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Argan oil improves surrogate markers of CVD in humans

Souad Sour[1], Meriem Belarbi[1], Darine Khaldi[1], Nassima Benmansour[1], Nassima Sari[1], Abdelhafid Nani[1], Farid Chemat[2] and Francesco Visioli[3]*

[1]*Natural Products Laboratory, Department of Biology, Faculty of Natural and Life Sciences and Sciences of Earth and the Universe, Abu Bakr Belkaid University, Tlemcen, Algeria*
[2]*Eco-Extraction Laboratory of Natural Products (GREEN), University of Avignon and Pays de Vaucluse, France*
[3]*Laboratory of Functional Foods, IMDEA-Food, Calle Faraday 7, 28049 Madrid, Spain*

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Abstract

Limited – though increasing – evidence suggests that argan oil might be endowed with potential healthful properties, mostly in the areas of CVD and prostate cancer. We sought to comprehensively determine the effects of argan oil supplementation on the plasma lipid profile and antioxidant status of a group of healthy Algerian subjects, compared with matched controls. A total of twenty healthy subjects consumed 15 g/d of argan oil – with toasted bread – for breakfast, during 4 weeks (intervention group), whereas twenty matched controls followed their habitual diet, but did not consume argan oil. The study lasted 30 d. At the end of the study, argan oil-supplemented subjects exhibited higher plasma vitamin E concentrations, lower total and LDL-cholesterol, lower TAG and improved plasma and cellular antioxidant profile, when compared with controls. In conclusion, we showed that Algerian argan oil is able to positively modulate some surrogate markers of CVD, through mechanisms which warrant further investigation.

Key words: Argan oil: *Argania spinosa*: CVD: Atherosclerosis: Cholesterol: Lipid oxidation: Mediterranean diet

Diet and its components play major roles in the onset and development of several degenerative diseases such as atherosclerosis, CVD, cancer and neurodegeneration[1]. In this respect, adherence to the Mediterranean diet is associated with lower incidence of CVD and cancer[2,3]. It must be emphasised that the term 'Mediterranean diet' encompasses several diverse dietary profiles typical of the Mediterranean basin[4,5]. Indeed, some differences, e.g. with respect to alcohol consumption or protein intake, exist among the various Mediterranean countries[5]. Among the various components of the Mediterranean diet, much attention is being paid to olive oil, because its consumption is associated with favourable cardiovascular outcomes[6]. However, some countries, namely those in the Maghreb area, also use argan oil for culinary and cosmetic applications[7]. Indeed, accumulating evidence suggests that argan oil might be endowed with potential healthful properties, mostly in the areas of CVD[8] and prostate cancer[9]. As an example, several recent studies reported hypolipidaemic, hypocholesterolaemic and antihypertensive effects of argan oil in the rat[7,10]. Argan oil is obtained from the fruit of *Argania spinosa* (Sapotaceae), an endemic tree which mostly grows in South-western Morocco. Argan trees also grow in Algeria, namely in the Tindouf countryside. While technological advancement, e.g. Soxlet's extraction, is changing argan oil production, most of its making still follows traditional procedures, i.e. hand-grounding and cold-pressing of the kernels, yielding yellowish, nutty-flavoured products[7]. The fatty acid profile of argan oil consists of approximately 45 % MUFA, approximately 35 % PUFA and approximately 20 % SFA. Moreover, virgin argan oil contains minor, bioactive components such as phenolic compounds, phytosterols and tocopherols[11,12].

Despite suggestive *in vitro* and animal evidence, the potential cardioprotective properties of argan oil have been the subject of very limited studies in humans[8,13]. Moreover, the *in vivo* effects of Algerian argan oil have never been investigated. Therefore, we sought to comprehensively determine the effects of argan oil supplementation on plasma lipid profile and antioxidant status of a group of healthy Algerian subjects, compared with matched controls.

Experimental methods

Materials and methods

Argan fruits were harvested in the Tindouf area of South-western Algeria. The argan oil used in the present work was

Abbreviations: CAT, catalase; LOOH, lipoperoxides; ORAC, oxygen radical absorbance capacity; TBARS, thiobarbituric acid-reacting substances.

* **Corresponding author:** F. Visioli, email francesco.visioli@imdea.org

Table 1. Composition of the argan oil adopted in this study

Fatty acid	%
C16 : 0	12·89
C18 : 0	4·83
C16 : 1*n*-9	0·4
C18 : 1*n*-9	45·01
C18 : 2*n*-6	35·39
C18 : 3*n*-3	0·2
Total SFA	17·72
Total MUFA + PUFA	81
MUFA/PUFA	1·27
α-Tocopherol (mg/kg)	56·34
Phenolic compounds (mg/kg)	52·36

produced from freshly picked seeds of a single harvest, using traditional hand-methods. The oil was kept at 4°C in a brown glass bottle until the beginning of the trial.

The fatty acid composition of the oil was determined by gas-phase chromatography (Applied Sciences Labs, State College, PA, USA). Fatty acid standards were from Nu-Check-Prep (Elysian, MN, USA). Vitamin E (α-tocopherol) was measured by HPLC according to Zaman *et al.*[14]. Polyphenols were extracted from the oil and quantified using the method of Pirisi *et al.*[15]. The composition of the oil at study is given in Table 1. More than 80 % of total fatty acids was composed of oleic and linoleic acids (45·01 and 35·39 %, respectively). Linolenic acid accounted for only 0·2 % of total fatty acids. SFA (mostly palmitic and stearic acids) accounted for 17·7 % of total fatty acids. This oil was devoid of erucic acid. Vitamin E (α-tocopherol) concentration was quite low, i.e. 56·34 mg/kg. Polyphenols were also scarcely present, i.e. 52·36 mg/kg.

Subjects

The present study conforms to the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects) and was approved by the local ethics committee. A total of forty healthy subjects aged 25–45 years with normal BMI and blood pressure were recruited from within the Abu Bakr Belkaid University (Tlemcen, Algeria) and written or oral informed consent to the study was taken from them. All participants were free of metabolic diseases such as hypercholesterolaemia, hypertriacylglycerolaemia, diabetes and hypertension, and were non-smokers and medication-free. We monitored the volunteers' lifestyle, e.g. physical activity, working hours and sleep duration, which did not markedly change throughout this study. A total of twenty subjects consumed 15 g/d of argan oil – with toasted bread – for breakfast, during 4 weeks (intervention group), whereas twenty matched controls followed their habitual diet, but did not consume argan oil. We found that provision of argan oil translated into lower consumption of habitual fat (oils and, to a lower extent, butter). It is noteworthy that, unexpectedly, consumption of olive oil in Algeria is quite low (approximately 1 kg/year), whereas milk consumption is higher than in neighbouring Maghreb countries[16,17].

We chose the dose of 15 g/d because (a) it was well tolerated by our volunteers and approximates habitual consumption and (b) Drissi *et al.*[8] previously reported on the healthful effects of this daily dose.

Both groups had similar anthropometric characteristics (Table 2). All subjects filled in a food questionnaire, in which they noted the quality and quantity of food consumed during the day before blood sampling, including their argan oil intake (which was nil in controls). The registered values were converted into energy and were estimated according to the Ciqual standard table of food composition[18]. All participants had similar physical activity and lifestyle.

Blood analyses

At days 0 (T_0), 15 (T_{15}) and 30 (T_{30}), venous blood was drawn into evacuated tubes, some of which contained disodium EDTA as the anticoagulant; other tubes did not contain anticoagulants to allow for the preparation of serum. Both serum and plasma were separated by centrifugation at 2100 ***g*** for 20 min at 4°C, aliquoted, and stored at −20°C. Erythrocytes were collected and washed three times in isotonic saline; then they were haemolysed by the addition of cold distilled water (1/4, vol/vol). Cellular debris was removed by centrifugation.

Lipoprotein and lipid determination

Plasma lipoproteins (LDL, $d < 1{\cdot}063$; HDL, $d < 1{\cdot}21$ g/ml) were separated by sequential ultracentrifugation. Serum total cholesterol (TC) and TAG were measured using enzymatic kits (Quimica Clinica Aplicada S.A., Amposta, Spain). HDL-cholesterol and LDL-cholesterol concentrations were also measured by enzymatic kits.

Determination of hydroperoxides

Both plasma and erythrocyte levels of hydroperoxides (lipoperoxides, LOOH) were measured by the ferrous ion oxidation-xylenol orange assay – using the specific LOOH reducer triphenylphosphine – according to the method of Nourooz-Zadeh *et al.*[19].

Determination of thiobarbituric acid-reacting substances

Thiobarbituric acid-reacting substances (TBARS) in plasma and erythrocytes were measured according to the method of Nourooz-Zadeh *et al.*[19].

Table 2. Anthropometric characteristics of the study subjects (Mean values and standard deviations)

	Argan oil (*n* 20)		Controls (*n* 20)	
	Mean	SD	Mean	SD
Age (years)	37·9	2·18	35·2	1·52
BMI (kg/m^2)	23·08	0·37	22·82	0·55
Glycaemia (mg/l)	931	20·0	926	18·7
SBP (mmHg)	117	2·59	115·5	2·32
DBP (mmHg)	73·5	1·29	70·5	1·7

SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 3. Dietary intakes at day 30

	Argan oil (*n* 20)				Controls (*n* 20)			
	g/d		%		g/d		%	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy intake								
kcal/d	2108·06		40·59		2100·14		93·28	
kJ/d	8820·12		169·82		8786·98		390·28	
Total protein	74·86	2·86	14·20	0·55	73·83	3·89	14·06	0·78
Total sugars	313·24	9·70	59·43	1·87	319·58	7·46	60·86	1·95
Simple sugars	100·85	2·64	32·19	1·42	104·05	4·84	32·55	1·75
Complex sugars	212·39	9·98	67·80	3·46	215·53	7·69	67·44	2·78
Total lipids	61·74	3·82	26·35	1·66	58·5	2·79	25·06	1·26
SFA	22·41	1·23	36·92	2·31	23·02	1·28	39·35	2·66
MUFA	20·75	1·74	33·60	3·26	20·78	1·17	35·52	2·43
PUFA	18·58*	1·17	30·09*	2·19	14·7	0·96	25·12	2·00
Cholesterol (mg/d)	244·82		15·58		257·72		34·74	

* Mean values were significantly different ($P< 0·05$).

Determination of carbonyl proteins

Carbonyl proteins were measured in plasma and erythrocytes by the 2,4-dinitrophenylhydrazine reaction described by Levine *et al.*[20].

Conjugated diene formation

The *in vitro* oxidisability of plasma lipoproteins induced by metals, i.e. copper sulphate, was analysed by monitoring, over time, the formation of conjugated dienes, as described by Esterbauer *et al.*[21].

Determination of plasma levels of vitamins A, E and C

Vitamins A (retinol) and E (α-tocopherol) were determined in the plasma of all volunteers by HPLC coupled with UV detection at 292 nm for vitamin E and 325 nm for vitamin A, according to Zaman *et al.*[14]. Vitamin C was measured in plasma by the method of Jagota & Dani[22].

Determination of catalase activity

Catalase (CAT; *EC* 1.11.1.6) activity was measured by spectrophotometric analysis of the rate of H_2O_2 decomposition at 240 nm, according to the method of Aebi[23].

Oxygen radical absorbance capacity

The total antioxidant ability of plasma (oxygen radical absorbance capacity, ORAC) was estimated by the capacity of erythrocytes to resist free-radical-induced haemolysis, according to the method of Blache & Prost[24].

Statistical analysis

Results are expressed as means and standard deviations. The Student's *t* test was used to compare data from the intervention group with those from controls. Statistical analysis was performed using Statistica (version 4.1, Statsoft, Paris, France). A *P* value less than 0·05 was considered as statistically significant.

Results

Dietary profile

Neither total daily energy intake nor consumption of proteins, carbohydrates and lipids significantly differed between the two groups during the experiment (Table 3). Consumption of SFA and MUFA was also similar in both groups; however, argan oil intake resulted in a higher overall consumption of PUFA (Table 3).

Table 4. Plasma lipid profile of argan oil-supplemented (A) and control (C) subjects, at different time points†

(Mean values and standard deviations)

	C		A-T_0		A-T_{15}		A-T_{30}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TAG (mg/l)	1210	80	1240	10	1170	90	980*	60
TC (mg/l)	1620	70	1640	60	1520	40	1400*	60
HDL-C (mg/l)	420	20	410	20	440	20	470	10
LDL-C (mg/l)	790	30	810	30	730	20	680**	20

T_0, day 0; T_{15}, day 15; T_{30}, day 30; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.

Mean values were significantly different from those of controls: *$P<0·05$, **$P< 0·001$.

† The plasma lipid profile of control subjects did not change significantly throughout the study: we present the average value of $T_0 + T_{15} + T_{30}$.

Table 5. Circulating and cellular markers of lipid and protein oxidation of argan oil-supplemented (A) and control (C) subjects, at different time points†

(Mean values and standard deviations)

	C		A-T_0		A-T_{15}		A-T_{30}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TBARS pl (μmol/l)	1·31	0·05	1·45	0·06	1·31	0·08	1·19*	0·06
TBARS erythrocytes (μmol/l)	8·67	1·57	8·70	1·71	7·89	1·30	6·65*	1·51
LOOH pl (μmol/l)	0·86	0·11	0·93	0·13	0·77	0·11	0·46**	0·05
LOOH erythrocytes (μmol/l)	9·98	1·40	10·3	1·33	9·42	1·37	8·09*	1·30
PC pl (nmol/l)	61·55	2·35	62·74	2·48	59·47	2·39	56·83*	2·03
PC erythrocytes (nmol/l)	90·97	5·18	94·93	6·31	87·85	5·02	81·05	4·83

T_0, day 0; T_{15}, day 15; T_{30}, day 30; TBARS, thiobarbituric acid-reacting substances; pl, plasma; LOOH, lipoperoxides; PC, protein carbonyls.

Mean values were significanty different from those of controls: *$P<0·05$, **$P<0·01$.

† The biomarkers of control subjects did not change significantly throughout the study: we present the average value of $T_0 + T_{15} + T_{30}$.

Circulating lipids

We recorded a significant improvement in the plasma lipid profile of subjects who were given argan oil as compared to controls, whose values did not significantly change during the study (data not shown). In particular, TAG decreased by 20·97 %, TC by 14·63 % and LDL-cholesterol by 16·05 % after 30 d of treatment (Table 4). We also recorded a concomitant non-significant increase in HDL-cholesterol.

Circulating and cellular markers of oxidation and antioxidant status

Circulating and cellular markers of lipid (TBARS and LOOH) and protein (carbonyls) oxidation are shown in Table 5. Argan oil significantly decreased the former (at T_{30}), but not the latter. In addition, susceptibility of LDL to copper-induced oxidation was decreased in argan oil-treated subjects, as indicated by the significantly increased lag phase and decrease of conjugated diene production (Table 6).

Plasma vitamin E concentrations were significantly increased (+18 % at T_{15}, $P< 0·05$ and +43 % at T_{30}, $P< 0·001$ when compared with controls; Table 7) by argan oil ingestion throughout the study. Conversely, vitamins A and C did not change significantly in either group. CAT activity also increased significantly in argan oil-treated subjects, whereas plasma ORAC remained unmodified.

Discussion

We performed the first trial of Algerian argan oil in human subjects and evaluated a wide array of surrogate markers of CVD. Our present study results show that daily consumption of feasible amounts of argan oil positively modulates such markers. Argan oil consumption is increasing in Europe and high-quality virgin argan oil can also be currently purchased in Japan and the USA, where it is mostly sold for its purported cosmetic properties. Thus far, due to its elevated price, the dietary use of argan oil is mostly limited to the areas where it is produced (for the most part Morocco, although West Algeria also contributes). As such, it contributes to the diet of selected population groups and is an integral part of the Maghrebian version of the Mediterranean diet.

Our results are in agreement with those of Drissi *et al.*[8] and of Cherki *et al.*[13] and fit with cumulated animal evidence of the anti-atherogenic potential of argan oil[7]. In particular, argan oil consumption – in feasible amounts – decreased total and LDL-cholesterol and TAG. Of note, our subjects had low mean cholesterolaemia, which reflects the average values found in countryside Algeria. Other cardiovascular parameters such as blood pressure were not modified by argan oil consumption, in contrast with the findings of Berrougui *et al.*[25] who reported hypotensive effects in normotensive Wistar rats. This discrepancy might be due to species specificity, dose or treatment duration.

Table 6. Susceptibility of LDL to oxidation of argan oil-supplemented (A) and control (C) subjects, at different time points†

(Mean values and standard deviations)

	C		A-T_0		A-T_{15}		A-T_{30}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
LP (min)	57·5	5·40	56·25	4·78	62·75	5·75	67·5*	6·45
MR (min)	122·5	6·45	123·25	7·05	124·55	7·27	126·75	8·16
IDP (nmol/l)	33·05	1·39	36·68	0·62	32·22	0·58	30·83	0·95
MDP (nmol/l)	40·83	4·61	43·25	4·97	38·30	4·53	33·7*	3·94

T_0, day 0; T_{15}, day 15; T_{30}, day 30; LP, lag phase; MR, maximal rate; IDP, initial conjugated diene production; MDP, maximum conjugated diene production.

* Mean values were significantly different from those of controls ($P< 0·05$).

† The susceptibility of LDL to oxidation of control subjects did not change significantly throughout the study: we present the average value of $T_0 + T_{15} + T_{30}$.

Table 7. Plasma antioxidant parameters of argan oil-supplemented (A) and control (C) subjects, at different time points

(Mean values and standard deviations)

	C		A-T_0		A-T_{15}		A-T_{30}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Vitamin C (μg/ml)	13·25	0·31	13·42	0·43	13·05	0·25	13·6	0·46
Vitamin E (μg/ml)	21·52	1·78	21·46	1·75	25·21*	1·92	30·66**	2·01
Vitamin A (μg/ml)	66·54	1·59	66·92	1·91	65·46	0·68	71·54	1·98
ORAC (AU)	2·22	0·11	2·1	0·10	2·43	0·11	2·97	0·12
CAT pl (U/ml)	0·23	0·02	0·22	0·02	0·26	0·03	0·28	0·03
CAT erythrocytes (U/ml)	81·21	2·64	80·12	1·95	82·05	1·71	88·88*	2·08

T_0, day 0; T_{15}, day 15; T_{30}, day 30; ORAC, oxygen radical absorbance capacity; AU, arbitrary; CAT, catalase; pl, plasma.
Mean values were significantly different from those of controls: *$P<0·05$, **$P<0·01$.

The exact mechanisms of action by which argan oil exerts its lipid-modulating and potentially anti-atherogenic actions remain elusive. However, we speculate that the high proportion of PUFA and MUFA in the argan oil we administered – and reflected in the overall dietary intake (Table 3) – played marked roles, as suggested by the available literature[26,27]. Other contributors to the hypolipidaemic effects of argan oil are – probably – sterols and saponins, which we did not measure in the present investigation due to technical limitations, but which have been previously investigated by Khallouki *et al.*[11] and Berrougui *et al.*[28]. Both classes of molecules interfere with intestinal cholesterol absorption and are currently exploited as functional food components[29].

We also evaluated circulating markers of oxidation and report that argan oil consumers had lower concentrations of LOOH and TBARS, but not of oxidatively-modified proteins. Total antioxidant capacity (evaluated as ORAC) also did not change. Finally, we recorded increased intracellular CAT activity. This finding is, indeed, counterintuitive (there should be no reason to activate an antioxidant enzyme if the antioxidant profile is augmented). However, several studies with antioxidants, e. g. polyphenols, reported similar increases in the activity of antioxidant enzymes, namely superoxide dismutase and CAT [30]. As far as argan oil is concerned, our results agreed with those of Benajiba *et al.*[31], which showed that the activities of cytosolic CAT were significantly higher in Wistar rats treated with argan oil in comparison with the untreated rats. We can conceivably attribute the observed antioxidant actions (including increased LDL resistance to oxidation, which confirms previous findings of Cherki *et al.*[13]) to the provision of vitamin E (which increased in consumers' plasma, Table 7) by argan oil. While Algerian argan oil appears to contain less vitamin E than, for example, the average Moroccan argan oil or extra-virgin olive oil, the contribution of tocopherols to the overall intake is, apparently, sufficient to elicit antioxidant effects. Other components such as polyphenols, namely ferulic acid [32], and sterols might have played a role. The precise nature of the cumulative positive effects on antioxidant profile, however, is still elusive and requires *ad hoc* investigations.

Our present study has several limitations – most of which are due to technical constraints – which we should acknowledge. As mentioned, we could not analyse the argan oil's content of sterols and saponins and, thus, we rely on the data of Khallouki *et al.*[11] and Berrougui *et al.*[28]. We also could not evaluate the modifications of plasma and lipoprotein lipid profile brought about by consumption of argan oil. Hence, we rely on food composition tables to estimate intakes. Finally, we assessed an array of lipid peroxidation indices, due to the current lack of robust biomarkers[33]; however, each of them has shortcomings[34].

In conclusion, we showed that argan oil is able to positively modulate some surrogate markers of CVD, through mechanisms which warrant further investigation. Moreover, based on these results, argan oil could prove to be an effective livelihood opportunity to diversify the options of small rural Berber producers and enhance their incomes.

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