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Basic nutritional investigation

A comparative study on the effect of argan oil *versus* fish oil on risk factors for cardio-vascular disease in high-fat-fed rats

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ABSTRACT

Objectives: The aim of this study was to investigate the effects of two different sources of polyunsaturated fatty acid—fish oil (FO) and argan oil (AO)—on some risk factors for cardiovascular disease, such as platelet aggregation, dyslipidemia, and oxidative stress.
Methods: To explore this, four groups of six male rats were fed with different diets: The first group received a standard diet (control); the second group received a high-fat diet; the third was fed with a high-fat diet supplemented with 5% FO, and the last group received a high-fat diet supplemented with 5% AO.
Results: After 8 wk of the diet, AO showed a decrease in plasma lipids similar to that of FO. However, unlike FO, AO had no significant effect on hepatic lipid levels. On the other hand, supplementation with AO and FO similarly reduced platelet hyperactivity induced by high-fat diet. Concerning the results of oxidative stress, AO showed an antioxidant effect in the tissues and platelets greater than that observed in the high-fat FO group.
Conclusions: For rats, the consumption of FO prevented the development of adiposity, restored insulin sensitivity, decreased plasma and liver lipid levels, and also prevented the prothrombotic effect. Intake of AO as a food supplement did not affect adiposity or liver lipid levels but decreased plasma lipid levels and improved oxidative status and platelet activity. FO and, to a lesser degree, AO thus represent promising nutritional tools in the prevention of cardiovascular disease.

Introduction

Dyslipidemia is a metabolic disorder that can induce several risk factors for cardiovascular diseases (CVD) such as hypertension, insulin resistance (IR), inflammation, and abdominal obesity [1]. Platelet activation is thought to play a crucial role in the initiation and development of atherosclerotic lesions [2]. The mechanism by which CVD risk factors increase platelet activity is related to the production of the oxidized low-density lipoprotein (LDL) as well as to the increased reactive oxygen species in patients [3]. The type of polyunsaturated fatty acid (PUFA) also may differentially affect the cardiovascular outcome. A high intake of fatty fish or long-chain ω-3 fatty acids (eicosapentaenoic acid [EPA; 20:5 ω-3] and docosahexaenoic acid [DHA; 22:6 ω-3]) present in fish oil (FO) has fairly consistently been associated with a lower incidence of CVD risk factors in prospective cohort studies and in animal studies [4]. In addition to containing EPA and DHA, fish is a good source of iodine, selenium, taurine, high-quality proteins, and vitamins D and B_{12} [5]. Hence, dietary guidelines recommend increasing the consumption of fish, with an emphasis on consuming fatty fish [6]. However, intake of lean fish is also recommended as it may reduce blood pressure (BP) [7] and lower triacylglyceride (TG) levels [8].

Omega-6 fatty acids and in particular their precursor, linoleic acid (18:2 ω-6), often have been shown to exert proinflammatory and prothrombotic effects [9]. On the other hand, long chain ω-6 PUFA, namely, γ-linolenic acid (18:3 ω-6), was reported to be protective against CVD risk factors [10]. Among vegetable oils, argan

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oil (AO) is traditionally used in Morocco against hypercholesterolemia and associated CVD. It is extracted from the almonds of the *Argania Spinosa*, an endemic Moroccan tree growing in semi-desert areas. This oil contains a high proportion of monounsaturated fatty acids (MUFAs; mainly oleic acid, 18:1 ω-9, 45%) and ω-6 PUFAs (35% 18:2 ω-6) and a very low ω-3 PUFA levels (0.1–0.3% 18:3 ω-3). It is also rich in antioxidants (e.g., tocopherol and plant sterols) and in several phenolic compounds (e.g., ferulic, syringic, and vanillic acids) [11,12]. As could be expected from its interesting composition, AO has demonstrated its pharmacologic effects in several studies. AO intake has been shown to decrease several CVD risk factors, such as atherogenic lipids [13], BP [14], and IR [15] in human and animal models. Haimeur et al. [16] recently demonstrated the antiplatelet and antioxidant effects of AO in dyslipidemic patients. In animals, Mekhfi et al. [17] reported that AO has antithrombotic activity in rats, which could be related to its antiplatelet aggregation rather than its anticoagulant activity. Other studies have demonstrated that AO can be used as a balanced dietary supply without marked adverse effects on immune cell function [18].

The present study was undertaken to compare the preventive effects of FO and AO intake on high-fat diet–induced metabolic disorder risk factors in rats. In this study, we used a high-fat diet containing 40% of lipids composed mainly of saturated fat (33%), and the effects of FO (rich in ω-3 PUFAs) and AO (rich in ω-6 and ω-9 PUFAs) on lipid levels, IR, platelet activity, and oxidative status in rats fed a high-fat diet were compared.

Methods

Animals and experimental protocol

All the experiments involving animals were approved by the institutional ethics committee (Direction des Services Vétérinaires de la Mayenne, France). Male Wistar rats weighing 120 ± 10 g (purchased from Centre d'Elevage Janvier, Le Genest Saint Isle, France) were randomly assigned to four groups (six animals per group) as follows: control group (C), fed a standard diet with 16 kcal% fat (SAFE, Augy, France); a high-fat (HF) group fed a high-fat diet with 64 kcal% fat (essentially coprah); an HFFO group that received the high-fat diet supplemented with 5% (w/w) FO (Polaris, Pleuven, France); and an HFAO group that was fed a high-fat diet supplemented with 5% (w/w) of AO (Argan Oil Company, Casablanca, Morocco). The composition of each diet is detailed in Table 1. All animals were housed in pairs in a room under standard conditions of temperature (22–24°C), humidity (40–60%), and 12-h light/dark cycle. They were fed ad libitum with free access to water.

The body weight (BW) gains of the rats were monitored at regular intervals, and their daily food intakes were estimated. Rats were fed the test diets for 7 wk and were anesthetized (with Diazepam/Ketamine 4 v/3 v) after an overnight fast on day 49, and sacrificed via the abdominal aorta. The liver and the visceral adipose tissue (AT) were removed, rinsed with ice-cold sodium chloride (0.9%), weighed, frozen in liquid nitrogen and kept for lipid and oxidative stress analysis.

Measurement of plasma and liver biochemical parameters

Glycemia was measured in rat tails using a glucometer (FreeStyle PAPILLON mini, Abbott, Rungis, France). However, plasma total cholesterol (TC) and TGs were measured using commercial enzyme kits (Biomérieux S.A, Marcy l'Etoile, France). The plasma insulin level was measured using a commercially available, enzyme-linked immunosorbent assay (ELISA) kit (Eurobio, Courtaboeuf, France). Hepatic cholesterol and TG levels were determined in an aliquot of the liver total lipid extract using commercial kits (Biomérieux S.A, Marcy l'Etoile, France).

Fatty acid analysis of plasma total lipids and liver phospholipids

The extraction of liver total lipids was conducted using the method of Folch et al. [20] Plasma total lipids were extracted following the method described by Bligh and Dyer [21]. Phospholipids analysis was conducted on the liver as described in Haimeur et al. [22].

Table 1
Composition of the experimental diets (g/kg diet)

Nutrient	C	HF	HFAO	HFFO
Caseine	230	230	230	230
Cornstarch	200	80	80	80
Glucose	360	150	150	150
Cellulose	60	60	60	60
Lard	50	50	-	-
Coprah	-	330	330	330
Argan oil	-	-	50	-
Fish oil	-	-	-	50
Corn oil	10	10	10	10
Rape oil	10	10	10	10
Mineral 205 B SAFE	70	70	70	70
Vitamine 200 SAFE	10	10	10	10

C, control diet; HF, high-fat diet; HFAO, high-fat diet supplemented with 5% (w/w) argan oil; HFFO, high-fat diet supplemented with 5% (w/w) fish oil.
Analysis was provided by SAFE (Scientific Animal Food & Engineering, Augy, France)
The mineral mixture provides the following amounts in mg/kg of diet: $CaHPO_4$, 17.2; KCl, 4000; NaCl, 4000; MgO, 420; $MgSO_4$, 2000; Fe_2O_3, 120; $FeSO_4$, 7 H_2O, 200; trace elements, 400.
Trace element mixture (mg/kg of diet): $MnSO_4$, H_2O, 98; $CuSO_2$, 5 H_2O, 20; $ZnSO_4$, 7 H_2O, 80; $CoSO_4$, 7 H_2O, 0.1; KI, 0.3. The vitamin mixture provides the following amounts per kg of diet: Retinol, 39.600 IU; cholecalciferol, 5000 IU; thiamin, 40 mg; riboflavin, 30 mg; pantothenic acid, 140 mg; pyridoxine, 20 mg; inositol, 300 mg; cyanocobolamin, 0.1 mg; ascorbic acid, 1600 mg; choline, 2.720 mg; folic acid, 10 mg; *P*-aminobenzoic acid, 100 mg; biotin, 0.6 mg.

Platelet aggregation

In vitro platelet aggregation was measured according to Born's [23] turbidimetric method on washed platelet suspension using a four-channel Apact aggregometer (LABiTEC, Ahrensburg, Germany). The platelet-rich plasma was isolated as described in detail in Haimeur et al. [22]. Platelet suspension concentrations were estimated using a Coulter cell counter (Beckman Coulter, Villepinte, France), and the concentrations were adjusted to 5×10^5 cells/μL using Tyrode buffer. A sample of washed platelets was preincubated at 37°C for 10 min in the cuvettes stirred at 1000g. Platelet stimulation was initiated by adding adenosine diphosphate (ADP) 5 μM (Sigma-Aldrich, Saint-Quentin Fallavier, France) or collagen 5 μg/mL (Kordia, Lille, France). The light transmission was recorded for 5 min after platelet stimulation. The platelet aggregation was quantified as the maximum change in light transmission through a washed platelet solution expressed as a percentage of the light transmission through the blank (Tyrode buffer).

Platelet thromboxane B_2 measurement

To determine the platelet thromboxane B_2 (TxB_2) level, isolated platelets were subjected to three successive freeze/thaw cycles to release the cell contents. The baseline TxB_2 level was determined using an ELISA kit (ADI-900-002/txb2-Elisa-kit, Enzo-Life Sciences, Exeter, UK) according to the manufacturer's instructions.

Oxidative status evaluation in liver and platelets

The redox status in liver and platelets was evaluated by determining the malondialdehyde (MDA) level and the glutathione peroxidase (GPx) activity. The MDA level was measured separately in the liver homogenate or washed-platelet solution using the method of Ohkawa [24] as previously described in Haimeur et al. [19]. The GPx activity was determined according to the method of Paglia and Valentine [25] as modified by Chaudière and Gérard [26].

Statistical analysis

All values were expressed as the mean ± SD. After analysis of variance, the mean values were compared using Fisher's least significant difference test (Statgraphics Plus 5.1, Manuguistics Inc., Rockville, MD, USA).

Results

Body weight, organ weights, and food intake

As shown in Table 2, final BW did not significantly differ among the control group and HF-fed rats. However, supplementation with FO significantly decreased final BW. Among measured organ

Table 2
Effects of supplementation with argan oil compared with fish oil on the body and organ weights, daily food intake plasma and organ biochemical parameters in rats fed a high-fat diet for 8 wk (mean values + SD for n = 6)

Groups	C		HF		HFAO		HFFO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight (g)	465.4 [a]	22.7	468 [a]	31.2	461.8 [a]	20.5	449.3 [b]	22.5
Liver weight (g)	12.4	0.7	11.8	1.7	12.5	0.7	12.4	1.1
Adipose tissue (g)	13.6 [b]	2.8	17.1 [a]	3	15.8 [a]	2.7	9.5 [c]	2.6
Food intake (g/d)	26.7 [a]	2.7	19.7 [b]	1.1	19.3 [b]	1	18.9 [b]	1.9
AT/BW (%)	2.7 [b]	0.7	3.3 [a]	1.2	3.4 [a]	0.9	2.2 [b]	1.3
Plasma (mmol/L)								
Glucose	3.17	0.17	3.11	0.28	3.46	0.31	3.35	0.23
Cholesterol	1.81 [a]	0.13	1.86 [a]	0.23	1.37 [b]	0.15	1.23 [b]	0.12
Triacylglycerols	0.74 [a]	0.11	0.82 [a]	0.22	0.64 [b]	0.15	0.58 [b]	0.17
Insulin (ng/mL)	0.64 [b]	0.16	1.12 [a]	0.22	0.99 [a]	0.18	0.61 [b]	0.12
HOMA-IR	2.21 [b]	0.21	3.74 [a]	0.32	3.12 [a]	0.34	2.26 [b]	0.25
Liver (mg/g)								
Cholesterol	5.67 [b]	0.98	22.32 [a]	1.95	20.45 [a]	1.87	4.73 [b]	0.76
Triacylglycerols	40.51 [b]	8.04	89.23 [a]	7.81	86.42 [a]	6.12	39.67 [b]	7.5

C, control diet; HF, high-fat diet; HFAO, high-fat diet supplemented with 5% (w/w) argan oil; HFFO, high-fat diet supplemented with 5% (w/w) fish oil; HOMA-IR, homeostatic model assessment-insulin resistance.
[a, b, c] Mean values with different superscript letters are significantly different ($P < 0.05$).

weights, only adipose tissues were affected by the dietary treatments. HF diet significantly increased the abdominal adipose tissue (AT) weight in the HF group compared with the control group ($P < 0.05$); thus the AT/BW ratio was significantly greater ($P < 0.05$) in the HF group than in the control group, confirming the development of obesity in this dietary model. The increased adiposity was prevented by FO but not by AO intake in HF-fed rats. During the experiment, the food intake of the rats fed the standard diet was greater than that of those fed with the HF diets, despite their equal overall energy intakes.

Plasma biochemical parameters

As shown in Table 2, the data showed no significant difference in plasma glucose and lipid levels between the control group and the HF mice. However, a significant increase in the plasma insulin level ($P < 0.05$) was observed in rats fed the HF diet versus the control animals. AO treatment significantly decreased ($P < 0.05$) cholesterol and TG levels in plasma compared with HF diet intake, but no significant effect on plasma insulin levels was observed in AO group ($P = 0.062$). However, FO treatment significantly decreased ($P < 0.05$) both plasma lipid and insulin levels compared with the HF diet.

Measurements of the hepatic TG and cholesterol levels revealed that HF intake significantly increases the hepatic lipid levels compared with control group. The increased hepatic lipid level was prevented by FO intake but AO intake appeared to have no significant effect on hepatic lipid metabolism.

Fatty acid composition of the plasma total lipid and the hepatic phospholipids

Rats fed the HF diet showed a higher saturated fatty acid (SFA) content in plasma total lipids and liver phospholipids (Tables 3 and 4, respectively) compared with control rats. We analyzed the composition of the fatty acids in plasma total lipids, which reflected the fat consumed in the diet. The data of the composition of the fatty acids in plasma total lipids (Table 3) showed a significant increase of the oleic acid (18:1 ω-9) and the linoleic acid (18:2 ω-6) levels in the HFAO group compared with the other experimental groups. Oleic and linoleic acids are the main unsaturated fatty acids in AO. Consequently, an increase of total MUFAs and ω-6 PUFAs was observed in rats fed the HFAO diet compared with the other experimental diets. On the other hand, the fatty acid composition of the total plasma lipids in the HFFO group showed increased ω-3 PUFA levels compared with the other groups. The increased ω-3 PUFA level concerns the EPA (20:5 ω-3), the main fatty acid in the FO used in this study. We also analyzed the composition of the fatty acids in the liver phospholipids to evaluate the incorporation of the fatty acids provided from the experimental diets in the membranes of the hepatocytes. The data (Table 4) showed a significant increase of the oleic acid (18:1 ω-9) and the linoleic acid (18:2 ω-6) levels in the HFAO group compared with the others experimental groups. A higher level of ω-3 PUFAs was observed in the liver phospholipids of the HFFO group, specifically, the EPA and DHA levels.

Table 3
Effect of supplementation with argan oil compared with fish oil on the fatty acid composition of plasma total lipids in rats fed a high-fat diet for 8 wk (means values + SD for n = 6)

Fatty acid %	C		HF		HFAO		HFFO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	0.6 [b]	0.2	2.6 [a]	0.8	2.5 [a]	0.3	2.7 [a]	0.5
16:0	16.3	4.1	17.2	5	14.4	0.6	15.5	1.7
18:0	6.5 [b]	1.2	11.1 [a]	2.3	11.7 [a]	1.8	11.9 [a]	2.1
SFA	23.4 [b]	4.4	31[a]	4.9	29.2 [a]	2.1	30.5 [a]	2.3
16:1	1.7	0.8	0.8	0.1	0.5	0.1	0.9	0.2
18:1 ω-9	17.9 [a]	7.4	14.3 [b]	7.6	17.1 [a]	5.3	6.3 [c]	0.9
18:1 ω-7	0.1	0	-	-	0.2	0.05	-	-
22:1	0.6 [b]	0.2	0.8 [b]	0.2	1.3 [a]	0.2	0.3 [b]	0.1
20:1 ω-9	0.2	0.1	0.2	0.1	0.2	0.03	0.3	0.1
24:1 ω-9	1.3	0.6	1	0.4	1.1	0.3	0.7	0.3
MUFA	20.5 [a]	4.5	18.1 [b]	3.7	21.0 [a]	3.1	9.1 [c]	1.2
18:2 ω-6	7.6 [b]	1.2	8.9 [b]	2.5	12.2 [a]	1.2	7.8 [b]	0.8
20:4 ω-6	18.2 [a]	2.7	19.6 [a]	3.7	18.1 [b]	2	12.4 [b]	2.1
ω-6	26.2 [b]	3.1	28.7 [b]	3	30.9 [a]	2.1	21.2 [c]	2.5
18:3 ω-3	0.4	0.1	0.3	0.1	0.2	0.05	0.3	0.1
20:5 ω-3	0.2 [b]	0.0	0.2 [b]	0.1	0.1 [b]	0.02	8.8 [a]	1.7
22:5 ω-3	0.4 [b]	0.1	0.5 [b]	0.1	0.4 [b]	0.1	2.1 [a]	0.3
22:6 ω-3	2.2 [b]	0.8	2.8 [b]	0.9	1.85 [b]	0.1	5.2 [a]	0.7
ω-3	2.8 [b]	0.9	3.4 [b]	1	2.7 [b]	0.2	15.9 [a]	1.1
ω-6/ω-3	10.1 [a]	2.2	8.8 [a]	1.3	10 [a]	0.2	1.5 [c]	0.2
MUFA/SFA	0.9 [a]	0.2	0.6 [a]	0.2	0.7 [a]	0.1	0.3 [b]	0.1

C, control diet; HF, high-fat diet; HFAO, high-fat diet supplemented with 5% (w/w) argan oil; HFFO, high-fat diet supplemented with 5% (w/w) fish oil; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids.
-, not detected.
[a, b, c] Mean values with different superscript letters are significantly different ($P < 0.05$).

Table 4
Effect of supplementation with argan oil compared with fish oil on the fatty acid composition of liver phospholipids in rats fed a high-fat diet for 8 wk (mean values + SD for n = 6)

Fatty acid %	C		HF		HFAO		HFFO	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	0.4 [b]	0.02	3.1 [a]	1	3.6 [a]	0.3	2.2 [a]	0.3
16:0	20.9	1.2	20.9	1.5	19.7	0.8	19.6	1.6
18:0	14.6	0.1	17.1	2.7	15.4	0.5	17	2.4
SFA	35.9 [b]	1.2	41.3 [a]	0.8	39.1 [a]	1	37.5 [a,b]	1.5
16:1	2.1 [a]	0.5	1.1 [b]	0.3	0.7 [b]	0.1	1.1 [b]	0.5
18:1 ω-9	22.4 [a]	3.1	19.9 [b]	2.7	21.5 [b]	1.2	8.9 [c]	2.5
18:1 ω-7	0.07	0.02	0.05	0.01	-	-	-	-
22:1	0.6 [b]	0.1	0.9 [b]	0.09	1.68 [a]	0.2	0.2 [c]	0.06
20:1 ω-9	0.2	0.04	0.2	0.04	0.3	0.02	0.3	0.01
24:1 ω-9	0.06	0.04	0.06	0.02	0.1	0.01	0.07	0.02
MUFA	25.4 [a]	3.2	22.3 [a]	3.1	25.1 [a]	1.8	11.0 [b]	0.7
18:2 ω-6	9.4 [b]	0.6	10 [b]	0.3	15.6 [a]	0.8	7.6 [c]	0.3
20:4 ω-6	18.3 [a]	1.7	15.3 [b]	2.8	15.1 [b]	0.6	10.1 [c]	0.5
ω-6	28.1 [b]	2.1	25.5 [b]	2.9	31.2 [a]	1	17.3 [c]	0.9
18:3 ω-3	0.3	0.04	0.3	0.07	0.3	0.02	0.4	0.06
20:5 ω-3	0.1 [b]	0.03	0.1 [b]	0.01	0.1 [b]	0.01	6.1 [a]	0.8
22:5 ω-3	0.3 [b]	0.06	0.4 [b]	0.07	0.5 [b]	0.1	6.7 [a]	0.8
22:6 ω-3	4.7 [b]	0.6	4.8 [b]	0.6	3 [b]	0.1	14.1 [a]	1.2
ω-3	5.2 [b]	0.7	5.5 [b]	0.6	4.1 [b]	0.5	28.5 [a]	1
ω-6/ω-3	5.4 [b]	0.6	4.6 [b]	0.3	7.5 [a]	0.3	0.6 [c]	0.1
MUFA/SFA	0.7 [a]	0.08	0.5 [a]	0.1	0.6 [a]	0.07	0.3 [b]	0.05

C, control diet; HF, high-fat diet; HFAO, high-fat diet supplemented with 5% (w/w) argan oil; HFFO, high-fat diet supplemented with 5% (w/w) fish oil; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; -, not detected.
[a, b, c] Mean values with different superscript letters are significantly different ($P < 0.05$).

Oxidative status of the liver

Results concerning the oxidative stress in the liver are shown in Figure 1. After 8 wk, rats fed the HF diet displayed greater oxidative stress than the control rats. This oxidative stress was marked by a significant increase in liver MDA level (Fig. 1A) and a significant decrease in GPx activity (Fig. 1B). AO intake significantly prevented HF-induced oxidative status in the liver by decreasing the MDA level and enhancing the GPx activity. However, FO intake showed a significant increase of the GPx activity, but no effect on the MDA level was observed.

Platelet activity

Results obtained on ADP and collagen-induced platelet aggregation are reported in Figure 2A. The data showed that significant platelet hyperaggregation occurred in rats fed the HF diet compared with the controls. AO intake significantly decreased platelet aggregation induced by ADP by ~33% and collagen-induced platelet aggregation was decreased by ~23% compared with the HF group. FO intake showed a similar effect on platelet aggregation as that observed in AO intake. The effects of the different experimental diets on platelet TxB_2 levels are reported in Figure 2B. The data showed that rats fed the HF diet displayed significantly more platelet TxB_2 than control rats. FO intake significantly prevented increased platelet TxB_2 levels but not AO intake. The effect of AO and FO on platelet oxidative status was investigated and the results are shown in Figure 3. The data reveal greater oxidative stress in the platelets of rats fed the HF diet than in control rats. AO intake significantly improved HF diet–induced oxidative stress in platelets by significantly reducing, the MDA level and enhancing GPx activity. However, FO supplementation significantly increased platelet GPx activity but also increased platelet lipid peroxidation by increasing platelet MDA level.

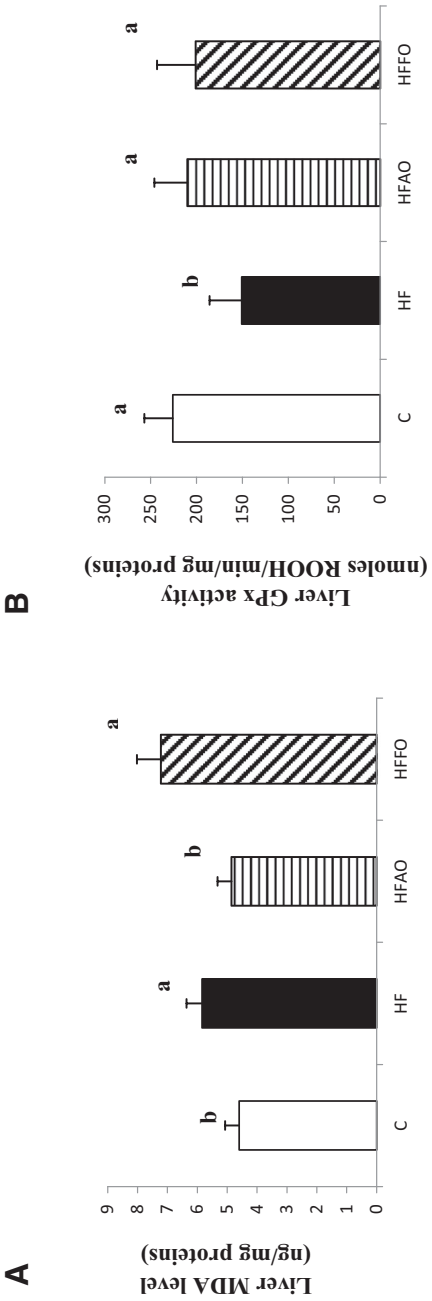

Fig. 1. Effects of supplementation with argan oil compared with fish oil on (**A**) the liver malondialdehyde (MDA) level and (**B**) the liver glutathione peroxidase (GPx) activity in rats fed a high-fat diet for 8 wk. Values are means + SD (n = 6), represented by vertical bars. [a, b] Mean values with different superscript letters are significantly different ($P < 0.05$). C, control diet; HF, high-fat diet; HFAO, high-fat diet supplemented with 5% (w/w) argan oil; HFFO, high-fat diet supplemented with 5% (w/w) fish oil. , control diet; , high-fat diet; , high-fat diet supplemented with argan oil; , high-fat diet supplemented with fish oil.

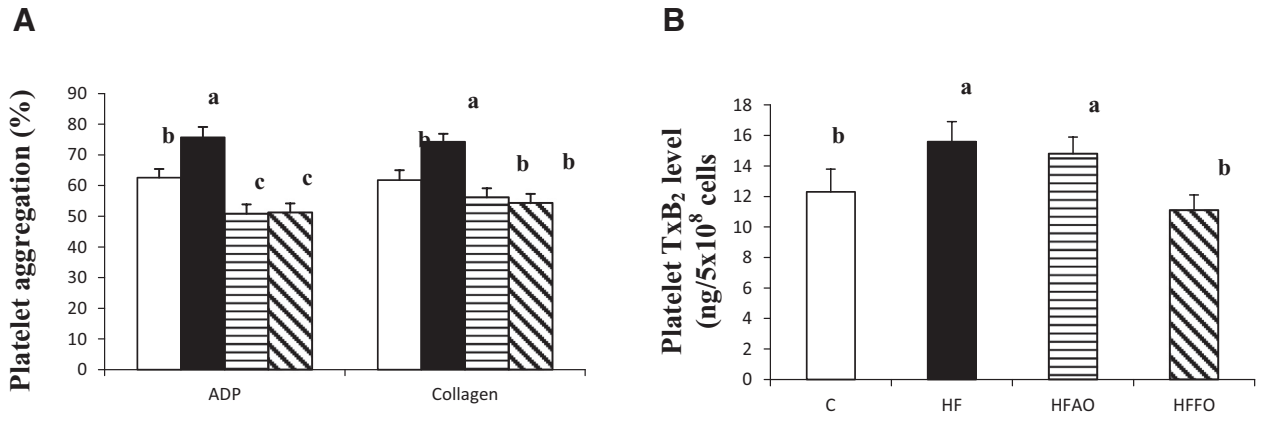

Fig. 2. Effects of supplementation with argan oil compared to that with fish oil on (**A**) platelet aggregation and (**B**) platelet thromboxane B_2 (TxB_2) level in rats fed a high-fat diet for 8 wk. Values are means + SD (n = 6), represented by vertical bars. [a, b, c] Mean values with different superscript letters are significantly different ($P < 0.05$). Rat platelets were isolated as described in material and methods section and stimulated with adenosine diphosphate (ADP) 5 μM or with collagen 5 μg/mL. C, control diet; HF, high-fat diet; HFAO, high-fat diet supplemented with 5% (w/w) argan oil; HFFO, high-fat diet supplemented with 5% (w/w) fish oil. □, control; ■, high-fat; ▤, high-fat supplemented with argan oil; ▨, high-fat supplemented with fish oil.

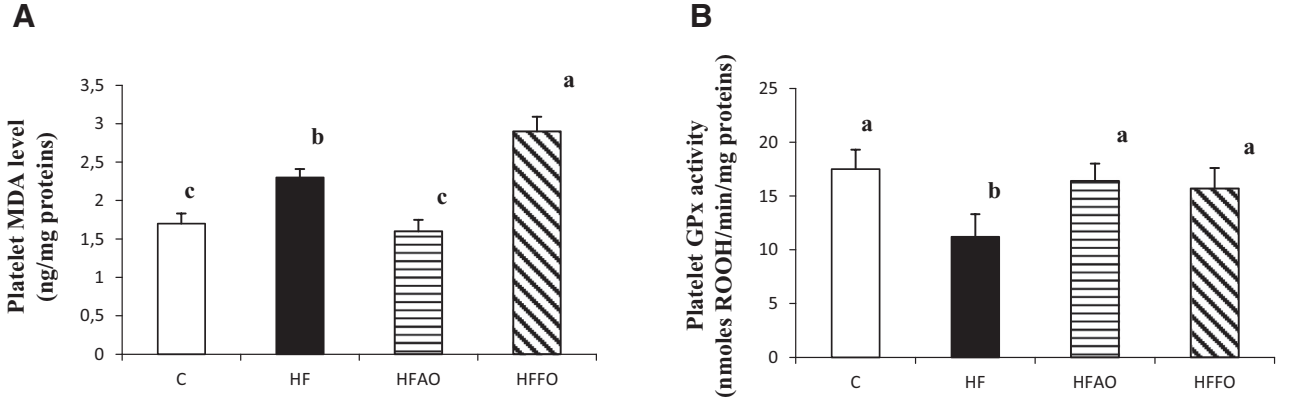

Fig. 3. Effects of supplementation with argan oil compared with fish oil supplementation on (**A**) platelet malondialdehyde (MDA) level and (**B**) platelet glutathione peroxidase (GPx) activity in rats fed a high-fat diet for 8 wk. Values are means + SD (n = 6), represented by vertical bars. [a, b, c] Mean values with different superscript letters are significantly different ($P < 0.05$). C, control diet; HF, high-fat diet; HFAO, high-fat diet supplemented with 5% (w/w) argan oil; HFFO, high-fat diet supplemented with 5% (w/w) fish oil. □, control diet; ■, high-fat diet; ▤, high-fat diet supplemented with argan oil; ▨, high-fat diet supplemented with fish oil.

Discussion

Results from the present study showed that the intake of either dietary FO or AO can improve some features of CVD risk factors in HF-fed rats, a well-known model of diet-induced metabolic disorders. The results with the FO diet confirm the results of previous studies concerning its beneficial action in CVD risk factors, notably by showing a reduction in adiposity [9,19]. Previous studies have demonstrated the beneficial effects of AO on blood lipid parameters in animals and humans [12–16,27]. AO can prevent platelet hyperactivity and oxidative stress in both platelets and the liver of HF-fed mice, confirming results previously published [16]. In the present study, rats fed the HF diet (40% fat, essentially saturated fats) developed several CVD risk factors such as central obesity, IR, and non-alcoholic fatty acid liver diseases (NAFLDs), but not dyslipidemia. We found that replacing 5% of the fat content of the HF diet with fish oil prevented many features of CVD risk factors in HF-fed rats. First, fish oil treatment prevented a gain in BW and AT weight induced in this dietary model. This is consistent with the data reported by Raclot et al. [28] but differs from other studies that either failed to observe this [29] or simply did not study adiposity evaluation [30]. In HFFO animals, final BW and AT weight were reduced compared with that of HF animals. Previous studies already reported that supplementing the diet with marine ω-3 PUFA extracted from FO has beneficial effects on parameters involved in CVD [31]. It has been established that consumption of a HF diet has the serious consequence of increased blood lipids. However, we did not observe any difference in blood lipids between the HF and control groups. Walrand et al. [32] showed that short-chain SFAs (with <12 carbon atoms) appear to be neutral with regard to blood levels of LDL cholesterol, high-density lipoprotein cholesterol, and TGs. After being ingested, these SFAs can be used immediately for β-oxidation to supply energy, and therefore do not have a direct effect on lipid metabolism [33]. The HF diet used in this study contained 40% of lipids, including 33% of hydrogenated coconut oil, which is known to be rich in short-chain SFAs, especially in lauric acid, which is almost totally catabolized. This could explain the blood lipid profile obtained in the HF group.

However, the HF diet did effectively trigger HF-induced CVD, insofar as we observed IR and NAFLD in the rats on the HF diet. Our data showed that the FO significantly decreased insulin and lipid levels in plasma.

In the liver, FO markedly reduced TG and cholesterol levels. Omega-3 PUFAs from marine sources are already known to be involved in managing CVD risk factors by reducing lipid levels in the blood and liver, and by enhancing insulin sensitivity. Accumulating evidence shows that FO supplementation reduces lipidemia and prevents hepatic steatosis by lowering lipid metabolism in the liver [33]. These effects of FO could be associated with the regulation of the hepatic gene involved in lipid metabolism. Stearoyl-coenzyme A desaturase (SCD) is an endoplasmic reticulum enzyme that catalyzes the biosynthesis of MUFAs from SFAs that are either synthesized de novo or derived from the diet. The regulation of this process plays a critical role in disorders such as obesity, diabetes, and atherosclerosis. SCD1, the main SCD isoform expressed in liver, is a key player in the regulation of lipid partitioning in the liver. The importance of SCD1 in neutral lipid synthesis in the liver has been confirmed by studies in SCD1−/− mice, in which an SCD1 deficiency leads to a fall in the hepatic TG and cholesterol ester contents and downregulates de novo fatty acid synthesis [34]. In addition to ω-3 PUFAs, fish oils contain some minor components such as vitamin D [5]. The decrease in atherogenic lipids observed in the HFFO group might be due to the effect of vitamin D. Indeed, an association between vitamin D deficiency and atherogenic dyslipidemia has been suggested [35]. However, the mechanisms by which vitamin D may affect the lipid metabolism are largely unknown [35]. Existing evidence suggested that treatment with 1,25-dihydroxyvitamin D [1,25$(OH)_2$ D], the active form of vitamin D, could increase the lipoprotein lipase expression and activity in cells [36,37]. Vitamin D deficiency has been reported to exert negative effects on β-cell function and insulin sensitivity, whereas it may be causally related to the development of type 2 diabetes mellitus [38].

The composition of the fatty acids in the liver phospholipids showed that FO intake is associated with enrichment in ω-3 PUFA, essentially in EPA, the main fatty acid in the FO used in the present study. The DHA level was also higher in liver phospholipids of HFFO-fed rats compared with the other groups. The increased DHA level could be due to a conversion of the EPA provided from FO into DHA, or provided directly from the FO intake. When the ω-3 PUFAs are ingested, the unsaturation of membrane fatty acids is increased as a result of the ω-3 PUFA incorporation. There may be an increased need for antioxidants (i.e., α-tocopherol) to prevent oxidative stress [39]. Indeed, this corroborates with our findings concerning the liver and the platelet lipid peroxidation. We found first that HF diet increases oxidative status in liver and platelets. Thus, FO intake showed no effect on MDA levels in tissues but enhanced GPx activity.

As a result, inhibitors of platelet aggregation are becoming important in the prevention and treatment of many atherothrombotic disorders [40]. The present results showed that HF diet intake increased platelet activity compared with standard diet intake. Thus, the platelet TxB_2 level, the stable form of the TxA_2, and the platelet oxidative status were higher in the HF group than in the control animals. FO intake showed a preventive effect against the HF diet–induced platelet hyperactivity and TxA_2 synthesis, and it also enhanced platelet antioxidant enzyme activity. Recent studies have demonstrated that the dietary intake of a marine source of ω-3 PUFAs can reduce the risk for coronary heart disease as a result of their antiatherogenic, anti-inflammatory, and antithrombotic effects [16]. Platelet aggregation is an early event in the onset of thrombosis and is initiated by TxA_2, a potent aggregation agent and vasoconstrictor [41]. Evidence from dietary intervention studies has shown that the consumption of marine ω-3 PUFAs reduces the production of TxA_2 [42], increases bleeding time [43], and decreases platelet aggregation in vitro [44]. EPA and DHA both reduce platelet aggregation in hypertensive individuals [45], patients with diabetes [46], and in healthy controls both in vitro and ex vivo [47]. However, consuming an excessive dose of fish oil may have a negative effect. Studies have shown that at high doses, EPA and DHA may have a pro-oxidant effect [47].

Vitamin D also exerts protective effects on endothelial activation/dysfunction, an inflammatory process that precedes atherosclerosis, through several mechanisms both genomic and nongenomic [48]. Among the main alterations ascribable to endothelial dysfunction are the reduced availability of nitric oxide (NO) and increased production of reactive oxygen species [49,35].

The present study demonstrated that the intake of dietary FO could improve IR and decrease platelet aggregation and hepatic lipid levels. This improvement could be associated with the different compounds of FO. It is well known that fish oil may contain an amount of vitamin D, in addition to EPA and DHA [5], that may play a beneficial role in improving some risk factors for CVD. However, recent studies have shown that fresh fish consumption can improve some risk factors for CVD better than ω-3 supplementation, and fish oil is not a substitute for fresh fish consumption [50]. Therefore, the beneficial effects of fish oil could be associated to a synergistic effect due to the different components of this oil.

Results with AO-fed animals were different from those of FO-fed mice. First, unlike fish oil treatment, AO supplementation did not affect adiposity and plasma insulin levels compared with the HF group. Similarly, AO effects on lipid metabolism were quite different from those of FO, with only improvements in plasma lipid level without any effect on liver lipid level, unlike fish oil intake. Sour et al. [51] and El Midaoui et al. [52] found different results than ours in some respects. Sour et al. [51] reported that AO consumption decreased BW gain, AT weight, and plasma levels of glucose and insulin. Thus, El Midaoui et al. [52] reported that treatment with AO reduced hyperglycemia, hyperinsulinemia, and IR of glucose-fed rats. These differences could be largely explained by the important difference in experimental protocols used in these two studies compared with ours. Indeed, concerning Sour et al.'s study [51], the duration of the diet was longer. The nature and percentage of lipids in the HF diet were different as well as the dose of AO tested. With regard to El Midaoui et al. [52], these authors tested the effect of AO in glucose-fed rats, using different doses of AO.

Nevertheless, AO-fed animals demonstrated a marked improvement in oxidative status in liver and platelets. Unlike FO, AO feeding decreased lipid peroxidation in both liver and platelets. On the other hand, AO decreased platelet aggregation similarly as shown with FO, but the antiaggregation mechanism of both oils seems to be different because FO decreased TxA_2 synthesis but not AO.

Several factors may explain the different responses of HF-fed animals to the oils used as dietary supplements in this study. Whereas the beneficial effects of FO can be mostly related to its ω-3 PUFA content, it is less clear whether the effects of AO can be linked to its fatty acid content, essentially oleic and linoleic acids. The effect of these abundant acids on CVD risk factors such as dyslipidemia, platelet aggregation, IR, or hypertension is rather controversial [13]. On the other hand, the preventive effects of AO could be attributed to the antioxidant properties of its tocopherols and polyphenols [22] because a reduction of oxidative stress is known to improve platelet activity [53] Indeed, the polyphenols extracted from AO have been shown in vitro to inhibit LDL oxidation and to stimulate the reverse transport of cholesterol [54]. Additionally,

there is convincing evidence for a beneficial effect of dietary AO on altered blood lipids [55]. It is, in fact, these positive considerations that prompted us to study the biological activity of AO in the context of obesity-induced CVD. Indeed, given the involvement of dyslipidemia, platelet activity and oxidative stress in the pathogenesis of the CVD and diabetes, these ameliorations by AO may thus also play a role in the preventive effects observed herein.

Conclusion

The present study clearly demonstrated that diets containing FO or AO can prevent a distinct number of deleterious effects of feeding an HF diet. More specifically, consuming FO prevents the development of adiposity, restores insulin sensitivity, decreases plasma and liver lipid levels, and also prevents the prothrombotic effect. However, the consumption of AO does not affect adiposity or liver lipid levels but decreases plasma lipid levels and improves oxidative status, and platelet activity. FO and, to a lesser degree, AO thus represent promising nutritional tools in the prevention of CVD.

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