



ORIGINAL ARTICLE

Evidence of hypolipemiant and antioxidant properties of argan oil derived from the argan tree (*Argania spinosa*)[☆]

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Received 27 June 2003; accepted 6 March 2004

KEYWORDS

Virgin argan oil;
Hypolipidemic;
Antioxidant;
Atherosclerosis

Summary Background: Virgin argan oil is of interest in cardiovascular risk prevention due to its fat composition and antioxidant compounds.

Aims: We investigated with Moroccan subjects the effect of regular virgin argan oil consumption on lipid profile and antioxidant status and the in vitro effect of argan oil minor compounds (tocopherols, sterols and polyphenols) on LDL peroxidation.

Design: Healthy subjects (20 men, 76 women) were studied. Sixty-two were regular consumers of argan oil and 34 were non-consumers.

Methods: Fasting plasma lipids, antioxidant vitamins and LDL oxidation susceptibility were analyzed. In vitro LDL oxidation by phenolic and apolar compounds of virgin argan oil were performed.

Results: Diet composition of argan oil consumers has a higher significant content of polyunsaturated fatty acids than that of non-consumers (8.8 ± 1.0 vs. 6.6 ± 0.9 g, $P < 0.05$). Subjects consuming argan oil have lower levels of plasma LDL cholesterol (12.7%, $P < 0.05$) and Lp(a) (25.3%, $P < 0.05$) compared with the non-consumers. In argan oil consumers, plasma lipoperoxides were lower (58.3%, $P < 0.01$) and molar ratio α -tocopherol/total cholesterol (21.6%, $P < 0.05$) and α -tocopherol

Abbreviations: ApoA1, apolipoprotein A1; Apo B, apolipoprotein B; DBP, diastolic blood pressure, LDLc, LDL cholesterol; LP, lag phase; Lp(a), lipoprotein (a); LPO, lipoperoxides, MDP, maximum diene production; MR, maximal Rate; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SBP, systolic blood pressure, SFA, saturated fatty acids, TG, triacylglyceride, TBARS, thiobarbituric acid-reactive substances; TC, total cholesterol.

[☆]This work was supported by a grant from Agencia Española de Cooperación Internacional (1999-2000, 13PRO/00), CNR-Morocco, Protars No. P1T2/11, FIS: PI021037, DGCYT SAF98-0084 and a grant from the FIS of the Instituto de Salud Carlos III, Red de Centros RCMN (C03/08), Madrid, Spain.

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concentration (13.4%, $P < 0.05$) were higher compared with the non-consumers group. In spite of higher levels of plasma antioxidant and lower levels of lipoperoxides in argan oil consumers, LDL oxidation susceptibility remained fairly similar. A strong positive correlation was observed between increasing phenolic extract, sterol and tocopherol concentrations and the LDL-Lag phase ($P < 0.05$).

Conclusions: Our findings suggest for the first time that regular consumption of virgin argan oil induces a lowering of LDL cholesterol and has antioxidant properties. This oil offers an additional natural food to reducing cardiovascular risk.

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Introduction

Dietary fat is a key factor in cardiovascular disease (CVD) prevention. It is known that saturated fatty acids increase the risk of coronary artery disease (CAD) while unsaturated fatty acids lower the risk. It is of interest to know the chemical and biological properties of argan oil. This oil is obtained from the argan fruit of *Argania spinosa* (Sapotaceae), an endemic tree located mainly in south-western Morocco.¹ Virgin argan oil, which is extracted from argan almonds by cold pression, is well known for its cosmetic, pharmaceutical and nutritional virtues. Its fat composition is 45% monounsaturated fatty acid (MUFA), 35% polyunsaturated fatty acid (PUFA), and 20% saturated fatty acids (SFA). The ratio alpha linolenic/linoleic acid is 0.003. This oil is rich in minor compounds such as phenolic compounds (3.3 mg/Kg), plant sterols (295 mg/100g) and tocopherols (637 mg/Kg)^{2,3} and these compounds have antioxidant effects as mentioned in many studies.⁴⁻⁶ These facts suggest that argan oil could play a role in CAD prevention by reducing lipid risk factors. We hypothesize that virgin argan oil can reduce total plasma and LDL cholesterol, and the high content in minor compounds could possess lipid antioxidant properties, which in turn

may lead to protection against the development of atherosclerosis.

Argan oil is mainly used in the diet of people living in the south-west of Morocco and its role in cardiovascular risk factors have never been studied. We were, therefore, interested in investigating the effect of regular consumption of virgin argan oil on lipid profile and antioxidant status in a group of healthy Moroccan subjects compared with non-consumers who live in the same area of the south-west of Morocco. We have also studied the in vitro effect of the unsaponified fraction of virgin argan oil (tocopherols, sterols and polyphenols) on the susceptibility of LDL oxidation.

Subjects and methods

Subjects

The study was conducted in a rural community of south-west Morocco. Ninety-six subjects (20 men, 76 women), non-smokers, with no chronic metabolic diseases (glycemia < 126 mg/dl) and not taking lipid affecting drugs, were recruited for this observational survey. The preponderance of women in our study may be explained by the emigration of men for long periods looking for work. Sixty-two subjects were regularly consuming argan oil in their diet (consumer group) and 34 were not consumers of this oil (non-consumer group).

Mean daily intake of argan oil in the consumers group was 15 g. Both groups had similar anthropometric characteristics (Table 1). All the subjects filled in a food questionnaire, in which they noted the quality and quantity of food consumed during the day before blood sampling, including specifically the argan oil intake. The registered values were converted into energy and were estimated according to the Ciqual standard table of food composition.⁷ All participants had similar physical activity and lifestyle.

Table 1 Anthropometric characteristics.

	Consumers (n = 62)	Non-consumers (n = 34)
Sex		
(%Male/	23/77	18/82
%Female)		
Age (y)	38.3 ± 12.6	37.1 ± 12.6
BMI (kg/m ²)	22.3 ± 3.8	22.3 ± 3.9
SBP (mmHg)	121 ± 14	120 ± 14
DBP (mmHg)	69 ± 10	66 ± 11

Results are expressed as mean ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

Lipid measurements and isolation of LDL

After a 12 h overnight fasting period, 20 ml of venous blood was taken into EDTA tubes and centrifuged immediately for 15 min at 4°C for 1500 × g. Plasma was immediately separated and stored at -70°C with the addition of BHT (10 μmol/L) to prevent autooxidation. Total cholesterol (TC), triacylglycerol (TG) and HDL cholesterol (HDL-c) were measured using enzymatic kits adapted for Cobas-Mira autoanalyzer (Roche Diagnostic, Germany). LDL cholesterol (LDL-c) was calculated by the Friedewald formula.⁸ Plasma apolipoprotein (apo) A-I, apo B100 and Lp(a) were measured using immunoturbidimetric assays adapted for the Cobas-Mira autoanalyzer (Roche, Basel, Switzerland). LDL (1.006–1.063 g/ml) from each sample was obtained by sequential preparative ultracentrifugation in a Kontron 45.6 rotor (Kontron instruments, Milan, Italy).⁹

Vitamin E measurement

The vitamin E (α -tocopherol) content was determined by high-performance liquid chromatography (HPLC) (Hewlett-Packard, 1050 series, Waldbronn, Germany) as described by Catignani and Bieri¹⁰ using tocopherol acetate as an internal standard (50 μg/ml). The column was Spherisorb ODS2, 125 × 4 mm, 5 μm (Teknokroma) and the mobile phase was methanol (100%). The HPLC instrument was equipped with a UV-visible detector and absorbance was recorded at 292 nm.

TBARS and lipoperoxide measurements

Thiobarbituric acid reactive substances (TBARS) were measured in plasma of all the subjects. TBARS were measured according to the method of Yagi,¹¹ using malondialdehyde (MDA) as standard. The results are expressed as malondialdehyde equivalent content (nmol MDA/ml).

The lipoperoxides (LPO) were measured in plasma of the subjects by an enzymatic method using a commercial kit (Wak-Chemie Medical GMBH, Germany).

Argan oil extraction

The virgin argan oil used in this work was extracted by a cold presson process¹² and its composition is listed in Table 2.³ The fruit originated from the Essaouira area in the south-west of Morocco. Argan oil was used in its rough state, without any preliminary processing. It was preserved at 4°C in

Table 2 Composition of virgin argan oil.³

Fatty acid	%
C16:0	13.4
C18:0	5.1
C18:1 n-9	44.8
C18:2 n-6	35.7
C18:3 n-3	0.1
Sterols	mg / 100 g oil
Schottenol	142
Spinasterol	115
Stigmasta-8,22-dien-3 β -ol	9
Others	29
Tocopherols	mg/kg oil
α	35
δ	122
γ	480
Phenolic compounds	μg/kg oil
Vanilic acid	67
Syringic acid	37
Ferulic acid	3147
Tyrosol	12

a bottle made of brown glass. In order to investigate the antioxidant effect of this oil, we were interested in analysing the unsaponifiable fraction that represent 1% of the total mass of the oil, for which we extracted three compounds: polyphenols, sterols and tocopherols. The polyphenols were obtained by a method using methanol.¹³ Sterols and tocopherols were obtained separately after the extraction of the unsaponifiable fraction, which was obtained by the hexane-extraction method. Using the thin layer chromatography, the purified fractions of sterols and tocopherols were reconstituted in chloroform solvent.^{2,14}

Conjugated diene formation

After extensive dialysis against PBS at 4°C, the conjugated diene formation in LDL (50 μg/ml) was analyzed by monitoring the change at 234 nm at 30°C in a Uvikon spectrophotometer 922 (Kontron) in the presence of 6 μmol/l of cupric chloride dehydrate. Absorbance was recorded every 5 min for at least 5 h. Conjugated diene formation was measured in the LDL fraction from each subject.

On the other hand, in the in vitro study, argan oil extracts were added to an LDL pool (50 µg/ml) obtained from a 15 normolipidemic subjects at concentrations ranging from 1 to 21 µg/ml of tocopherols (1.55 mg/ml); from 2 to 20 µg/ml of polyphenols (7.5 mg/ml) and from 12 to 96 µg/ml of sterols (3.5 mg/ml). The Lag phase (LP), maximal rate of dienes production (MR) and maximum dienes production (MDP) of LDL were analyzed.

Statistical analysis

Differences between means of consumers and non-consumers of argan oil were analyzed by the ANOVA. The relationship between parameters of LDL conjugated diene formation and increasing concentration of argan oil extracts was evaluated using regression analyses. Statistical significance was set at $P < 0.05$. Statistical analyses were performed with the Statistical Package for the Social Science software package version 6.1 (SPSS). Results are expressed as means \pm SD.

Results

Dietary content of nutrients

Table 3 shows the results of the dietary consumption analysis. The total daily energy intake was not different in both groups. The diet was rich in carbohydrates and low in lipid content. Regular argan oil intake was associated with a higher intake of PUFA in the consumer group with respect to the non-consumer group ($3.4 \pm 1.5\%$ vs. $2.6 \pm 1.2\%$, $P < 0.05$).

Argan oil consumption and lipid profile

The values of lipid, lipoprotein and apolipoprotein parameters in plasma between argan oil consumers and non-consumers are presented in Table 4. LDL cholesterol levels were significantly lower in the consumers group as compared to the non-consumers (2.47 ± 0.81 mmol/l vs. 2.83 ± 0.77 mmol/l, $P < 0.05$). Lp(a) concentrations were lower in the consumers group (25.14 ± 17.73 mg/dl vs. 33.67 ± 20.01 mg/dl, $P < 0.05$). Plasma TC and apo B levels were lower in consumers without reaching

Table 4 Plasma lipid profile for the virgin argan oil consumers and non-consumers.

	Consumers (n = 62)	Non-consumers (n = 34)
Plasma		
TC (mmol/l)	4.10 ± 0.89	4.37 ± 0.98
TG (mmol/l)	0.95 ± 0.40	0.88 ± 0.34
LDLc (mmol/l)	$2.47 \pm 0.81^*$	2.83 ± 0.77
Lp(a) (mg/dl)	$25.14 \pm 17.73^*$	33.67 ± 20.01
HDLc (mmol/l)	1.19 ± 0.33	1.14 ± 0.36
Apo B (mg/dl)	56.47 ± 24.58	65.31 ± 32.65
Apo AI (mg/dl)	122.42 ± 32.63	122.16 ± 38.82
LDL		
Apo B (mg/dl)	$41.14 \pm 13.89^*$	54.96 ± 27.35
HDL		
Apo AI (mg/dl)	99.79 ± 22.11	96.54 ± 17.68

Results are expressed as mean \pm SD. TC, total cholesterol; TG, triacylglyceride; LDLc, LDL cholesterol; Lp(a), lipoprotein (a); Apo B, apolipoprotein B; ApoAI, apolipoprotein AI. Significantly different from non-consumers: $^*P < 0.05$.

Table 3 Diet composition analysis of virgin argan oil consumers and non-consumers.

		Consumers (n = 62)	Non-consumers (n = 34)
Energy intake (kcal/day)		2337 ± 608	2292 ± 669
Carbohydrates	g	382.1 ± 17.8	394.2 ± 18.1
	%	65	69
Proteins	g	96.4 ± 5.9	90.5 ± 5.7
	%	16	16
Lipids	g	45.7 ± 5.9	37.4 ± 5.9
	%	18	15
SFA	g	15.6 ± 2.8	12.9 ± 2.9
	%	6	5
MUFA	g	15.3 ± 2.4	12.5 ± 2.5
	%	6	5
PUFA	g	$8.8 \pm 1.0^*$	6.6 ± 0.9
	%	3.5	2.5

SFA; saturated fatty acid, MUFA; monounsaturated fatty acid, PUFA; polyunsaturated fatty acid. $^*P < 0.05$ using ANOVA test.

Table 5 Plasmatic oxidant and antioxidant parameters for the virgin argan oil consumers and non-consumers.

	Consumers (n = 62)	Non-consumers (n = 34)
LPO (nmol/ml)	16.53 ± 15.80**	39.68 ± 28.28
TBARS (mmol/l)	3.46 ± 1.19	4.17 ± 2.51
α-Tocopherol (mg/l)	11.09 ± 3.42*	9.78 ± 3.46
α-Tocopherol/ TC (mg/mmol)	2.59 ± 0.72*	2.13 ± 0.83

Results are expressed as mean ± SD. LPO, lipoperoxides; TBARS, thiobarbituric acid reactive substances. Significantly different from non-consumers: * $P < 0.05$, ** $P < 0.01$.

Table 6 Susceptibility of LDL oxidation for the virgin argan oil consumers and non-consumers.

	Consumers (n = 62)	Non-consumers (n = 34)
LP (min)	40.92 ± 12.93	41.92 ± 80.95
MR (mol diene/ mol LDL/min)	4.90 ± 1.14	5.14 ± 1.68
MDP (mol diene/mol LDL)	310.50 ± 75.15	309 ± 68.73

Results are expressed as mean ± SD. LP, Lag phase; MR, Maximal Rate; MDP, Maximum diene production.

statistical significance. A significant reduction of apo B in LDL particles was observed in the consumers group (41.14 ± 13.89 mg/dl vs. 54.96 ± 27.35 mg/dl, $P < 0.05$).

Argan oil consumption and antioxidant status

Plasma LPO and TBARS, as markers of lipid peroxidation, and the antioxidant vitamin α-tocopherol concentrations in both groups were listed in Table 5. There were lower levels of plasma LPO in the consumers group (16.53 ± 15.80 mmol/l vs. 39.68 ± 28.28 mmol/l, $P < 0.01$). However, we did not observe significant differences in TBARS levels between groups. A higher α-tocopherol concentration (11.09 ± 3.42 mg/l vs. 9.78 ± 3.46 mg/l, $P < 0.05$) and also a higher molar ratio (α-tocopherol/TC) were observed in the consumers group (2.59 ± 0.72 mg/mmol vs. 2.13 ± 0.83 mg/mmol, $P < 0.05$).

Table 6 shows the conjugated diene formation in LDL samples from each subject in both groups. No

significant differences were observed in LDL-LP, LDL-MR and LDL-MDP parameters between groups.

Effect of Argan oil extracts on LDL oxidation in vitro

The kinetics of LDL diene formation with and without the addition of different concentrations of argan oil unsaponifiable fractions are shown in Fig. 1. A clear antioxidant effect on LDL oxidation can be seen with increasing amounts of tocopherols, polyphenols and sterols. This effect was corroborated by the strong positive correlation between increasing amounts of tocopherols, polyphenols and sterols in the incubation buffer and LDL-LP ($r^2 = 0.8443$, $P = 0.0096$; $r^2 = 0.9913$, $P < 0.0001$; $r^2 = 0.800$, $P = 0.041$, respectively).

Discussion

In the present study, we demonstrate that consumption of virgin argan oil is associated with significantly low levels of plasma LDL cholesterol in healthy subjects compared with non consumers living in the south-west of Morocco. In addition, a higher plasma content of vitamin E accompanied with lower LPO suggests an antioxidant effect of the studied oil. These differences are not due to energy differences because both groups have a similar energy intake and dietary composition except for the higher PUFA intake observed in the usual argan consumer group. We have also shown that argan oil has an antioxidant effect on LDL oxidation in vitro. These results indicate and demonstrate for the first time that argan oil consumption could reduce lipid risk factors against CAD in humans.

The peculiarity of argan oil is that contains 45% of MUFA and 35% PUFA. It is reported that diets enriched with MUFA or PUFA positively correlated with a reduced risk of cardiovascular mortality.^{15,16} Also, this oil is rich in minor compounds such as phenolic compounds (3.3 mg/kg), sterols (295 mg/100 g) and tocopherols (637 mg/kg).^{2,3} These compounds are known for their antioxidant effects as mentioned in numerous studies.⁴⁻⁶ Because of that, this chemical composition of argan oil is interesting in regard to cardiovascular risk prevention. Plasma LDL cholesterol were significantly lower in consumers than in non-consumers of argan oil and these lower levels may be due to the presence of not only unsaturated fatty acid but also minor compounds, such as plant sterols, in this oil. In fact, the molecular structure of sterols is very

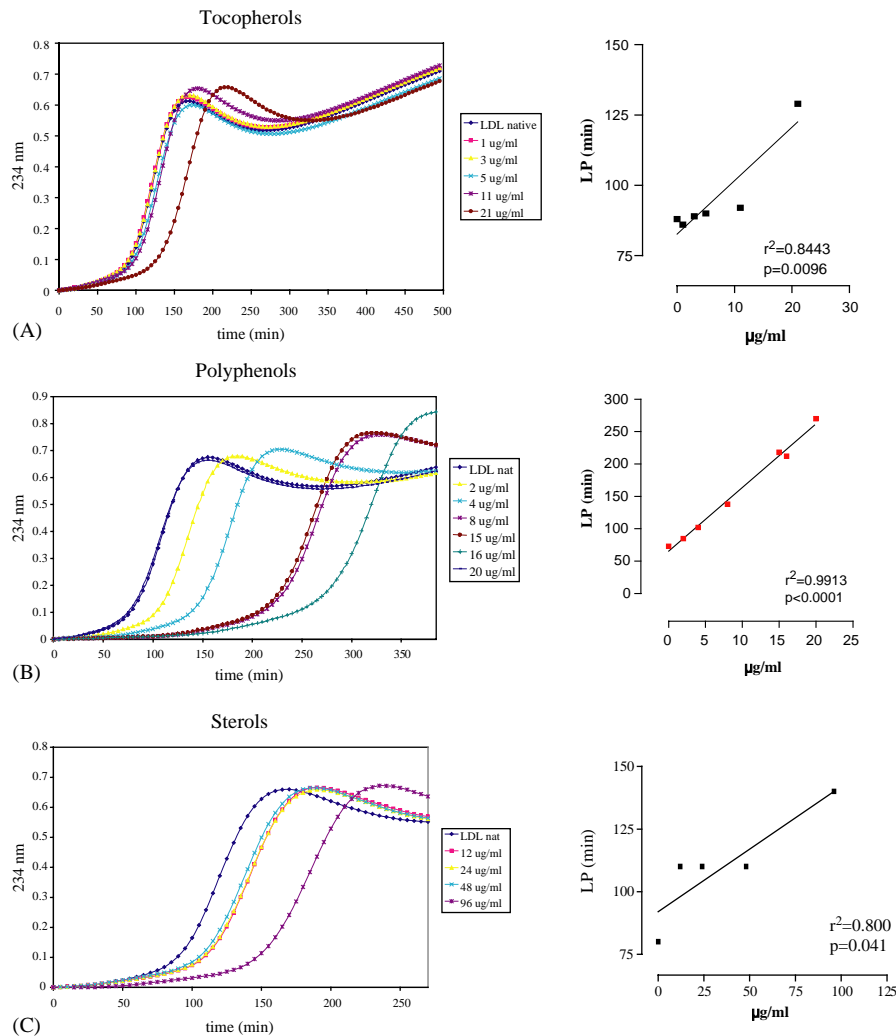


Figure 1 Conjugated diene formation in LDL incubated with Cu^{2+} in the presence of increasing concentration of argan oil extracts, tocopherols: 0, 1, 3, 5, 11 and 21 $\mu\text{g/ml}$ (A), polyphenols: 0, 2, 4, 8, 15, 16 and 20 $\mu\text{g/ml}$ (B) and sterols: 0, 12, 24, 48 and 96 $\mu\text{g/ml}$ (C) and linear regression analysis of the parameters of diene formation in LDL (A, B and C). Results are expressed as a mean of 4 determinations.

similar to that of human cholesterol, so they reduce cholesterol absorption by mixing with the micelles and blocking cholesterol from doing so.¹⁷ Controlled clinical trials have proven that increasing the amount of sterols in the diet changes blood lipid profile. Supplementation with 2–3 g of stanol-sterol/day has been shown to reduce 10–15% of LDL cholesterol.^{5,18} Also, we have found that apo B concentration in LDL particles decreased in the consumers group. Our results were interesting since it is well established that elevated plasma LDL cholesterol and LDL apo B are known to play a major role in the development of atherosclerosis and subsequent CAD.¹⁹ Many clinical studies on Lp(a) have shown that a decrease in plasma Lp(a) concentration could offer protection from the risk of cardiovascular disease.²⁰ We have shown that

plasma concentration of Lp(a) was closely decreased in argan oil consumers suggesting that this group could be protected against atherosclerosis complications. It is known that some food habits and also drug treatment can influence the levels of Lp(a)^{20,21} although genetic determinants play an important role.

Our results indicate that regular consumption of argan oil improves the plasma lipoprotein profile in our study population.

Concerning the oxidative parameters, our results show a significant decrease in plasma LPO in the consumers group. Therefore, we showed a significantly higher α -tocopherol and also molar ratio of α -tocopherol/TC observed in the consumers of argan oil. Although the main tocopherol vitamer that we can find in argan oil is the γ -tocopherol,³

we have shown that the difference of plasma tocopherol levels between consumer and non consumer groups is mainly due to α -tocopherol. This result could be consequence of the eventual conversion from γ to α because of the close similarity between chemical structure of both molecules.²² These results suggest that their plasma is more protected against the oxidation process. A number of studies in animals and humans have demonstrated that natural antioxidants from foods inhibit the development of atherosclerosis.^{23,24} It has been suggested that the oxidative modification of LDL is believed to be a key event in the development of atherosclerosis.²⁵ Argan oil is rich in minor compounds such as polyphenols and tocopherols, which are powerful antioxidants. We also observed that plant sterols can increase the resistance to LDL oxidation showing an antioxidant effect of these compounds. Our results show a consistent dose-dependent antioxidant effect of tocopherols, polyphenols and sterols, extracted from argan oil, by measuring the resistance of LDL to oxidation. Previous studies in humans have shown that vitamin E^{26,27} and phenolic compounds^{6,28,29} extracted from extra-virgin olive oil, cacao, tea and red wine significantly inhibited LDL oxidation. A similar increase in LP during the oxidation of LDL was noted in a recent study with dietary virgin olive oil phenols.^{4,30} It would be interesting to confirm by interventional studies the protective cardiovascular effects of argan oil.

In conclusion, our findings show for the first time that usual consumption of virgin argan oil has a LDL-cholesterol lowering effect together with antioxidant properties. This oil offers an additional product to reduce cardiovascular risk factors thus retarding the onset of the atherosclerosis process.

Acknowledgements

We thank Anna Cabré, Mercedes Heras and Silvia Olivé for their excellent technical support.

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