant capacity** (%)	**Antioxidant capacity** (%)	**M ao les**
	Baseline	12th week	[?]**	P[1]	Baseline	12th week	Changes	P[1]	12 we
Group I (Standard) (n=4)	0.77±0.07	0.57± 0.10	-0.21±0.01	0.01*	74.38±7.28	72.50±1.89	-1.87±7.63	0. 40	0
Group II (Olive oil) (n=4)	0.53±0.12	0.63±0.15	0.10±0.05	0.05	70.25±2.90	64.25±8.01	-6.00±9.03	0.27	0
Group III (Cholesterol)[2]	1.15±0.22	5.62±0.18	4.47±0.63	<0.001*	64.70±5.09	56.40±12.19	-8.38±16.44	0.30	3.6 0.3

Group IV (Cholesterol + Olive oil)[2]	0.95±0.48	2.06±0.64	1.10±0.60	0.05	61.95±15.24	43.00±3.44	-19.34±8.75	0.04*	*2.4*
P Between	0.18	<0.001*	<0.001*	-	0.40	0.10	0.74	-	<0

All values are mean±SEM.

[2] n=6 at baseline and n=5 at 12th week.

** P<0.05 indicates a significant difference in related variables between groups. P Between was obtained from ANOVA. P[1] was obtained from paired sample t test.*

*** differences between 12 week and baseline levels; *** p value that is related to comparison of aortic lesion was obtained from Kruskal- Wallis test.*

Table 4. Comparision of serum lipid profile, oxidative stress factors and aortic lesion degree between cholesterol and cholesterol+olive oil groups at the end of the experimental period.[1]

Variable Group	[?]Total cholesterol (mg /dl)	[?]HDL – C (mg /dl)	[?]Triglyceride (mg/dl)	[?]M D A (mg/dl)	[?]Antioxidant Capailty(%)	Mean Aortic Lesion
Group III (Cholesterol)	1698.6±149.7	8.4±6.2	113.8 ±47.37	4.47±0.28	-8.38±16.44	3.66±0.33
Group IV (Cholesterol + Olive oil)	2837±423.86	15.2±7.53	627.8±111.3	1.1±0.6	-19.34±8.75	2.4±0.6
P_ Value	0.02	0.5	0.007	0.003	0.57	0.02*

-All values are mean±SEM.

- Values are the degree of aortic lesion and P was obtained from nonparametric tests (Mann–Whitney U).

** P<0.05 indicates a significant difference in related variables between two groups. P was obtained from independent-samples t-test*

Figure legends

Figure 1. The samples of aortic cross sections among four groups of rabbits.

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