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Consumption of argan oil may have an antiatherogenic effect by improving paraoxonase activities and antioxidant status: Intervention study in healthy men

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KEYWORDS

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Summary *Background and aim:* Due to its high antioxidant and mono- and polyunsaturated fatty acid content virgin argan oil (VAO) could play a beneficial role in cardiovascular prevention. We were therefore interested in determining whether the consumption of VAO could improve plasma paraoxonase (PON1) activities and antioxidant status in healthy men.

Methods and results: Sixty young men were included in this interventional study. They were given a controlled diet for 2 weeks as baseline and then received 25 g/day of butter. The group was randomised to two diet group periods of 3 weeks each. The VAO group received 25 ml/day of oil and the extra virgin olive oil (EVO) group received the same quantity of EVO as control group. Plasma PON1 activities, antioxidant vitamins and LDL susceptibility to oxidation were measured. The analysis of the results shows that PON1 activities increase significantly in both groups and that lipoperoxides and conjugated dienes formation decreases significantly in VAO and EVO groups compared to baseline values ($P = 0.001$ and $P = 0.014$, respectively). Vitamin E concentration increases significantly only in

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VAO group ($P = 0.007$). Susceptibility of LDL to lipid peroxidation shows a significant increase in lag phase and a significant decrease in maximum diene production in VAO ($P = 0.005$) and EVO groups ($P = 0.041$ and $P = 0.005$, respectively).

Conclusions: Our findings confirm the beneficial effect of EVO on plasma antioxidant status and show for the first time the same effect for VAO supplementation in man. Thus, VAO offers an additional natural food supplement to reduce cardiovascular risk.

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Introduction

The main risk factors for cardiovascular disease (CVD), high blood pressure, high cholesterol level and smoking are traditionally linked to an affluent lifestyle. Several studies suggest that oxidative modification of low-density lipoprotein (LDL) represents the major step in the development of atherosclerotic lesion [1]. The susceptibility of LDL to oxidation is dependent, in part, on its content of mono- and polyunsaturated fatty acids (MUFA and PUFA), as well as on its content of endogenous antioxidants principally vitamin E [2,3]. Indeed, it is well documented that dietary lipids are known to modulate the incidence and severity of CVD and that diets rich in antioxidant compounds reduce the susceptibility of LDL to lipid peroxidation and therefore have protective effects against atherosclerosis progression [2].

HDL are antiatherogenic lipoproteins that are implicated in the protection of LDL against oxidation [4]. Although the mechanism of this protection is not clear, increasing attention is being focused on the potential antioxidant of serum enzymes, associated with HDL. Paraoxonase 1 (PON1) has been targeted as the principal enzyme contained within HDL and responsible for their antioxidant effect [5]. Several studies proposed the implication of PON1 as an independent risk factor for cardiovascular diseases and some of them have demonstrated an effect of dietary composition in fatty acids (MUFA and PUFA) on PON1 activity [6–12].

The dietary sources of MUFA, PUFA and antioxidant compounds are numerous and mainly found in different plant oils such as sunflower, olive and others. Argan tree (*Argania spinosa*) is endemic to south-western Morocco, where it plays a major ecologic and socio-economic role. The fruit of this tree is fat rich. Argan oil is very interesting because of its particular chemical properties. It is composed of 45% of MUFA, 35% of PUFA and 20% of saturated fatty acids. Moreover, this oil is rich in minor components such as phytosterols, tocopherols and phenolic compounds [13]. Our previous prospective study [14] showed

that usual consumption of virgin argan oil has an LDL-cholesterol lowering effect together with antioxidant properties. These facts suggest that argan oil could play a role in CVD prevention by reducing susceptibility of LDL to oxidation. Thus, we were interested knowing if argan oil consumption could affect HDL enzymes (paraoxonase and arylesterase activities), plasma antioxidant status and susceptibility of LDL peroxidation after a 3-week period of intervention using randomly selected groups of 60 Moroccan normolipidemic men.

Methods

Subjects

This study was conducted on student nurse volunteer Moroccan men in military hospital (Meknes, Morocco) and was approved by the local Ethics Committee. All participants rigorously adhered to the dietary instructions and signed the informed written consent. The protocol and objectives of this study were explained to the participants in detail. A total of 66 healthy subjects, without any chronic metabolic diseases (hypercholesterolemia, hypertriglyceridemia, diabetes, hypertension) and not taking lipid-affecting drugs, were recruited for this study. Sixty participants completed the study and 6 dropped out because of personal reasons. The average age of the participants was 23.42 ± 3.85 years. Body mass index ($21.798 \pm 1.71 \text{ kg/m}^2$), diastolic ($69.3 \pm 6.6 \text{ mmHg}$) and systolic ($118.4 \pm 8.05 \text{ mmHg}$) blood pressures, total plasma cholesterol ($156.88 \pm 38.26 \text{ mg/dl}$), plasma triglycerides ($76.31 \pm 33.09 \text{ mg/dl}$) and plasma glucose ($90.01 \pm 11.53 \text{ mg/dl}$) were in normal range. During this study, the daily habits of the participants such as physical activities, number of sleeping hours and working time were not changed.

Study design and experimental diets

There were two diet periods. In diet period I (baseline diet), all the subjects consumed 25 g/day

of butter with toasted bread for breakfast for 2 weeks. In diet period II, the subjects were randomised to two diet groups: one group of 30 subjects in which 25 g/day butter was substituted by 25 ml/day of virgin argan oil (VAO group) taken in a single dose with bread at breakfast and the second group of 30 subjects consuming 25 ml/day of extra virgin olive oil (EVO group) as control, at breakfast for 3 weeks. The daily assessment of food intake was required. The argan and olive oils supplied and distributed to the participants were from the same origin. All meals were served at the same canteen school in order to better control the diet during the study period.

The diets consisted of common foods such as fruits, vegetables, meat, fish, starch and dairy products. The number of daily servings of each diet period was similar. The composition of the three diets was estimated using the Ciqual standard table of food composition [15] and described in Table 1.

Biological material

The fruit of “*A. spinosa*” is original of the Essaouira area in the southwest of Morocco. The VAO and EVO used in this study were extracted by industrial process [16]. Their fatty acid and minor components composition were similar to the one presented in Table 2 [13].

Plasma samples

At the end of each diet period, venous blood was collected into draw tubes after 12 h fast. Plasma was obtained by centrifugation for 12 min at 4 °C for 4000 rpm. Plasma samples were stored at –80 °C until analysis. Two milliliters of plasma

obtained from each subject was immediately used for LDL separation.

LDL isolation

LDL fraction from each sample was isolated by sequential preparative ultracentrifugation using a Beckman ultracentrifuge as described by Sattler et al. [17]. After centrifugation at 100 000 rpm during 2 h at 15 °C in a Beckman TLA 100.4 rotor, the LDL fractions were collected with a syringe and protein composition was measured by commercial assay (Pierce method, Rockford, IL, USA) using bovine serum albumin as a standard.

PON1 activities

Plasma paraoxonase (PON) activity was measured by the initial velocity of paraoxon hydrolysis at 25 °C, to yield *p*-nitrophenol at 412 nm ($\epsilon_{412 \text{ nm}} = 18\,290 \text{ M}^{-1} \text{ cm}^{-1}$) as already described by Gan et al. [18]. A PON activity of 1 U/l was defined as 1 μM of *p*-nitrophenol formed per minute. Plasma arylesterase activity was measured using phenylacetate as substrate at pH 8, by the method of Gan et al. [18]. Initial rates of hydrolysis were determined spectrophotometrically at 270 nm ($\epsilon_{270 \text{ nm}} = 1310 \text{ M}^{-1} \text{ cm}^{-1}$). A unit of arylesterase activity is defined as 1 μmol phenylacetate hydrolyzed per minute, under the above assay conditions. PON1 phenotyping of each subject was determined by the dual substrate method as proposed by Eckerson et al. [19].

Vitamin E measurement

Plasma vitamin E content was assayed as α -tocopherol by reverse-phase high-performance liquid chromatography (HPLC) with UV and electrochemical detection [20]. α -Tocopherol-acetate was used as internal standard.

Table 1 Composition of diets used in the study

	Butter diet	VAO diet	EVO diet
Total energy (kJ/day)	10614.81 \pm 1021.73	10715.22 \pm 1029.26	10709.36 \pm 1058.55
Proteins (g/day)	85.74 \pm 15.58	84.79 \pm 14.99	84.66 \pm 15.01
% Proteins	13.49 \pm 1.91	13.23 \pm 1.96	13.29 \pm 1.91
Carbohydrates (g/day)	339.44 \pm 44.2	391.66 \pm 46.12	390.29 \pm 48.01
% Carbohydrates	62.01 \pm 12	60.99 \pm 2.88	61.04 \pm 2.85
Fats (g/day)	68.97 \pm 12	73.33 \pm 11.51	72.66 \pm 11.48
% Fats	24.51 \pm 3.56	25.84 \pm 3.62	25.70 \pm 3.49
% SFA	41.63 \pm 2.43	26.97 \pm 1.38	26.39 \pm 3.43
% MUFA	30.22 \pm 1.94	36.45 \pm 1.38	43.87 \pm 1.24
% PUFA	28.16 \pm 3.61	36.54 \pm 4.53	29.73 \pm 3.87

VAO, virgin argan oil; EVO, extra virgin olive oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 2 Chemical composition of virgin argan oil and extra virgin olive oil used in the study [13]

	VAO	EVO
<i>Fatty acids (%)</i>		
C16:0	13.4	10.4
C18:0	5.1	2.76
C18:1 n-9	44.8	71.0
C18:2 n-6	33.7	12.9
C18:3 n-3	0.1	1.04
<i>Sterols (mg/100 g oil)</i>		
Schottenol	142	0
Spinasterol	115	0
Stigmasta-8,22-dien-3 β -ol	9	0
β -Sitostérol	0	156
Campesterol	0	12
Others	29	151
Total	295	319
<i>Tocopherols (mg/kg oil)</i>		
α	35	190
δ	122	42
γ	480	26
Total	636	257
<i>Phenolic compounds (μg/kg oil)</i>		
Vanillic acid	67	359
Syringic acid	37	0
Ferulic acid	3147	51
Tyrosol	12	19 573
Others	0	773 000
Total	3263	792 983

VAO, virgin argan oil; EVO, extra virgin olive oil.

Conjugated diene and lipid peroxide measurements

Conjugated diene formation was determined spectrophotometrically by the measure of the absorbance at 234 nm (U-3000 spectrophotometer, Hitachi) as previously described [20]. Plasma was diluted (1/100) with phosphate buffer before each measure. Lipid peroxides (LPO) were measured on plasma of all subjects by the method of El-Saadani et al. [21].

Evaluation of LDL susceptibility to oxidation

LDL at concentration of 100 μ g/ml underwent in vitro oxidation induced by incubation with 10 μ M CuSO₄ at 37 °C. The kinetics of conjugated diene formation was continuously monitored by measuring the absorbance at 234 nm, every 15 min for at least 5 h. Lag phase (LP (min)), maximal rate (MR, (mol conjugated diene/mol LDL/min)) and maximum diene production (MDP, as mol conjugated diene/mol LDL) were determined as described by Kleinveld et al. [22]. For the two last parameters, we used the molar absorbance coefficient of

conjugated dienes ($\epsilon_{234 \text{ nm}} = 29.500 \text{ M}^{-1} \text{ cm}^{-1}$) to calculate diene production [22].

Statistical analysis

The results are reported as means \pm SD. The Mann–Whitney test was used for the comparison between VAO and EVO groups in each diet periods (in time) and in diet-period interaction effects. Difference of lipid and antioxidant parameters between baseline and 3 weeks after diet in VAO and EVO groups was analyzed by the Wilcoxon signed rank test. Value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed with a statistical Package for the Social Science (SPSS, version 11.0).

Results

Plasma markers of lipid peroxidation

Plasma lipid peroxide (LPO), conjugated diene (CD) and vitamin E contents, used as an oxidative stress markers, were determined for each subject either at baseline and after 3 weeks under a diet rich in VAO or EVO. Results are presented in Table 3. Plasma LPO and CD concentrations decreased significantly in subjects on VAO and EVO-rich diet ($P = 0.001$ and $P = 0.001$ for VAO and $P = 0.001$ and $P = 0.01$ after EVO diet, respectively). However, plasma vitamin E contents significantly increased from baseline only on VAO-rich diet ($P = 0.007$). There was no significant difference in LPO, CD and vitamin E concentrations between the two groups when compared at baseline or at the end of the study. As reported in Table 1, the daily energy intake during the two diet periods was not changed and subsequently did not affect body weight in either group.

LDL susceptibility to lipid peroxidation

LDL from each subject were isolated and submitted to oxidation induced by CuSO₄ in order to assess the effect of VAO and EVO administration on their susceptibility to oxidation. Three weeks of diet enrichment in VAO or EVO induced a significant increase in the lag phase ($P = 0.005$ for VAO and $P = 0.041$ for EVO) which explain a reduction of LDL susceptibility to lipid peroxidation (Table 4). Also, the total quantity of CD as well as LPO formed was reduced for the two groups of subjects ($P = 0.005$ for VAO and EVO groups) after the period of intervention.

Table 3 Plasma oxidation markers in participants at baseline and at the end VAO and EVO diets

	VAO group				EVO group				Periods of comparison between both groups	
	Baseline	After 3 weeks	Percentage	<i>P</i>	Baseline	After 3 weeks	Percentage	<i>P</i>	Baseline	After 3 weeks
Vitamin E (μM)	24.92 \pm 8.49	29.80 \pm 11.47	+16.37	0.007	26.52 \pm 11.47	27.38 \pm 11.01	+3.15	0.185	0.906	0.46
LPO (μM)	2.84 \pm 1.21	2.33 \pm 0.76	-17.95	0.001	2.96 \pm 1.23	2.56 \pm 0.98	-13.51	0.001	0.75	0.69
CD (234 nm)	2.88 \pm 0.56	2.64 \pm 0.48	-8.3	0.001	2.95 \pm 0.81	2.61 \pm 0.54	-11.52	0.01	0.683	0.741

Mean \pm SD. *P*, significantly different from baseline. LPO, lipid peroxides; CD, conjugated dienes; VAO, virgin argan oil; EVO, extra virgin olive oil.

Table 4 Kinetic of conjugated dienes formation from oxidized LDL of participants at baseline and at the end of VAO and EVO diets

	VAO group				EVO group				Periods of comparison between both groups	
	Baseline	After 3 weeks	Percentage	<i>P</i>	Baseline	After 3 weeks	Percentage	<i>P</i>	Baseline	After 3 weeks
LP (min)	56.60 \pm 16.29	61.50 \pm 17.05	+8	0.005	46.20 \pm 14.95	60.3 \pm 24.98	+23.3	0.041	0.218	0.529
MR (mol diene/ mol LDL/min)	6.10 \pm 2.65	5.23 \pm 1.78	-14.26	0.059	6.22 \pm 2.65	5.96 \pm 1.92	-4.18	0.646	0.912	0.481
MDP (mol diene/ mol LDL)	744.91 \pm 99.97	649.59 \pm 102.32	-12.96	0.005	709.12 \pm 161.54	649.53 \pm 136.44	-8.40	0.005	0.853	0.796

Mean \pm SD. *P*, significantly different from baseline. LP, lag phase; MR, maximal rate of dienes production; MDP, maximum dienes production; VAO, extra virgin argan oil; EVO, extra virgin olive oil.

Effect of VAO and EVO on HDL antiatherogenic effect

The effect of VAO as well as EVO consumption towards the antiatherogenic property of HDL was evaluated by investigating the activities of PON1 at baseline and after 3 weeks of VAO and EVO supplementation. PON1 activities, basal paraoxonase, salt-stimulated paraoxonase and arylesterase activities were significantly increased in both VAO and EVO groups (Table 5). No difference was observed in plasma PON1 activities between the VAO and the EVO groups in time or in diet-period interaction.

Discussion

Argan oil has been traditionally known for its various pharmacological properties and used in traditional medicine like a natural remedy for several centuries. This is probably related to its fat composition, 45% MUFA and 35% PUFA, and to its minor compounds such as polyphenols, phytosterols and tocopherols [13]. Due to its high contents of γ -tocopherols, squalene and phenolic compounds, argan oil could have a cancer chemopreventive effect [13]. Only a few studies have reported some pharmacological effects of this oil on animals regarding the cardiovascular status [23,24]. However, no evidence of such effects has been reported in man. Recently, the first prospective study made in subject consuming regularly argan oil in their diet proved the hypolipidemic and the antioxidant effects of this oil [14]. In spite of the high concentration of argan oil in different antioxidants, little is known about its action in the modulation of in vivo oxidative stress.

In this study, we aimed to investigate by an interventional trial whether the consumption of argan oil could improve some oxidative stress plasma markers as well as its effect towards HDL paraoxonase activity in normolipidemic men in comparison with olive oil. Concerning the oxidative parameters, our results show beneficial effects on plasma LPO, conjugated diene and vitamin E concentrations. In isolated LDL the LP was increased in both groups reflecting an increase in the resistance of LDL to lipid peroxidation. Therefore, daily consumption of VAO or EVO oils offers a good protection to plasma and LDL against peroxidation. It is noteworthy that there is an increase in resistance of LDL to copper-induced oxidation after both diets as evaluated by an increase in LP and a decrease in MDP. These findings could be mainly related to an antioxidant content of VAO and EVO

Table 5 Plasma PON1 activities in participants at baseline and at the end VAO and EVO diets

	VAO group				EVO group				Periods of comparison between both groups					
	Baseline		After 3 weeks		Baseline		After 3 weeks		Baseline	After 3 weeks				
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	P	P				
PON1 (U/ml)	122.03	± 71.21	171.97	± 91.98	+29.03	0.01	121.02	± 61.22	165.61	± 85.40	+26.23	0.01	0.403	0.607
PON1-NaCl (U/ml)	187.42	± 57.64	273.85	± 103.91	+31.5	0.001	196.32	± 90.66	272.45	± 123.89	+27.94	0.004	0.13	0.48
Arylesterase (U/ml)	40.47	± 31.18	73.82	± 37.81	+45.17	0.001	49.86	± 32.38	76.40	± 33.17	+34.73	0.001	0.06	0.23

Mean \pm SD. P, significantly different from baseline. PON1, paraoxonase 1; VAO, virgin argan oil; EVO, extra virgin olive oil.

oils. Indeed, these oils are rich in minor compounds such as polyphenols, tocopherols and sterols, which are powerful antioxidants [14,25]. Thus, LDL might be enriched with different antioxidant on both VAO and EVO diets, which might induce a reduction in their susceptibility to lipid peroxidation [26,27]. Our data on EVO are in agreement with the previous studies of Gimeno et al. [28] suggesting that an intake of 25 ml/day of EVO could increase the resistance of LDL to oxidation. Similarly, several *in vitro* and *in vivo* studies in humans as well as in animals have demonstrated that vitamin E and phenolic compounds extracted from EVO inhibited oxidation of LDL [3,29–30]. At the same time, our data do not exclude that the reduction of LDL susceptibility to lipid peroxidation, with both VAO and EVO oils, could be explained, at least in part, by an enrichment in monounsaturated fatty acids [31]. The marked increase in plasma vitamin E only in the VAO group may be due to mean difference in vitamin E content between the two oils (Table 2). Although, the main tocopherol present in VAO the γ -tocopherol [13], we have found that plasma α -tocopherol levels are increased in the VAO group. This result could be consequence of the conversion from γ - to α -tocopherol because of the close similarity between the chemical structures of both molecules [32]. Also, γ -tocopherol supplementation simultaneously increases the levels of γ - and α -tocopherol.

Vitamin E is the major antioxidant among those present in LDL; thus it is considered as the first line defence against oxidation [3]. This was confirmed by the finding of Nigdikar et al. [33], which showed that the increase in LP with vitamin E was significant, being four- to fivefold greater than with red wine polyphenols. Also, Berra et al. [34] showed that LDL oxidation was decreased by the addition of tyrosol or an extract of minor components of EVO. Visioli et al. [29] also found that LDL oxidation was strongly inhibited by hydroxytyrosol from EVO. More recently, Drissi et al. [14] showed that sterols, tocopherols and phenol compounds from argan oil increase the resistance of LDL to oxidation.

It is well known from epidemiological data that HDL exerts cardioprotective properties. Also, its antioxidant activity is largely due to the presence of paraoxonase (PON1) [35]. In the present study, we showed an increase in PON1 paraoxonase and arylesterase activities after 3 weeks of VAO or EVO diets. There is evidence that dietary habits, particularly the amount of dietary fat, have been reported to affect paraoxonase and arylesterase activities [36,37]. However, little is known about the association between diet and PON1 activity in addition there are some discrepancies between

studies on the effect of diet on PON1 activity. Indeed, Kleemola et al. [36] observed that a lower PON1 activity is associated with a high vegetable intake. However, antioxidants, such as flavonoid quercetin, were shown to reduce the amount of lipoprotein-associated lipid peroxides and preserved PON1 activity [38]. Also, meals rich in oxidized fat used for deep-frying have been shown to reduce PON1 activity in plasma [39], while olive oil-rich meals are accompanied with an increase in postprandial plasma PON1 activity [40]. The increase in PON1 activities seems to be associated with unsaturated fatty acid as mentioned by Nguyen and Sok [41]. In their study, they showed that monoenoic acids or their phospholipid derivatives play a beneficial role in protecting PON1 from oxidative inactivation as well as in stabilising PON1. The minor polar components of argan oil or olive oil could also be involved in this change. Thus, increasing plasma PON1 activities by argan oil is potentially antiatherogenic.

In conclusion, the results of this intervention study confirm the antioxidant effects of EVO and show for the first time the powerful antioxidant effects of VAO in man. Thus, argan oil may be used as an antiatherogenic oil.

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